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In-vitro evaluation of three herbal oils against larval stages of *Lyperosia-Haematobia* complex (Diptera: Muscidae)

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Abstract
The present study was conducted to evaluate the efficacy of Neem (*Azadirachta indica* A. Juss.*)*, Nilgiri (*Eucalyptus globules* Labill.) and Karanja (*Pongamia glabra* L.) crude oils against the field collected larvae of *Lyperosia-Haematobia* complex (Diptera: Muscidae). For the present experiment field collection of larvae was done from dung pits belonging to different buffalo shed nearby Parbhani city. In the laboratory few larvae were processed for identification using the standard keys. All the three oils in the study were tested at five different concentrations *viz.* 1, 1.5, 2, 3 and 5 percent aqueous solution and were sprayed on the faecal culture seeded with ten larvae per thirty gram of faeces in petridish. The oils were dissolved in the 5 ml of acetone and then in distilled water as per the desired concentration. Control group was maintained for each herbal oil as 10 larvae in 30 gram of faeces and sprayed with acetone mixed distilled water. All the trials were repeated thrice and average mortality count was recorded as assessment criteria for judging the efficacy of oils. The observations were taken till all the larvae in control group molted to pupal stage. The median lethal concentration (LC_{50}) values of the Neem oil, *Eucalyptus* oil and Karanja oil against the larvae of *Lyperosia-Haematobia* complex were 3.63, 2.69 and 3.04% respectively. Thus the present experiment concluded that Neem oil, *Eucalyptus* oil and Karanja oil have potent action as knockdown effect against larval stages of *Lyperosia-Haematobia* complex and can be inducted in integrated pest management program after *in-vivo* evaluation.

Keywords: *Lyperosia-Haematobia* complex flies**,** *Azadirachta indica, Eucalyptus globules, Pongamia glabra*

Introduction
The *Lyperosia* - *Haematobia* complex are one of the most economically important and obligate blood-feeding ectoparasites of cattle and buffaloes worldwide including India. *H. irritans* (horn fly) causes annual losses of 700 million to 1 billion US$ in the United States. Also for control of these flies an additional US$ 60 million annually expended.[1] Heavy infestation of these flies put stress on animals and grazing affects which results in reduced milk yield and loss of weight in beef cattle.[2] Each fly takes 24 to 38 bites/blood meals per day.[3] Continuous biting results in substantial blood loss, damage to hide and result in poor quality of leather. It is considered as one of the most economically important ectoparasite of the beef cattle in USA.

Lyperosia - *Haematobia* complex of muscid flies contains several species and in taxonomy, cited by several authors [4-7], there appears to be little controversy over the validity of genus *Lyperosia* and *Haematobia*. Many books describe these two genera as *at par*. Most of the control strategies for *Lyperosia* - *Haematobia* complex involve chemical insecticides which are often toxic or mutagenic to vertebrates. Insecticide resistance, insecticide residues and non-availability of new insecticides are serious threats for the livestock industry. An alternative to conventional insecticides is use of herbal products. Herbal oils consist of many volatile substances that are natural candidates for the development of new products for controlling insect pests [8]. Owing to this fact, the present study was designed with the objectives to evaluate the three different herbal oils Neem, *Eucalyptus* and Karanja oil against larval stages of *Lyperosia* - *Haematobia* complex.

Material and Methods
Collection of larvae
For the present experiment field collection of larvae was done from the faecal samples, which were collected from dung pits belonging to different buffalo shed of private dairy owners

nearby Parbhani city. In the laboratory faecal samples were diluted with three times water quantity, filtered with strainer and from the processed samples larvae were individually picked up with moist Camelin hair brush of size 1. Few larvae were processed for identification under zoom stereoscopic microscope by applying the keys described by Baker [9] Fitzpatrick and Kaufman [10] before being introduced in the experiment.

In-vitro trials of herbal oils against larvae of _Lyperosia_ - _Haematobia_ complex

Table 1: Different herbal oils used in the present experiment

Sr No	Herbal crude oils	Scientific name of the herbal oils	Concentration used in experiment
1.	Neem crude oil	*Azadirachta indica* A. Juss.	Commercial products of these crude oil were purchased from the market. Individual oil at five different concentrations such as 1, 1.5, 2, 3 and 5 % were used in the study
2.	Eucalyptus crude oil (Nilgiri)	*Eucalyptus globules* Labill.	
3.	Pongamia crude oil (Karanj)	*Pongamia glabra* L.	

