Annexure -IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Final Report of the work done on the Major Research Project.

1. Project Report No. 1st/2nd/3rd/Final: **Final**

2. UGC Reference No. F. **42-551/2013 (SR) Dt. 14/03/14**

3. Period of report: **From 14-03-2014 to 31-03-2017**

4. Title of Research: **Assessment of efficacy of some indigenous plant extracts against myiasis causing larvae of *Chrysomya bezziana* (Diptera: Calliphoridae).**

5. (a) Name of the Principal Investigator: **Dr. Amandeep Singh**

   (b) Department: **Department of Zoology**

   (b) College where work has progressed: **Khalsa College Amritsar, Punjab**

6. Effective date of starting the project: **14-03-2014**

7. Grant approved and expenditure incurred during the period of report:

   (a) Total Amount Approved **: Rs. 1058467/-**

   (b) Total Expenditure **: Rs. 982900/-**

   (c) Report of the work done:

(i) Brief objective of the project:

The use of synthetic anti-myiatic compounds have limited efficacy in reducing myiasis infestation and is often accompanied by serious drawbacks, including environmental contamination and contamination of milk and meat products with drug residues resulting in serious adverse effects on human population. Development of an eco-friendly strategy for the control of myiasis is the urgent need of the hour so as to prevent huge economic losses to the farmers as well as to eliminate the adverse effects of synthetic anti myiatic agents on human population. Through developing an herbal control strategy for myiasis causing larvae, the present study will definitely be of great significance for society as well as for maintaining the delicate ecological balance.

(ii) Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication:

Work Done **(Attached Annexure I)**

Papers Published:

1. **Singh, A.,** Kaur, J. (2016). The bioefficacy of crude extracts of *Azadirachta indica* (Meliaceae) on the survival and development of myiasis causing larvae of *Chrysomya bezziana* (Diptera: Calliphoridae). ***Tropical Animal Health and Production***, 48(1): 117-124. **(Springer- Impact Factor: 0.8)**

2. **Singh, A.,** Kaur, J. (2016). Toxicity of leaf extracts of *Ricinus communis* L. (Euphorbiaceae) against third instar larvae of *Musca domestica* (Diptera: Muscidae). ***American Journal of BioScience***, 4(1-3): 5-10. (ISSN: 2330-0159)

3. **Singh, A.,** Kaur, J. (2017). Activity of foliage extracts of *Ricinus communis* L. (Euphorbiaceae) against myiasis causing larvae of *Chrysomya bezziana* Villeneuvae (Diptera: Calliphoridae). ***Journal of Veterinary Medicine and Research,*** 4(1): 1070. (ISSN: 2373-931X).

(iii) Has the progress been according to original plan of work and towards achieving the objective? if not, state reasons:

Yes, the project has been completed as planned and all objectives were met.

(iv) Please indicate the difficulties, if any, experienced in implementing the project: **NIL**

(v) If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet: **NA**

(vi) If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission.

(vii) Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

(a) Manpower trained: 01

(b) Ph. D. awarded: Nil

(c) Publication of results: 03

PRINCIPAL INVESTIGATOR **PRINCIPAL**

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. TITLE OF THE PROJECT: Assessment of efficacy of some indigenous plant extracts against myiasis causing larvae of *Chrysomya bezziana* (Diptera: Calliphoridae).

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR: Dr. Amandeep Singh, Department of Zoology, Khalsa College Amritsar (Punjab).

3. NAME AND ADDRESS OF THE INSTITUTION: Khalsa College Amritsar

4. UGC APPROVAL NO. AND DATE **:** 42-551/2013 (SR) Dt. 14/03/14

4. DATE OF IMPLEMENTATION **: 14/03/14**

5. TENURE OF THE PROJECT **: 03 Years (36 Months)**

6. TOTAL GRANT ALLOCATED **: Rs. 1058467/-**

7. TOTAL GRANT RECEIVED **: Rs. 9,82,900/-**

8. FINAL EXPENDITURE **: Rs. 8,42,890/-**

10. OBJECTIVES OF THE PROJECT:

Following objectives were proposed.

- To evaluate the efficacy of following plant extracts on the survival and development of larvae of *Chrysomya bezziana.*
  (a) Azadirachta indica (Neem),
  (b) Ricinus communis (Arind)
  (c) Ocimum sanctum (Tulsi)
- To determine the percentage mortality of larvae resulting from treatment with plant extracts.
- To calculate LC50 of above mentioned plant extracts against larvae.
- To compare the effectiveness of different plant extracts for control of larvae of *Chrysomya bezziana.*
- An attempt will be made with a near pure product from the selected plant on the larvae for determining their efficacy.

11. WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAILS)

Yes, all above mentioned objectives were fully achieved.

12. ACHIEVEMENTS FROM THE PROJECT:

The plant extracts resulted into considerable larval and pupal mortalities as well as reduced adult emergence, thereby indicating their possible future use as myiasis control agents. Moreover, the plant derived materials being biodegradable can prove to be better alternative to synthetic myiasis control agents, which generally cause the contamination of dairy products with their residues, resulting into severe health hazards among human population.

13. SUMMARY OF THE FINDINGS:

This project evaluated the efficacy of indigenous plant extracts like *Azadirachta indica*, *Ricinus communis* and *Ocimum sanctum* against the myiasis causing larvae of the fly-*Chrysomya bezziana*, using dipping and thin film technique. The results showed that the extract of the plants in all the solvents had a toxic effect against the third instar larvae of *C. bezziana* in both the methods. The plant extracts resulted into considerable larval and pupal mortalities as well as reduced adult emergence among exposed third instar larvae. The choice of dipping technique is based on 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.

In case of *Azadirachta indica*, the highest mortalities were recorded in methanol extract followed by chloroform, petroleum ether and ethyl acetate extracts with LC50 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 dipping method. In the thin film technique, 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. 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 in dipping method. Accordingly, the effect of extracts were in the order of methanol>chloroform>ethyl acetate>petroleum ether in thin film technique.

LC_{50} values on exposure to extracts of *Ricinus communis* 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/cm2, 0.9 mg/cm2, 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.

The LC_{50} values recorded in case of *O. sanctum* in dipping method as 0.4g/100ml, 0.2g/100ml, 0.5g/100ml, 0.6 g/100ml in petroleum ether, chloroform, ethyl acetate and methanol extract respectively. Thus, according to larval mortalities the effect of extracts of *O. sanctum* on larvae of *C. bezziana* can be arranged as chloroform> petroleum ether >ethyl acetate> methanol. Similarly, the LC_{50} values in case of thin film technique were recorded as 0.2mg/cm^2,0.07mg/cm^2, 0.4mg/cm^2, 0.5 mg/cm^2 in petroleum ether, chloroform, ethyl acetate and methanol extract respectively. So, according to LC_{50} values the effects of the extracts were in the order chloroform > petroleum ether> ethyl acetate > methanol.

It is concluded that the crude extracts of the plants tested in the present study can be useful in controlling myiasis-causing larvae of *C. bezziana*. The outcomes of this study are promising as this point towards the potential of the plants 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.

