



Efficacy of Herbal Fly Repellent Product (Keetguard Liquid) to Control *Musca Domestica* Population in Poultry Egg Layer Farm

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ABSTRACT

The housefly *Musca domestica* L. is recognized as a public health pest causing a serious threat to human and livestock by vectoring many infectious diseases. Chemical control method commonly used against this pest, though effective, has some major disadvantages, such as development of insect resistance and bioaccumulation. Therefore the efficacy of a herbal fly repellent Keetguard Liquid (supplied by M/S Ayurvet Limited, Baddi, H.P., India) was evaluated in vivo on the layer farm as well as in vitro. For in vivo trial the keetguard was used at the concentrations of 1:20 (Group-I recommended for application on body) in one shed and 1:40 (Group-II, recommended for application in premises) in another shed. Another shed kept as control (Group-III) was sprayed with plain water. The herbal fly repellent product was assessed for fly repellent and larvicidal effect and for its efficacy to minimize the count of larvae. In both the treatment groups, the fly repellency recorded during first 2-6 hrs was satisfactory. However, the fly population observed at the end of the week was encouraging in group-I due to its larvicidal effect. The probit analysis to draw Effective Concentration 50 (EC50) showed 43.66 ml/lit as EC50. The in vitro comparative efficacy study was conducted on third stage larvae of *Musca* spp. divided equally in 4 groups and exposed to 1:20, 1:40 (two different concentrations) of KEETGUARD LIQUID, a standard pyrethroid insecticide 'Cypermethrin (1%)' and control (plain water) respectively as group-I: 1:20 concentration of Keetguard liquid Group-II: 1:40 concentration of Keetguard liquid, group-III: standard pyrethroids insecticide 'Cypermethrin (1%) and group-IV: control (plain water). In invitro study, higher efficacy (95 %) was recorded in group-I as compared to group-II (66.66%) and the standard insecticide-Cypermethrin-1% (100%) while similar trend of efficacy (90.69% in G-I and only 65.76% in G-II) was observed during in vivo field experimentation. The plant based herbal product Keetguard is efficacious as a fly repellent against house fly *Musca* spp. in egg layer poultry farm in both 1:20 & 1:40 dilution. In addition, it also has larvicidal potential against 3rd stage of larvae of *Musca* spp. considering the detrimental effects of chemical fly repellents and insecticides on the environment and human health, Keetguard Liquid was found better option for control of fly population in and around poultry layer farms.

Key words: keetguard liquid, fly repellent, larvicidal, ectoparasitocidal, herbal

INTRODUCTION

The flies are not only nuisance to the health of the birds in the farm but also become annoyance not only to the workers of the farm but also to the people residing in the villages nearby the farms. The manure accumulated in commercial caged-layer houses are the major source of attraction for the flies (*Musca* spp.). These flies act as a vector to various disease causing organisms and thus possess a great threat to human and confined poultry health. *Musca* spp. can prosper in wide range of environmental conditions with high reproductive rate and can breed throughout the year (Crespo *et al.*, 1998). Thus control of these potential vectors of disease is a serious concern. The house fly control is largely based upon the use of chemical insecticides such as organochlorines, organophosphates, pyrethroids. However, use of chemical insecticide is not only detrimental to environment and have undesirable effects on non-target organisms, but its long term use also leads to development of resistance among insects (Thomas and Jespersen, 1994). Injudicious use of these insecticides on large scale particularly in proximity to human food may prove to be toxic to man (Bhatia *et al.*, 2006). House flies are notorious for their ability to develop behavioral and metabolic mechanisms to avoid and detoxify chemical insecticides. Resistance to DDT was noticed within a few years of its introduction (Varzandeh *et al.*, 1954; Perry, 1958). House flies (*Musca domestica* L.) have resisted human attempts to control them since antiquity, and the global problem of fly resistance to conventional insecticides has resulted in renewed interest in biopesticides as alternative management tools to conventional insecticides (Geden, 2012). In search of environment friendly and effective insecticides, essential oils from plants could be a good approach (Kant and Bhatt, 1994). Presently, bioinsecticides, especially those derived from plant origin, have been increasingly evaluated in controlling insects. Plants contain bioactive organic chemicals in the form of metabolites and plant extracts have been used locally in herbal preparations to cure ailments even before the advent of orthodox medicine in many developing countries (Oyedokun *et al.*, 2011). Flavonoids, Alkaloids, Saponins, Sesquiterpenes, Limonoids, Phenols, Stilbenes and Coumarins of plant origin have been reported to possess toxic, growth regulating and anti-feedant effects against a host of insect pests (Sunita and Lalijs, 2008). The knowledge and use of plants as well as their extracts as protectants against grains and other foodstuffs had been in existence since time immemorial (Dales, 1996; Isman, 2000). Therefore, considering the detrimental effects of chemical fly repellents and insecticides on the environment and human health the present study was undertaken to evaluate the *in vitro* and *in vivo* acaricide efficacy of herbal Fly repellent product (Keetguard liquid).