For *in-vitro* trial of herbal oils, fresh faecal sample were collected from the buffalo farm of College of Veterinary and Animal Sciences, Parbhani. Care was taken that, faecal samples used for growth of larvae in the laboratory were free of larvae. Isolated 10 larvae of *Lyperosia-Haematobia* complex were inoculated on each faecal pod in petridishes containing 30 grams of faeces. Such five petri dishes were inducted for each concentration of individual herbal oil. The crude oils used in the present experiment was purchased from authorized shop. All the three oils in the study were used in five different concentrations *viz.* 1, 1.5, 2, 3 and 5 percent aqueous solution and sprayed on the faecal culture seeded with 10 larvae per 30 gram of faeces in petridish. The requisite quantity of herbal oils were dissolved in the 5 ml of acetone first and then distilled water added to achieve desired concentration. The acetone was used as emulsifying agent and it was also recorded by undertaking pilot experiment that alone acetone was not harmful to the larvae of *Lyperosia* - *Haematobia* complex. Control group was maintained for each herbal oil as 10 larvae in 30 gram of faeces and sprayed with 5 percent acetone in distilled water (fig.1). All the trials were repeated thrice and average mortality count was recorded as assessment criteria for judging the efficacy of oils. The observations were taken till all the larvae in control group molted to pupal stage. The individual larvae were considered as dead when it showed no movement even after pricking gently with pinhead. The mortality data were tabulated and efficacy was worked out in terms of per cent mortality. The mortality data were subjected to Probit analysis and LC_{50} and LC_{90} were worked out as per the method described by Finney [11] and compared with LD_{50} values of acute toxicity in rats referred from literature.

Results and Discussion

Larvicidal effect of Neem oil

The larvicidal effect of Neem oil against larvae of *Lyperosia* - *Haematobia* complex depicted in Table 2. The highest mortality occurred at 5% concentration solution. LC_{50} and LC_{90} value were found as 3.63 and 8.88 % respectively (Table 5, fig.2). These findings are congruent with the study conducted by Miller and Chamberlain [12] on azadirachtin (bioactive component of Neem) as a larvicide against the horn fly (*Haemaobia irrians*). They estimated an LC_{50} of 0.096 and an LC_{90} of 0.133 ppm azadirachtin in the manure. Also an emulsifiable concentrate formulation Margosan-O (3mg azadirachtin/ml), showed an LC_{50} and an LC_{90} of 0.151 ppm and 0.268 ppm respectively. Although neem produces a variety of compounds, extract efficacy is mainly attributed to azadirachtin, a nortriterpenoid, that acts as an insect growth regulator against larval insects by disruption of molting, growth inhibition and malformation that can result in mortality. Neem extract effects result from disruption of endocrine activity, including the downregulation of hemolymph ecdysteroid levels that block release of prothoracicotropic hormone or delay ecdysteroid production, an action which inhibits molting in insects including horn fly larvae [13]. In an *in-vitro* study with ethanolic extract of ground neem seed (2.7 mg azadirachtin/g) blended with cow manure, showed LC_{50} and an LC_{90} for horn fly larvae of 0.096 and 0.133 ppm azadirachtin, respectively [14].

Azadirachtin is the predominant insecticidal active ingredient found in neem oil. It is highly interrelated with its bioactivity against variety of insects. Neem products are capable of producing various effects in insects such as antifeedancy, growth regulation, fecundity suppression and sterilization, oviposition repellency and changes in biological fitness.[14] In the present study also, many larvae failed to pupate and showed structural abnormality may be because of insecticidal activity of azadirachtin present in the neem oil (Fig.3).

Table 2: Larvicidal effect of Neem oils against larvae of *Lyperosia-Haematobia* complex

Concentration of Neem oil (%)	Log concentarion	Mean corrected mortality (%)	Probit Mortality
1	0	2.5	3.04
1.5	0.176	6.66	3.49
2	0.301	26.66	4.38
3	0.477	53.33	5.08
5	0.699	56.66	5.17

Larvicidal effect of *Eucalyptus* oil

The larvicidal effect of *Eucalyptus* crude oil against larvae of *Lyperosia* - *Haematobia* complex shown in Table 3. The highest mortality occurred in 5% concentration solution. LC_{50} and LC_{90} value were found 2.69 and 5.91 % respectively (Table 5, fig.4). Similar type of larvicidal effect of *E. globulus* oil were observed by Kumar *et al.* [15] against *Musca domestica* larvae which belongs to same family of *Lyperosia-Haematobia* complex. They found LC_{50}, of *E. globulus* oil varied between 2.73 and 0.60 µl/cm^2 for different observation days while LC_{90} for the same was between 4.65 and 1.32 µl/cm^2. Juan *et al.* [8] extracted oils from various species of *Eucalyptus* and found that essential oil of *E. polybractea* had the highest knockdown activity of 50% (KT_{50}) at 3.4 min in an enclosed chamber and a significant correlation was detected between the content of 1,8-cineole in the *Eucalyptus* species and toxicity to horn flies.