14. CONTRIBUTION TO THE SOCIETY (GIVE DETAILS):

The crude extracts of the plants can be applied locally to myiasis affected 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 and eco-friendly control strategy. The plant extracts will be useful for dairy farmers and livestock owners in controlling infestations of *C. bezziana* on their livestock under normal working conditions.

15. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT:

No

16. NO. OF PUBLICATIONS OUT OF THE PROJECT: (PLEASE ATTACH RE-PRINTS)

Papers Published: 03 (Re-prints attached)

1. **Singh, A.,** Kaur, J. (2016). The bioefficacy of crude extracts of *Azadirachta indica* (Meliaceae) on the survival and development of myiasis causing larvae of *Chrysomya bezziana* (Diptera: Calliphoridae). ***Tropical Animal Health and Production***, 48(1): 117-124. **(Springer- Impact Factor: 0.8)**

2. **Singh, A.,** Kaur, J. (2016). Toxicity of leaf extracts of *Ricinus communis* L. (Euphorbiaceae) against third instar larvae of *Musca domestica* (Diptera: Muscidae). ***American Journal of BioScience***, 4(1-3): 5-10. (ISSN: 2330-0159)

3. **Singh, A.,** Kaur, J. (2017). Activity of foliage extracts of *Ricinus communis* L. (Euphorbiaceae) against myiasis causing larvae of *Chrysomya bezziana* Villeneuvae (Diptera: Calliphoridae). ***Journal of Veterinary Medicine and Research,*** 4(1): 1070. (ISSN: 2373-931X).

(PRINCIPAL INVESTIGATOR) **PRINCIPAL**

Annexure-III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG NEW
DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator- **Dr. Amandeep Singh**

2. Deptt. of College **: Deptt. of Zoology, Khalsa College Amritsar.**

3. UGC approval No. and Date: **42-551/2013 (SR) Dt. 14/03/14**

4. Title of the Research Project: **Assessment of efficacy of some indigenous plant extracts against myiasis causing larvae of *Chrysomya bezziana* (Diptera: Calliphoridae).**

5. Effective date of starting the project: **14-03-2014**

6. a) Period of Expenditure: **From 14-03-2014 to 31-03-2017**

   b) Details of Expenditure:

S.No.	Item	Amount Approved Rs.	Amount Received Rs.	Expenditure Incurred Rs.
i.	Books & Journals	40,000/-	40,000/-	39,266/-
ii.	Equipment	1,75,000/-	1,75,000/-	1,75,588/-
iii.	Contingency	1,00,000/-	90,000/-	82991/-
iv.	Field Work/Travel	1,00,000/-	90,000/-	41159/-
v.	Hiring Services	1,00,000/-	90,000/-	68948/-
vi.	Chemicals & Glassware	1,50,000/-	1,35,000/-	93029/-
vii.	Overhead	87,800/-	87,800/-	66,809/-
viii.	Honorarium to Project Fellow	3,05,667/-	2,75,100/-	2,75,100/-
Total		**1058467/-**	**9,82,900/-**	**8,42,890/-**

c) Staff

Date of appointment:-

S.No.	Items	From	To	Amount Approved (Rs.)	Amount Received (Rs.)	Expenditure Incurred (Rs.)
1	**Project Fellow @14000/- per month**	**06-06-14**	**20-02-16**	3,05,667/-	2,75,100/-	2,75,100/-

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.

2. If as a result of check or audit objective, some irregularly is noticed, later date, action will be taken to refund, adjust or regularize the objected amounts.

3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

4. It is certified that the grant of Rs. 9,82,900/- (Rupees Nine Lac Eighty Two Thousands and Nine Hundred Only) received from the University Grants Commission under the only scheme of support for Major Research Project entitled **Assessment of efficacy of some indigenous plant extracts against myiasis causing larvae of *Chrysomya bezziana* (Diptera: Calliphoridae)** vide UGC letter No. F. **42-551/2013 (SR) Dt. 14/03/14**. Out of received grant a sum of Rs. **8,42,890**/-(Rupees Eight Lac Forty Two Thousands Eight hundred and Ninety only)) has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

PRINCIPAL INVESTIGATOR **PRINCIPAL**

Work Done

Introduction

Myiasis is the infestation of live human and other vertebrate animals with dipterous larvae which at least for certain period feed on the host's dead or living tissue, liquid body substances or ingested food (Zumpt, 1965). The term myiasis was first proposed by Hope (1840) to refer to the disease of man and animals originating specifically with dipterous larvae. The dipterous larvae, often called 'maggots', complete or at least for a certain period continue their normal development on or in the vertebrate body. Myiasis is common in domestic and wild animals all over the world especially in the tropical countries. In man, it is frequent in rural and slum areas where unhygienic conditions are prevalent and domestic animals are in close human contact. The gravid flies may be attracted to open wounds or even natural body openings such as eye, nose, ear, vagina, anus etc. to lay eggs. On hatching, the larva invade the broken skin and with their chitinous mandibular oral hooks either burrow in the dermal layers or pre-existing wounds resulting into enlargement of wounds.

The old world screwworm fly- *Chrysomya bezziana* (Diptera: Calliphoridae) is known for causing myiasis among man and animals in tropical regions all over the world. *C. bezziana* is an obligatory myiasis causing species infesting generally wild and domestic animals. It occasionally infests the neglected wounds of man where unhygienic conditions are prevalent and domestic animals are in close vicinity. Myiasis is having a widespread incidence in a country like India which fulfils all the favourable conditions for the abundant growth of myiasis causing flies and their larvae. The gravid flies are attracted to neglected wounds or even natural body openings such as eye, ear, nose, vagina, anus etc. to lay their eggs. On hatching, the larvae invade the broken skin and start feeding on the underlying tissue. Animals infested by larvae of *C. bezziana* show restlessness, irritation at the site, dermatitis and purulent infection which retard the growth of the animal, decrease the body weight and result in to loss in milk and meat production. In severe cases it may result into death, if the vital organs like brain or lungs are invaded. **Disease as well as mortality among domestic animals leads to the economic losses to the farmers in a country like India where a major component of the population depends upon agricultural income for their survival. Development of a strategy for the control of myiasis is the urgent need of the hour so as to prevent huge economic losses to the poor farmers. 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. Plant derived materials being biodegradable, are strongly considered the alternate remedy to synthetic products in the control of myiasis causing larvae. Flora grown in the Indian subcontinent includes 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.

There are large numbers of studies available in the literature regarding the use of plant products in control of various agricultural pests as well as parasites of domestic animals. However, limited reports were found regarding the use of plants for the control of some myiasis causing species. **No report was found for the herbal control of obligatory myiasis causing species *Chrysomya bezziana*.** Keeping in view the threat posed to the health of domestic animals and apprehension of considerable economic loss to the poor farmers, the present study has been planned to be undertaken at the Department of Zoology, Khalsa College, Amritsar. The study basically aims to evaluate the efficacy of crude extracts of these plants in the control of *Chrysomya bezziana.*

Interdisciplinary relevance:-

The present study is of great importance in medical and veterinary fields as infestations with maggots cause severe pain and mental agony among humans besides hammering economic loss significantly among domestic animals.