MATERIAL AND METHOD

Experiment design

The experiment was conducted in a farm near village Yewat, Dist. Pune having huge fly population suitable to undertake the experiment on efficacy of herbal fly repellent Keetguard liquid. The environmental temperature and relative humidity at farm premises was recorded during the period of experiment between 26.6-30.8 °C and humidity between 45-67 per cent. At this farm, three layer sheds caged with 10 thousand layer birds placed wide apart (100 feet) from each other with sufficient quantity of fresh manure underneath and having approximately identical fly population were selected for the present study. The repellents were applied to the area under (dropping area) and around the sheds by using power spray so that the spray reaches to the side mesh and entrances and tested against *Musca* spp. on the droppings, mesh, grills, grass and other objects and places where these flies were flying freely in the area as shown in figure 1.



Figure 1. Thick layer of droppings accumulated under the shed which is an ideal breeding place for flies and Spraying of the repellent in area around the shed

In vivo study

Experimental groups

The three selected sheds were marked as three different groups for the study

Group I: Shed treated with Keetguard liquid @ 1:20

Group II: Shed treated with Keetguard liquid @ 1:40

Group III: Control group (Shed treated with plain water)

The product Keetguard liquid comprises of oil of herbs viz. *Eucalyptus globulus*, *Cedrus deodara*, *Pinus longifolia* & many others in a fixed concentration, which has got proven insecticidal, repellent and larvicidal efficacy.

Selection of fly base/bait

A day before starting actual experiment, a pilot trial was conducted to select a best fly base (viz. paper sheets, white gonies, grass sticks etc.) or bait (viz. coloured sugar, egg albumin baits, syrup-soap baits etc), its position (viz. vertical/horizontal/ hanging/ fixed etc.) and location (viz. under/ sides of the shed) around the shed to assess maximum fly population per unit area at three different timings at an interval of 3 hrs throughout a day. Finally, syrup-based baits were preferred and applied on 8 the plain papers / fly sheets. These fly sheets were hanged at the side mesh of each shed so as to get the maximum access to flies in the premises and those escaping – newly emerged imagoes. The method was adopted on the basis of preference by the flies.

Preparation of test solution (Keetguard liquid) for spray

The stock herbal preparation which is sticky, brownish-orange coloured liquid presented in 250 ml plastic bottle was first poured in a plastic jar containing 1 lit of clean water and mixed thoroughly with a clean wooden stick to ensure proper and complete mixing of the drug. Then such stock solution was used to prepare working / spray solution of desired concentration i.e. 1:20 and 1: 40 respectively.

Parameters Estimated

Following parameters were estimated to evaluate the efficacy of the product.

Fly Repellent efficacy against adult Fly population

Total six baited fly sheets (three on either side) were hanged around the shed and kept undisturbed for one hour to settle on the flies as shown in figure 2. The snap shots of all six fly sheets, hanged around each shed, and were taken after 1 hour of its application. Fresh fly sheets were applied and the procedure was repeated 1 hour before every observation. The observations were recorded at 1 hour, 4 hours, 6 hours, 1 day, 3 days, 5 days and 1 week after the treatment. The numbers of flies present on each sheet at the end of an hour were counted on the 15'' screen of LCD monitor (Kirby, 2008).



Figure 2. Fly sheets to assess the fly population per unit area before and after treatment.

In Vitro study

The larvicidal activity was assessed by *in vitro* and on field studies.

In vitro larvicidal activity of Keetguard liquid was undertaken to evaluate two parameters viz. EC 50 and comparative efficacy.

I. Probit analyses of EC50

The *in vitro* study was conducted on twenty number of 3rd stage larvae of *Musca* spp. to evaluate effective concentration of Keetguard liquid by exposing the larvae to a series of 3 concentration of the compound in distilled water (25, 50, 75 ml/L). The larvae were exposed to different concentrations for 60 minutes and percentage mortality was calculated. The probit analyses test was performed to find out the EC 50 value on the basis of log regression and graph method (Finney.D.J and Stevens. W.L, 1948 and Finney. D.J, 1952)

II. In vitro comparative efficacy

The *in vitro* comparative efficacy study was conducted on third stage larvae of *Musca* spp. divided equally in 4 groups and exposed to 1:20, 1:40 (two different concentrations) of KEETGUARD LIQUID, a standard pyrethroid insecticide 'Cypermethrin (1%)' and control (plain water) respectively as

Group-I: 1:20 concentration of Keetguard liquid

Group-II: 1:40 concentration of Keetguard liquid

Group-III: standard pyrethroids insecticide 'Cypermethrin (1%)'

Group-IV: control (plain water)

These larvae were later kept for pupation and the number of flies emerged were recorded as shown in Figure 3. Cypermethrin is a synthetic pyrethroid used as standard insecticide in large-

scale commercial agricultural applications as well as in consumer products for domestic purposes (Kirby, 2008).