Sukontason *et al.* [16] conducted a bioassay of larvae, using the dipping method on the third instar, showed the LD_{50} and LD_{95} of *M. domestica* were 101 and 239 µg/µl, respectively. The

LD_{50} of *M. domestica* was significantly lower indicating that the muscid flies larvae are more susceptible to larvicidal action of *Eucalyptus* oil. In the present study similar type of susceptibility of *Lyperosia-Haematobia* complex larvae found to *Eucalyptus* oil.

Abdel Halim and Morsy [17] evaluated the insecticidal activity of *E. globulus* essential oil against the 3rd larval instar of *M. domestica* and reported 100% larval mortality at a concentration as low as 0.7%.

The main constituents in the eucalyptus oil are Eucalyptol or 1, 8-cineole, γ –Terpinene, α –Pinene and Globulol. These components are responsible for various morphological, physiological and biochemical changes in the larvae resulting in their mortality. [18] According to the primarily lipophilic property, several monoterpenoids have been reported as having toxic capability to many insects and were therefore considered as a potential, alternative biopesticide. [16]

Table 3: Larvicidal effect of *Eucalyptus* oils against larvae of *Lyperosia- Haematobia* complex

Concentration of Eucalyptus oil (%)	Log concentration	Mean Corrected Mortality (%)	Probit Mortality
1	0	2.5	3.04
1.5	0.176	13.33	3.89
2	0.301	53.33	5.08
3	0.477	63.33	5.34
5	0.699	76.66	5.73

Larvicidal effect of Karanja oil

The larvicidal effect of Karanja oil against 3rd instar larvae of *Lyperosia - Haematobia* complex presented in Table 4. The highest mortality occurred in 5% concentration solution. LC_{50} and LC_{90} value were found as 3.04 and 6.29 % respectively (Table 5, fig.5). There was no study found in the literature regarding effect of Karanja oil against *Lyperosia - Haematobia* complex larvae to compare with the results of present study. Pant *et al.* [19] studied the larvicidal effect of neem and Karanja oil encapsulated calcium alginate beads against *Aedes aegypti* (Diptera: Culicidae) and observed that LC_{50} of Karanja oil as 4 g/lit and Neem oil as 5.1g/lit. Combination of 30% Neem oil and 70% Karanja oil was found to be more effective with LC_{50} of 3.1g/lit.

Parmar *et al.* [20] found *Pongamia* oil and Karanjin have been active against the housefly (*Musca domestica*). Singh & Kataria [21] observed the toxicity of the chloroform extract of bark of *P. glabra* to third instar larvae of *Culex fatigens* at 31.2 ppm concentrations. The toxicity was attributed mainly to Karanjin and the furano flavonoids present in the oil. Juvenomimetic effects of karanjin on the larval development of flesh fly, *Sarcophaga ruficornis* was studied by Mathur *et al.* [22] who found three types of morphogenic forms; larval-pupal intermediates, pupal-adult intermediates and deformed adults. Larval mortality recorded in larvae at higher doses indicated that karanjin blocks the metamorphosis abruptly. Karanjin is known as the main active principle and effective against large number of insects. All these studies points out that active ingredients present in Karanja oil responsible for larval mortality of *Lyperosia-Haematobia* complex found in the present study.

Table 4: Larvicidal effect of Karanja oils larvae of *Lyperosia-Haematobia* complex

Concentration of Pongamia oil (%)	Log concentration	Mean Corrected Mortality (%)	Probit Mortality
1	0	3.33	3.16
1.5	0.176	2.5	3.04
2	0.301	30	4.48
3	0.477	70	5.52
5	0.699	73.33	5.62

Table 5: Lethal concentrations of the different herbal oils against larval stages of *Lyperosia- Haematobia* complex at 95% confidence limits

Herbal oil	LC_{50}	95% Fiducial CI		LC_{90}	Slope±SE	χ^2	n
		Lower	Upper				
Neem oil	3.63	2.72	4.88	8.88	3.340±0.064	0.870	30
Eucalyptus oil	2.69	2.11	3.42	5.91	3.896±0.054	0.726	30
Karanja oil	3.04	2.37	3.79	6.29	4.231±0.052	0.748	30