Review of Research and Development in the Subject:-

International Status:-

Biological effects of white mustard oil, *Brassica alba* against the cotton leafworm, *Spodoptera littoralis* was studied by El-Aziz *et al.* (1997) from Berlin, and concluded that spray of white mustard oil on cotton plant can reduce the egg deposition as well as viability of *S. littoralis* eggs and can protect the cotton plant from being attacked by the same. Similarly Schmidt *et al.* (1997) studied the effect of *Melia azadarach* extract on larval development and reproduction parameters of *Spodoptera littoralis and Agrotis ipsilon* (Lepidoptera: Noctuidae).

Morsy *et al.* (1998a) conducted a study regarding the larvicidal activity of solvent extracts of three medicinal plants, *Symbopogon ctratus, Artemisia cinae* and *Punica granatum*

against third instar larvae of *Chrysomyia albiceps* in Egypt and reported them to be having considerable effects for the same.

Morsy *et al.* (1998b) studied the effect of volatile oil of *Chenopodium ambrosioides* and *Thymus vulgaris* against larvae of *Lucilia sericata* from Egypt and reported their effectiveness in biological control of myiasis causing larvae in the region.

El-Khateeb *et al.* (2003) carried out a study regarding the insecticidal effects of Neem seeds and vegetable oils on larval and pupal stages of sheep blowfly *Lucilia sericata* (Diptera: Calliphoridae) in Egypt and reported them to be effective biological control agents against the same.

Bright *et al.* (2004) conducted a study in Brazil regarding the activity of *Ricinus communis* (Euphorbiaceae) against the leaf cutting ant *Atta sexdens rubropilosa* (Hymenoptera-Formicidae) and illustrated the lethal effects of Ricinine, one of the components of *Ricinus communis* on the same.

Siriwattanerungsee *et al.* (2008) from Thailand carried out a study regarding estimation of efficacy of *Azadirachta indica* against blowfliy and housefly. They concluded that incorporation of Neem extract in larval food of both the categories of flies could lead to greater inhibition of growth and fecundity in subsequent generations.

Abdel- Shafy *et al.* (2009) from Egypt conducted a study to assess the efficacy of some wild medicinal plants extracts like *Artemisia monosperma, Artemisia herba-alba, Euphorbia aegyptica* and *Francoenria crispa* on the survival and development of third instar larvar of *Chrysomyia albiceps* (Diptera: Calliphoridae) and concluded that the extracts of these plants can be effectively use in control against 3rd instar larvae of *Chrysomyia albiceps.*

Atawodi *et al.* (2009) from Nigeria reported *Azadirachta indica* as a plant of multiple biological and pharmacological activities and concluded that numerous opportunities presented by the same can be harnessed to solve various problems including those of agricultural, health and economic sectors.

Dad *et al.* (2011) determined LC_{50} of Chloropyriphos and Neem extracts on 3rd instar larvae of house flies- *Musca domestica* and their effect on fecundity in Pakistan. Neem extract was found to be responsible for highest mortality among the third instar larvae of *Musca domestica.*

National Status:-

Anees (2008) studied the larvicidal avtivity of *Ocimum sanctum* against Dengue vector- *Aedes aegypti* and filariasis vector *Culex quinquefasciatus* in Tamil Nadu and reported it to be effective biological control agent against the same.

Bagavan *et al.* (2009) from Tamil Nadu studied the larvicidal and nymphicidal potential of extracts of plants like *Citrus sinensis, Ocimum canum, Ocimum sanctum* and *Rhinancanthus nasutus* against 4th instar larvae of Malaria Vector, *Anopheles subpictus* Grassi, Japanese encephalitis vector *Culex tritaeniorhynchus* Giles and nymphs of cotton pests, *Aphis gossypii* Glover. They reported the plants to be potential biological control tools against the above mentioned species.

Bansal *et al.* (2009) evaluated the larvicidal efficacy of *Solanum xanthocarpum* against 4th instar larvae of *Anopheles culicifacies, A. stephensi, A. aegypti*, and *Culex quinquefasciatus* in North-Western Rajasthan.

Dua *et al.* (2009) studied the larvicidal activity of Neem oil (*Azadirachta indica*) against 3rd and 4th instar larvae of mosquitoes *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* in Delhi and found that the Neem oil formulation was effective in controlling mosquito larvae in different breeding sites under natural field conditions.

Rahuman *et al.* (2009) conducted a study to evaluate the efficacy of plant extracts of *Calotropis procera, Canna indica, Hibiscus rosa sinensis, Ipomea carnea* and *Sarcostemma brevistigma* against 2nd and 4th instar larvae of filariasis vector mosquito species, *Culex quinquefasciatus* in Tamil Nadu. They suggested that these plants can effectively be used against control of filariasis vector.

Zahir *et al.* (2009) carried out a detailed study from Tamil Nadu regarding laboratory determination of efficacy of various indigenous plant extracts like *Achyranthus aspersa, Anisomeles malabarica, Gloriosa superba, Ricinus communis* and *Solanum trilobatum* against the larvae of cattle tick-*Rhipicephalus microplus*, sheep internal parasite- *Paramphistomum cerv*i and 4th instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus*. They concluded that these plant extracts have the potential to be used as ideal eco-friendly approach for the control of aforesaid parasites.

Zahir *et al.* (2010) from Tamil Nadu conducted a study regarding evaluation of plant extracts like *Anisomeles malabarica, Gloriosa superba, Ricinus communis* and *Solanum trilobatum* against cattle tick- *Haemaphysalis bispinosa* and Hematophagus fly *Hippobosca maculata* and concluded that the plants had considerable potential for control of these parasites.

Maheswaran *et al.* (2012) from Chennai carried out a study to evaluate the efficacy of a novel herbal formulation from *Azadirachta indica* against dengue vector mosquito- *Aedes aegypti* and *Aedes albopictus* and reported it to be having 100% ovicidal, larvicidal and oviposition deterrent activities.

Significance of the Study

The use of synthetic anti-myiatic compounds have limited efficacy in reducing myiasis infestation and is often accompanied by serious drawbacks, including environmental contamination and contamination of milk and meat products with drug residues. Myiasis being one of the most widespread parasitosis affecting domestic animals, results in heavy production losses leading to huge economic losses to the farmers. **Development of a strategy for the control of myiasis is the urgent need of the hour so as to prevent huge economic losses to the poor farmers. 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. Plant derived materials being biodegradable, are strongly considered the alternate remedy to synthetic products in the control of myiasis causing larvae.**

Potential contribution to knowledge in the field of social relevance or national importance

Problem of myiasis is a matter of great concern among medical and veterinary fields. At the same time it is of great economic importance in an agriculture based country like India, where the economic status of a big chunk of population depends on the cattle industry. Myiasis has long been recognized as a cause of decreased productivity in the cattle industry due to pathological effects and management costs. It has been posing a major threat to livestock industry in India. **The need of the hour is to develop a herbal control strategy for myiasis causing larvae, so that the menace can be controlled both among man and domestic animals.**

(iii) Objectives

The objectives of the present study are as follows:-

- To evaluate the efficacy of following plant extracts on the survival and development of larvae of *Chrysomyia bezziana.*
  (d) Azadirachta indica (Neem),
  (e) Ricinus communis (Arind)
  (f) Ocimum sanctum (Tulsi)
- To determine the percentage mortality of larvae resulting from treatment with plant extracts.
- To calculate LC50 of above mentioned plant extracts against larvae.
- To compare the effectiveness of different plant extracts for control of larvae of *Chrysomya bezziana.*
- An attempt will be made with a near pure product from the selected plant on the larvae for determining their efficacy.