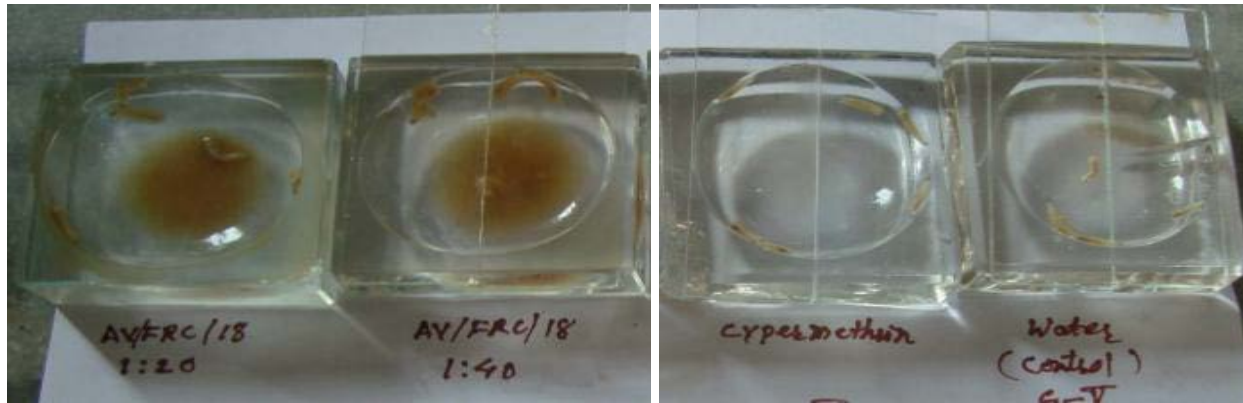


Figure 3. In vitro comparative efficacy study

On field larvicidal efficacy

The droppings from middle layer of manure from all the three sheds under study were collected in a plastic jars of the volume of 3”x3”x3” and this volume was considered as unit volume. The samples from all groups under study were collected before an hour and after 24 hrs of application of the test compound. After collection, these droppings were mixed with sufficient quantity of water and filtered to isolate the larvae as shown in figure 4. The larval population of the *Musca* spp. per unit volume of the droppings were counted and compared.



Figure 4. Larvicidal efficacy experiment

Data recording & analysis

The data of fly counts was properly recorded, organized and analysed statistically by using completely randomized design (CRD-equal) described by Snedecor and Cochran (1989) and software designed by Jangam and Thali (2001) WASP - Web Agri Stat Package (<http://www.icargoa.res.in/wasp/index.php>) to draw the conclusions and interpret the results.

RESULT AND DISCUSSION

A. Fly Repellent efficacy against adult Fly population

Fly repellent activity against *Musca* spp. after first application

The results for the effect of compound on the population of flies after First application on day 1 (1 hr, 4 hrs, 6 hrs and 24 hrs), day 3, day 5 and day 7 are summarized in Table 1

The pre count of fly population in the poultry layer sheds under study were 14.17+0.99, 14.00+2.03 and 13.83+2.21 in the Group-I, Group-II and Group-III respectively which was approximately similar with no statistically significant difference.

The post treatment count of flies on day 1 was found to be 1.67, 3.00, 4.50 and 5.83 per unit area after 1 hr, 4 hrs, 6 hrs and 24 hrs and 12.67, 10.33 and 6.33 at day 3, 5 and 7 post applications respectively for Group I. In Group-II and III, the count was 1.83, 3.67, 4.50, 9.00, 13.50, 17.33, and 19.83 per unit area and 13.00, 13.16, 13.50, 15.67, 19.83, 24.00 and 26.00 flies/UA at 1, 4, 6, 24 hrs, 3rd, 5th and 7th day post application respectively.