Fig 1: *In-vitro* trial of different herbal oils in the laboratory against larvae of *Lyperosia-Haematobia* complex

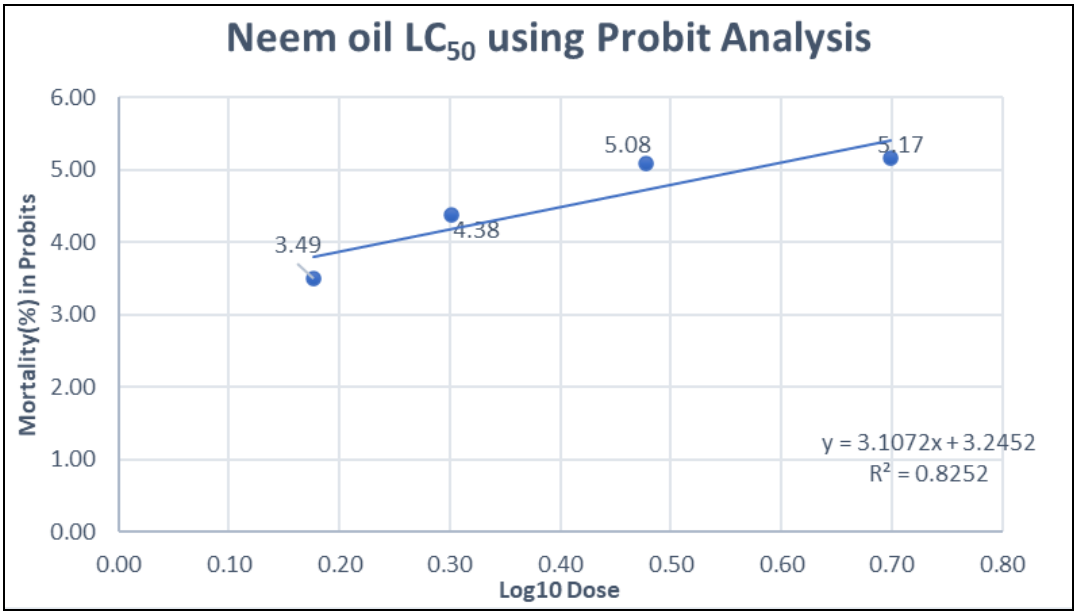

Fig 2: Probit analysis (dose-response curve) of Neem oil against *Lyperosia-Haematobia* complex larvae

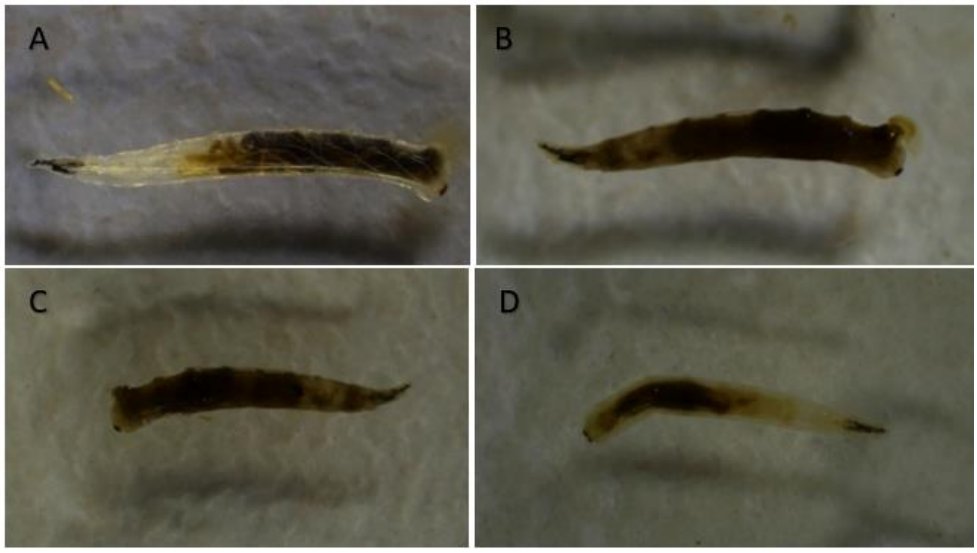

Fig 3: Larvae of *Lyperosia-Haematobia* complex. A. Normal larva from control group B. Larva from Neem oil treated group. C. Larva from Karanj oil treated group D. Larva from *Eucalyptus* oil treated group.