(iv) 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

Aerial parts of indigenous plants; *Azadirachta indica* (neem), *Ricinus communis* (Euphorbiaceae) and *Ocimum sanctum* (Labiatae) 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 totaling 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 totaling 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 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:

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

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). LC50 values were calculated using probit analysis (Finney 1971). Values with $P<0.05$ were considered to be statistically significant.

Results and Discussion

(a) *Azadirachta indica*

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 and 2. Figure 1 shows the toxicity in terms of LC50 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 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.

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 LC50 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 ($P<0.05$).

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. LC50 values were recorded as methanol 0.4 mg/cm^2, chloroform 0.6 mg/cm^2, ethylacetate 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.

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 active constituents may penetrate into the body of 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 dam-

age 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 maxi- mum 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 LC50 values of 79.17 and 63.17 ppm and LC90 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.

(b) *Ricinus communis*

The effects of crude leaf extracts of *Ricinus communis* in four solvents on larval survival and development by dipping and thin film method are shown in tables 3 and 4. Figures 3 and 4 shows the toxicity in terms of LC50 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. LC50 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/cm2, 0.9 mg/cm2, 1.2 mg/cm2 and 2.1 mg/cm2 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.

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 this 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 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. The chloroform extract showed highest larval and pupal mortality at lowest concentration. 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 LC50 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 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 LC50 144.11 ppm, followed by methanol extract with LC50 at 91.62 ppm. The petroleum ether extract was the least efficient with LC50 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 neuroendocrine 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*.

(c) *Ocimum sanctum*

Tables 5 and 6 shows the percentage larval and pupal mortalities of *C. bezziana* when exposed to crude extract *O. sanctum* of in four different solvents viz. petroleum ether, chloroform, ethyl acetate and methanol in both dipping and thin film technique. The lethal concentration (LC_{50}) values in different solvent of *O. sanctum* in the methods were shown in figure 5 and 6. 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 as 0.4g/100ml, 0.2g/100ml, 0.5g/100ml, 0.6 g/100ml in petroleum ether, chloroform, ethyl acetate and methanol extract respectively. Thus, according to larval mortalities the effect of extracts of *O. sanctum* on larvae of *C. bezziana* can be arranged as chloroform> petroleum ether >ethyl acetate> methanol. Larvae who from death pupated normally but all of them did not emerge in all concentrations showing pupal mortality. Similarly, the LC_{50} values in case of thin film technique were recorded as 0.2mg/cm^2,0.07mg/cm^2, 0.4mg/cm^2, 0.5 mg/cm^2 in petroleum ether, chloroform, ethyl acetate and methanol extract respectively. According to LC_{50} values the effects of the extracts were in the order chloroform > petroleum ether> ethyl acetate > methanol. In thin film technique method the larval mortalities were almost higher as compared to dipping method as shown in table 6. The mean larval mortality of the *C. bezziana* increases as

the concentration of the extract increases from 0.156g/100ml to10g/100ml in case of dipping method and 0.075mg/cm^2 to 1.78 mg/cm^2 in case of thin film technique in different solvents.

Conclusion

It is concluded that the crude extracts of the plants tested in the present study can be useful in controlling myiasis-causing larvae of *C. bezziana*. The crude extracts of the plants can be applied locally to myiasis affected 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 and eco-friendly control strategy. It is further recommended that the extracst 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 plant-based products on myiasis-causing flies.

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 *Chrysomyia albiceps* (Wied) (Diptera: Calliphoridae). *Tropical Animal Health and Production*, 41: 1741-1753.

Abdel-shafy, S., Soliman, M.M.M.and Habeeb, S.M., 2007. In vitro acaricidal effect of some crude extracts and essential oils of wild plants against certain tick species. *Acarologia*, 47 (1-2): 31-42.

Alouani, A., Rehimi, N., and Soltani, N., 2009. Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in the Republic of Algeria. *Jordan Journal of Biological Sciences*, 2 (1): 15 -22.

Anees, A.M., 2008. Larvicidal activity of *Ocimum sanctum* Linn. (Labiatae) against *Aedes aegypti (L.)* and *Culex quinquefasciatus. Parasitology Research*, 103: 1451-1453.

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.

Bagavan, A., Kamaraj, C., Rahuman, A.R., Elango, G., Zahir, A.A. and Pandiyan, G., 2009. Evaluation of larvicidal and nymphicidal potential of plant extracts against *Anopheles subpictus* Grassi, *Culex tritaeniorhynchus* Giles and *Aphis gossypii* Glover. *Parasitology Research*, 10: 1109-1117.

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.

Boadu, K.O., Tulashie, S.K., Anang, M.A. and Kpan, J.D., 2011. Production of natural insecticide from Neem leaves (*Azadirachta indica*). *Asian Journal of Plant Science and Research*, 1 (4): 33-38.

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

Dad, I., Yousuf, M.J. and Anjum, S.I., 2011. Determination of LC_{50} of Chloropyriphos and Neem extracts on third instar larvae of house flies and their effect on fecundity. *Journal of Basic and Applied Science*, 7 (2): 169-174.

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.

Dela, M.A., Koffivi, K.G., Komina, A., Arnaud, A., Philippe, G. and Adole, G.I., 2014. Evaluation of neem leaves –based preparations as insecticidal agents against the green peach aphid, *Myzus persica* (Sternorrhyncha: Aphididae). *African Journal of Agricultural Research*, 9 (17): 1344-1352.

Dua, V.K., Pandey, A.C., Raghavendra, K., Gupta, A., Sharma, T. and Dash, A.P., 2009. Larvicidal activity of Neem oil *(Azadirachta indica*) formulation against mosquitoes. *Malaria Journal*, 8:124-130.

El-Aziz, S.E.A. and Sharaby, A.M., 1997. Some biological effects of white mustard oil, *Brassica alba* against cotton leafworm, *Spodoptera littoralis* (Boisd.). Anz. Schadlingskde, Pflanzenschutz Umweltschutz, 70: 62-64.

Esparza-Diaz, G., Juan, A.V.J., Lopez-Collado, J. and Daniel, A.R.L., 2010. Azadiracttin 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*, 3:, 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.

James, M.T., 1947. The flies that causes myiasis in man. United State Department of Agriculture. Misc. Pub, No. 63 Washington.

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.

Maragathavalli, S., Brindha, S., Kaviyarasi, N.S., Annaduari, B. and Gangwar, S.K., 2012. Mosquitoes larvicidal activity of leaf extract of neem (*Azadirachta indica*). *International Jouranal of Advance Research in Biological Sciences*, 2 (1): 138-142.

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., 1998. The larvicidal activity of solvent extracts of three medicinal plants against third instar larvae of *Chrysomyia albiceps*. *Journal of Egyptian Society of Parasitology*, 28 (3): 699-709.

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., Jessinta, and Nour, A.H., 2012. larvicidal activity of extract from different parts of neem (*Azadirachta indica)* against *Aedes aegypti* mosquito larvae. Scientific Research and Essay, 7 (31): 2810-2815.

Rahuman, A.A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir, A.A., and Elango, G., 2009. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research*, 104: 1365-1372.

Ribeiro, B.C.C., Sanavria, A., Helena, H., Monteiro, M.S., Oliveira, M.O. and De Souza, F.S., 2003. An enquiry of cases of myiasis by *Dermatobia hominis* in dogs (*Canis familiaris*) of the Northern and Western zones of Rio de Janeiro city in 2000. Brazilian *Journal of Veterinary Research and Animal Sciences*, 40 (1): 144-150.

Salako, E.A., 2002. Plant protection for the resources- poor farmer. A keynote address at Nigerian Society for plant Protection. (University of Agriculture Abeokuta).

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

Shah Biren. and Seth, A. K., 2013. Textbook of Pharmacognosy and Phytochemistry. (Elsevier India), pp 525-526.

Sharma, T., Qamar, A., and Khan, A.M., 2010. Evaulation of Neem (*Azadirachta indica*) extract against the eggs and adults of *Dysdercus cingulatus* (Fabricius). *World Applied Science Journal*, 9 (4): 398-402.

Singh, S. and Singh, R.P., 1998. Neem (*Azadirachta indica*) Seed kernel extracts and Azadrachtin as oviposition deterrents against the melon fruit fly (*Bactrocera cucurbitae*) and Oriental fruit fly (*Bactrocera dorsali*s) *Phytoparasitica*, 26 (3): 191-197.

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.

Siriwattanarungsee, S., Sukontason, K.L., Olson, J.K., Chailpakul, O. and Sukontason, K., 2008. Efficacy of Neem extract against the blowfly and housefly. *Parasitology Research*, 103: 535-544.

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

Sukontason, K.L., Boonchu, N., Sukontason, k., Choochote, W., 2004. Effects of Eucalyptol on House fly (Diptera:Muscidae) and Blow Fly (Diptera:Calliphoridae). *Journal of the São Paulo Institute of Tropical Medicine* , 46 (2): 97-101.

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

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 beetle*) 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.

Table 1

Plant species	Family	Plant material used	Target Arthropods	References
Cymbopogon citratus *Artemisia cina* *Punicia granatum*	Poaceae Asteraceae Lythraceae	Aerial parts	*Chrysomyia albicepes*	Morsy et al. 1998a
Chenopodium ambrosioides *Thymus vulgaris*	Solanaceae Lamiaceae	Aerial parts	*Lucilia sericata*	Morsy et al.1998b
Nerium oleander	Apocynaceae	Aerial parts	*Chrysomya albicepes*	El-Shazly et al 2000
Eucalyptus	Myrtaceae	Aerial parts	*Musca domestica* *Chrysomya megacephala*	Sukontason et al. 2004
Azadirachta indica	Meliaceae	Aerial parts	*Musca domestica* *C. megacephala*	Siriwattanarungee et al. 2008
A. indica	Meliaceae	Aerial parts	*Culex pipiens*	Alouani et al. 2009
A. indica	Meliaceae	Neem fruit	*Culex quinquefaciatus*	Batabyal et al. 2009
A. indica	Meliaceae	Neem oil	*Culex quinquefaciatus,* *Anopheles stephensi,* *Aedes aegypticus*	Dua et al. 2009
Artemisia monosperma	Asteraceae	Aerial parts	*Chrysomya albiceps*	Abdel-Shafy et al. 2009
Artemisia herba - alba	Asteraceae			
Euphorbia aegytiaca	Euphorbiaceae			
Francoeuria crespa	Apocynaceae			
A. indica	Meliaceae	Leave, rhizome	*Culex gelidus* , *Culex quinquefaciatus*	Kamaraj et al. 2010
A. indica	Meliaceae	Aerial parts	*Dysdercus cingulatus*	Sharma et al. 2010
A. indica	Meliaceae	Aerial parts	*Musca domestica*	Dad et al. 2011
Ocimum suave	Labiatae	Aerial parts	*Musca domestica*	Ojianwauna et al. 2011
Aloe vera	Xanthorrhoeaceae	Aerial parts	*Musca domestica*	Jesikha 2012
A. indica	Meliaceae	Aerial parts	*Aedes Aegypti,* *Culex quinquefaciatus*	Maragathavalli et al. 2012
Aloe zebrina	Asphodelaceae	Aerial parts	*Lucilia cuprina,* *Chrysomya marginalis*	Mukandiwa et al. 2012
Aloe marlothii	Xanthorrhoeaceae			
Calpurnea aurea	Fabaceae			
Clausena anisata	Rutaceae			
Erythrina Lysistemon	Fabaceae			
Psydrax livida	Rubiaceae			
Spriostachys africana	Euphorbiaceae			
A. indica	Meliaceae	Whole plant	*Aedes aegypti*	Nour et al. 2012
Calotropis procera	Apocynaceae	Leaf, stem, root	*Musca domestica*	Islam and Akhtar 2013
Piper longum	Piperaceae			
Polygonum hydropiper	Polygonaceae			
Piper beetle	Piperaceae	Aerial parts	*Chrysomya bezziana*	Wardhana et al. 2013
A. indica	Meliaceae	Aerial parts	*Myzus persica*	Dela et al. 2014

Table 1 Studies showing the use of various plant extracts against parasitic Arthropods.

Table 2

Solvant	Conc. (g/100 ml)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
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	100 ± 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	100 ± 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	100 ± 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	100 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.001

Table 2 Effect of crude extracts of *A. indica* on development of third instar larvae of *C. bezziana* using Dipping Method

Table 3

Solvant	Conc. (mg/cm^2)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
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	100 ± 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	100 ± 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	100 ± 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	100 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.001

Table 3 Effect of crude extracts of *A. indica* on development of third instar larvae of *C. bezziana* using Thin Film Technique

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 4 Effect of crude extracts of *R. communis* on development of third instar larvae of *C. bezziana* using Dipping Method

Table 5

Solvents	Conc. (mg/cm^2)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergence (%)
Petroleum 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

Table 5 Effect of crude extracts of *R. communis* on development of third instar larvae of *C. bezziana* using Thin Film Application Method