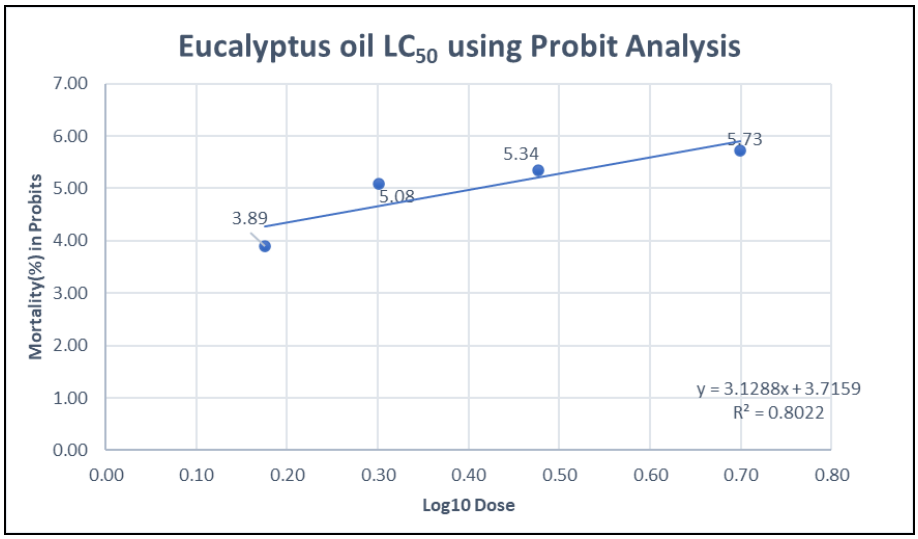

Fig 4: Probit analysis (dose-response curve) of *Eucalyptus* oil against *Lyperosia-Haematobia* complex larvae

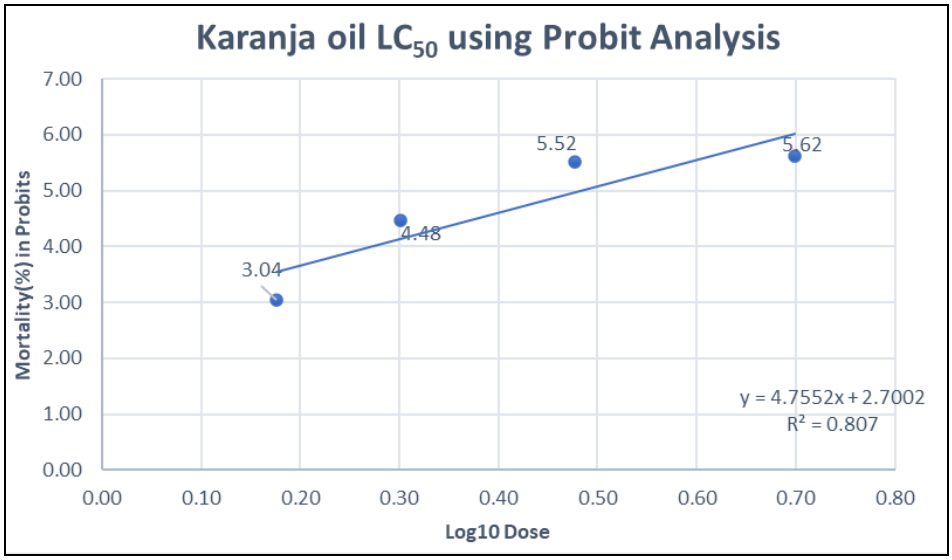

Fig 5: Probit analysis (dose-response curve) of Karanja oil against *Lyperosia-Haematobia* complex larvae

Conclusion

The present investigation showed the potential of all the three herbal oils *viz.* Neem oil, Karanj oil and *Eucalyptus* oil as natural insecticides against larval stages of *Lyperosia-Haematobia* complex. Out of these three oil, *Eucalyptus* oil performed better with Lc50 (2.69%) followed by Karanja oil (3.04%) and then Neem oil (3.63%) as potent action of knockdown effect against larval stages of *Lyperosia-Haematobia* complex. Although herbal compounds are not complete solution for control of *Lyperosia- Haematobia* complex flies, these can offer a cost effective, eco-friendly alternatives to chemical insecticides. These herbal agents have the potential to delay the resistance occurring to chemical insecticides and thus can be inducted in integrated pest management program after *in-vivo* evaluation. The most important finding of the study was the ease of the method of application which is suitable and economical for controlling fly larvae in dung pats (their breeding sites). Further research is needed to isolate and identify the key larvicidal active ingredient in the herbal oils.

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