Table: 6

Solvant	Conc. (g/100 mi)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
Petroleum ether	2.5	69.60± 5.55	30.66 ±5.42	92.00 ± 1.33	7.99± 1.33
	1.25	65.46 ±5.00	34.53± 4.95	90.66 ± 1.63	9.33± 1.63
	0.625	53.33± 3.65	46.66 ± 3.65	81.33 ± 3.89	18.66± 3.89
	0.3125	48.33± 3.65	51.67 ± 3.65	90.66 ± 1.63	9.33 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.001	0.000	0.000	0.001
Chloroform	1.25	85.33±3.89	14.66± 3.89	92.00 ± 1.33	7.99 ± 1.33
	0.625	73.73±5.29	26.66± 5.16	88.00± 2.49	12.00 ± 2.50
	0.3125	65.46 ±5.00	34.53± 4.95	78.66± 2.49	21.33 ± 2.49
	0.156	43.33 ±3.65	56.66 ± 3.65	90.66 ± 1.63	9.33 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.001	0.000	0.001
Ethyl Acetate	3.5	86.66 ±2.98	13.33± 2.98	92.00 ± 1.33	7.99 ± 1.33
	1.75	74.93 ±5.80	25.33± 5.73	90.66 ±1.63	9.33 ± 1.63
	0.875	58.33 ±3.89	41.66 ± 3.89	90.66 ±1.63	9.33± 1.63
	0.437	44.66 ±2.98	55.34 ± 2.98	89.33 ±1.63	10.66 ± 1.63
	Control	0.00 ± 00	0.00 ± 00	100 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.001
Methanol	3	85.33 ±3.89	3.89 ± 8.69	90.66 ± 1.63	9.33 ± 1.63
	1.5	78.00± 3.89	21.99± 3.89	90.66± 1.63	9.33 ± 1.63
	0.75	58.46 ±5.00	41.54± 4.95	89.33± 1.63	10.66±1.63
	0.375	40.66±2.98	59.34± 2.98	90.66 ± 1.63	9.33±1.63
	Control	0.00 ± 00	0.00 ± 00	100 ± 00	100 ± 00
	P value	0.000	0.001	0.000	0.001

Table 6 Effect of crude extracts of *O. sanctum* on development of third instar larvae of *C. bezziana* using Dipping Method

Table 7

Solvant	Conc. (g/100 mi)	Larval Mortality (%)	Pupation (%)	Pupal mortality (%)	Adult Emergence (%)
Petroleum ether	1.25	82.93 ± 3.35	17.20 ±3.31	90.66 ±1.63	9.33 ± 1.63
	0.6	72.26 ± 6.55	28.00 ± 6.46	89.33 ±1.63	10.66 ±1.63
	0.3	58.66 ± 2.49	41.95 ± 2.03	90.66 ±1.63	9.33 ±1.63
	0.15	44.44± 2.35	55.56± 1.63	90.66± 1.63	9.33 ±1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.001	0.000	0.000	0.001
Chloroform	0.6	85.46 ± 3.84	14.53 ± 3.79	92.00 ± 1.33	7.99 ± 1.33
	0.3	82.93 ± 3.35	17.20 ± 3.31	90.66 ± 1.63	9.33± 1.63
	0.15	64.13 ± 5.51	35.86 ± 5.47	88.00 ± 2.49	12.00 ± 2.50
	0.075	46.13 ± 5.51	53.87 ± 5.47	89.33 ± 1.63	10.66 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.002	0.000
Ethyl Acetate	1.78	81.33 ± 2.49	18.66 ± 2.49	90.66 ± 1.63	9.33 ± 1.63
	0.89	74.80 ± 6.49	25.33 ± 6.46	90.66 ± 1.63	9.33 ± 1.63
	0.445	58.00 ± 1.63	42.00 ± 6.46	89.33 ± 1.63	10.66 ± 1.63
	0.222	41.66 ± 2.49	58.34 ± 1.63	90.66 ± 1.63	9.33 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.001	0.002	0.000	0.000
Methanol	1.5	78.93 ± 2.53	21.07 ± 2.35	92.00 ±1.33	7.99 ± 1.33
	0.75	62.66 ± 4.52	37.06 ± 4.68	90.66 ±1.63	9.33 ± 1.63
	0.375	53.33± 2.49	46.67 ± 1.63	88.00 ± 2.49	12.00 ± 2.50
	0.1875	36.66± 1.63	63.34 ± 1.63	90.66± 1.63	9.33 ± 1.63
	Control	0.00 ± 00	100 ± 00	0.00 ± 00	100 ± 00
	P value	0.000	0.000	0.000	0.000

Table 7 Effect of crude extracts of *O. sanctum* on development of third instar larvae of *C. bezziana* using Thin Film Technique

Figures

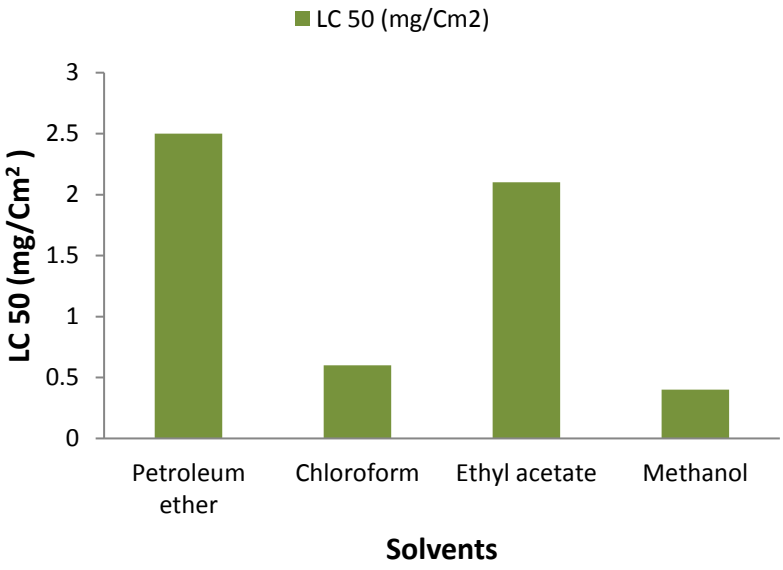

Fig.1 Toxicity of *A. indica* extracted with different solvents against third instar larvae of *C. bezziana* using Dipping Method

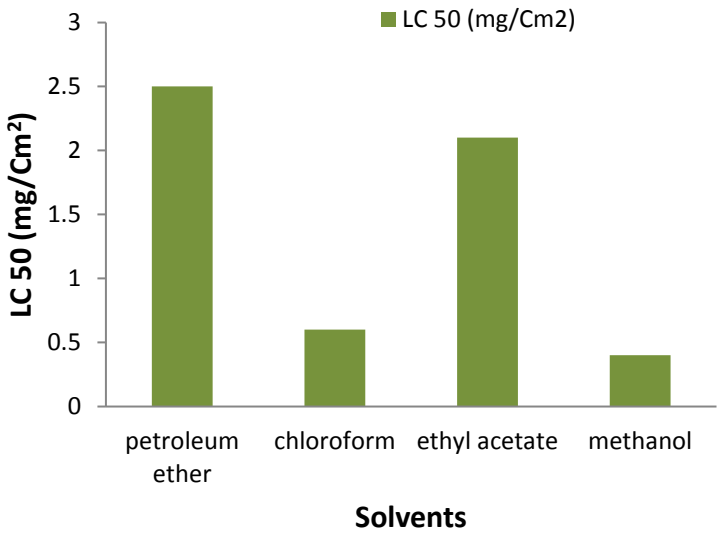

Fig.2 Toxicity of *A. Indica* extracted with different solvants against third instar larvae of *C. bezziana* using Thin Film Technique

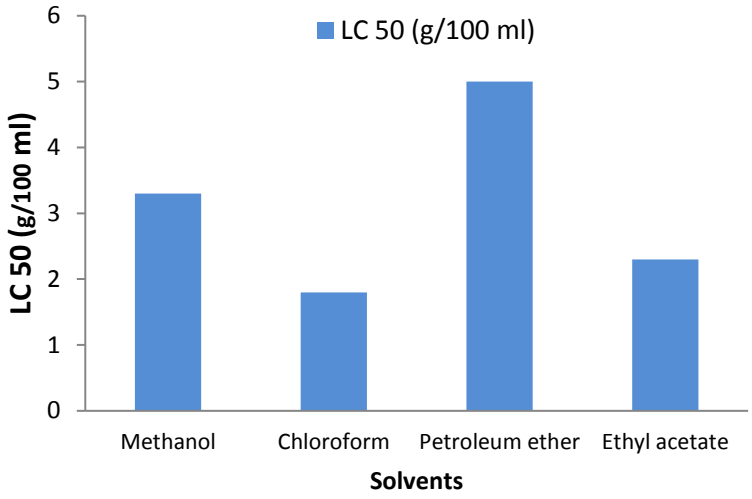

Fig. 3 Toxicity of *R. communis* extracted with different solvents against third instar larvae of *C. bezziana* using Dipping Method

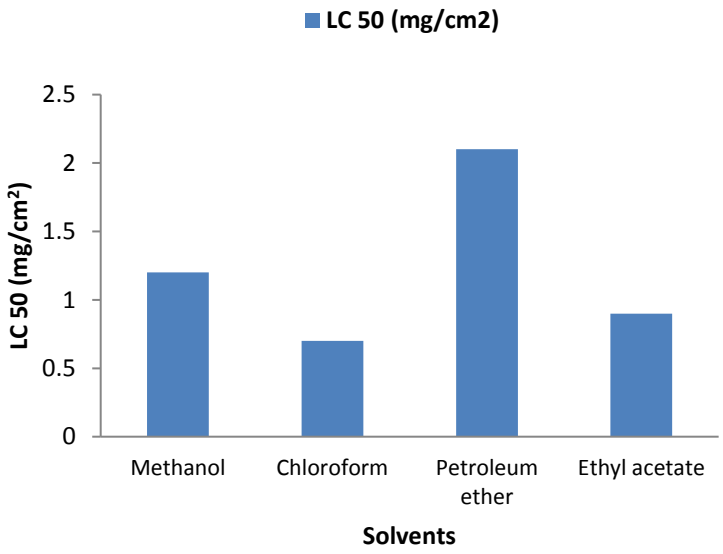

Fig. 4 Toxicity of *R. communis* extracted with different solvents against third instar larvae of *C. bezziana* using Thin Film Technique

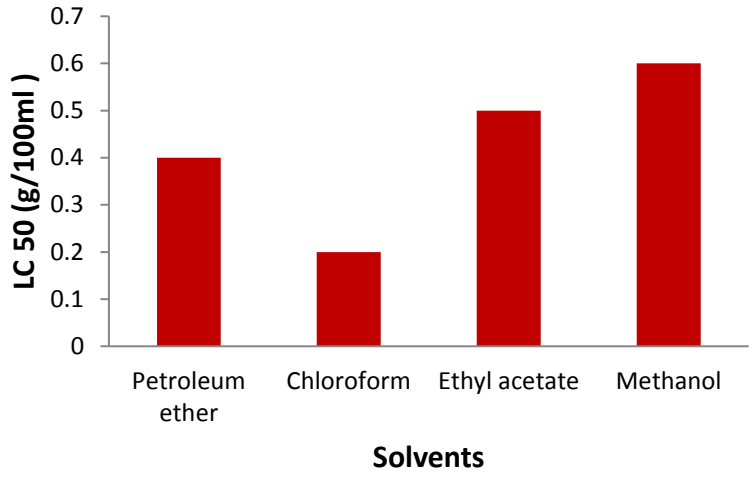

Fig. 5 Toxicity of *O. sanctum* extracted with different solvents against third instar larvae of *C. bezziana* using Dipping Method

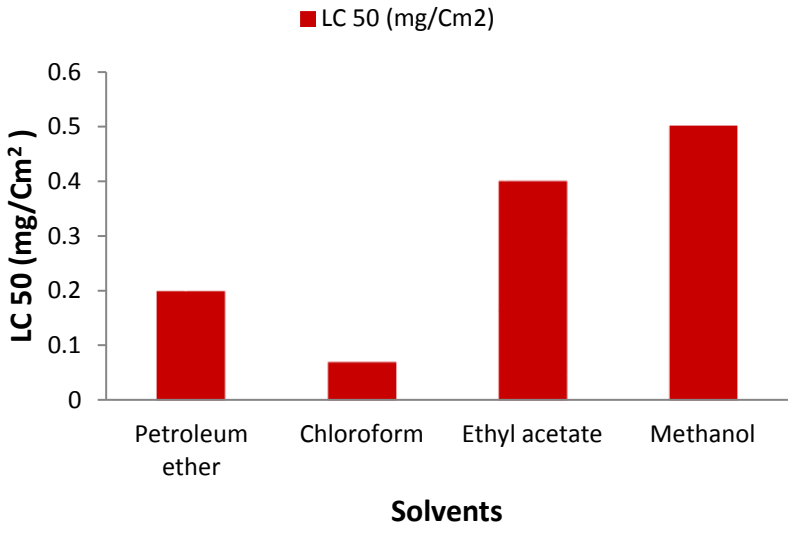

Fig. 6 Toxicity of *O. sanctum* extracted with different solvents against third instar larvae of *C. bezziana* using Thin Film Technique

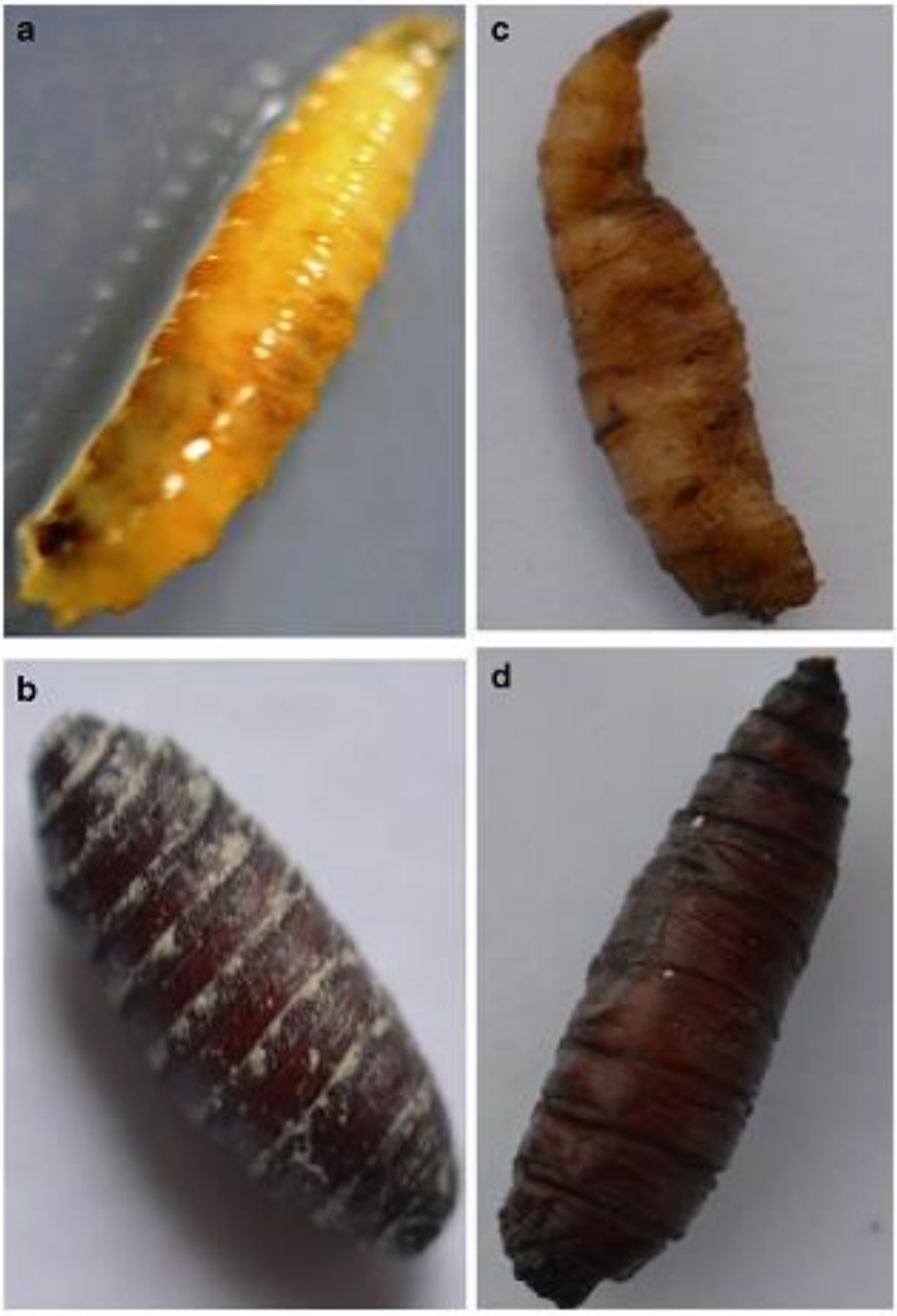

Fig. 7 The effect of plant extracts on the development of the third instar larvae of *C. bezziana*: (a) control larva and (b) control puparium have normal appearance. (c) Treated dead larva, shrunk and dark coloured (d) Dead puparium with anterior portion resembling normal larva.

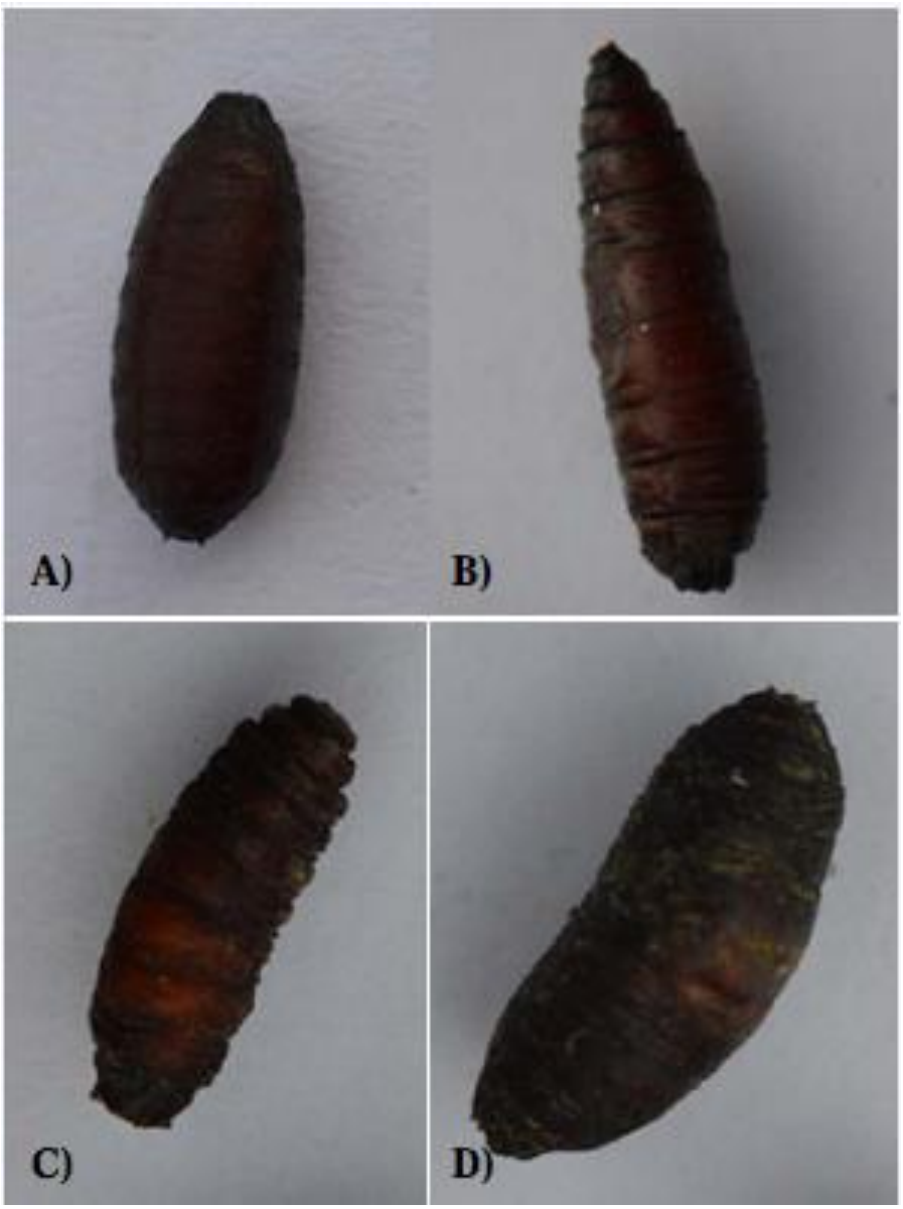

Figure 8. Different forms of pupae emerged from larvae of *C. bezziana* exposed to leaf extracts of *Ricinus communis* (A) Control (normal) puparium (B) larviform (C) segmented (D) distorted.