Table 1: *Musca* spp. population around poultry shed after first application

Groups	Statistics	Pre-treatment	Post-treatment count						
		count	1 hr	4 hrs	6 hrs	24 hrs	3 rd day	5 th day	7 th day
G I	Mean	14.17 ^a	1.67 ^d	3.00 ^{cd}	4.50 ^{bcd}	5.83 ^{bc}	12.67 ^a	10.33 ^a	6.33 ^b
	% Redn.	--	88.21	78.83	68.24	58.86	10.59	27.10	55.33
G II	Mean	14.00 ^b	1.83 ^e	3.67 ^e	4.50 ^{de}	9.00 ^{cd}	13.50 ^{bc}	17.33 ^{ab}	19.83 ^a
	% Redn.	--	86.93	73.79	67.86	35.71	3.58	+22.57	+41.64
GIII	Mean	13.83 ^d	13.00 ^d	13.16 ^d	13.50 ^d	15.67 ^{cd}	19.83 ^{bc}	24.00 ^{ab}	26.00 ^a
	% Redn.	--	6.01	4.84	2.39	13.30	+43.38	+73.53	+87.99

Values in the parenthesis indicate range of observations (n=6). Redn. = Reduction.
Different superscripts indicate that the values differ significantly in a row (P<0.01).

With earlier concentration, the repellency was high on the day of application particularly during first 4-6 hrs both in case of group I and group II as only the available adult population was exposed to the repellent. The level of aroma of the herbal formulation was gradually decreased after 6 hours of application and the fly population gradually started increasing after 6 hrs both in Group I and II but was still considerably lower as compared to pre-treatment values. There was significant (P<0.01) decrease in the flies' population in group I and group II after 24 hrs, but the population increased significantly (P<0.01) in Group III.

The fly population started increasing gradually on day second onwards. The increase was more significant in group II as Compared to group I. This may not be due to the return of the flies which already repelled away, but may be due to synchronized effect of two factors viz. continuous emergence of new flies as a major part and decreased level of aroma of formulation. However, at 5th day and at the week end (7th day) after application, the level of fly population was again decreased in Group I. This may be the result of the larvicidal effect of the formulation which was sprayed on the manure (on droppings of the birds) under the sheds. The larvae came in contact with the formulation might have failed either to moult or pupate. The batch of flies expected to be emerged during this phase failed to develop. Thus it finally resulted in lowering the fly population. However, the population of flies increased significantly (P<0.01) in Group II and Group III. The increase in population may be due to decreased aroma in Group II. Certain plant leaves have fly repellent and feeding deterrent activity against *Musca domestica* in Ethiopia (Wimalaratne *et al.* 1996). It has direct impact of mortality, also several secondary impacts on oviposition, repellence and antifeedancy (Pavela 2008). Similar results were found

against mosquitoes & flies (Watanabe *et al.*, 1993), livestock ticks (Lwande *et al.*, 1999), house flies (Singh *et al.*, 1991).

B. Larvicidal efficacy

In vitro larvicidal activity of Keetguard liquid was undertaken to evaluate two parameters viz. EC 50 and comparative efficacy.

I. Probit analyses of EC 50

The *in vitro* study was conducted to evaluate effective concentration Keetguard liquid by exposing twenty number of 3rd stage larvae of *Musca* spp. to a series of 3 concentrations of the compound in distilled water. The highest mortality of 85 % was observed at the 75 ml/L concentration followed by 55 % mortality at 50 ml/L concentration, 15 % mortality at 25 ml/L concentration of the test compound, as observed after 60 minutes. The probit analysis test was applied to find out EC 50 value on the basis of log regression and graph method and given in Table 2. The EC 50 calculated by using probit analysis was found to be 43.66 ml/L.

Table 2: Probit values drawn on the basis of log regression of different concentrations of Keetguard liquid.

Concentration	Log ₁₀ Conc.	Total No. of larvae	Dead Larvae	% mortality	Probit value
0	0.00	20	00	00	--
25	1.39	20	3	15	3.96
50	1.69	20	11	55	5.13
75	1.87	20	17	85	6.04

II. *In vitro* comparative analyses of larvicidal efficacy

The *In-vitro* larvicidal efficacy of Keetguard liquid on larval count of *Musca* spp. and its further development is represented in Table 3. Four larvae from group-I, one from group-II and 5 from group-III were found dead after one hr of treatment while there was no mortality in control group-IV.

The larvicidal efficacy on the basis of fly emergence was assessed which was 95 % in group-I (1:20), 66.66% in group-II (1:40), 100 % in group-III (Cypermethrin 1% as Standard insecticide) while only 5.54 per cent in the control group.

Table 3: *In-vitro* larvicidal efficacy of Keetguard liquid on larval count of *Musca* spp. and its further development

Conc. of the test compound	No. of larvae of <i>Musca</i> spp.		No. of imagoes emerged	Percent reduction in population
	Exposed to treatment	Died after treatment		
Keetguard liquid (1:20)	18	4	1	95
Keetguard liquid (1:40)	18	1	6	66.66
Cypermethrin 1%	18	5	Nil	100
water	18	nil	17	5.5

On field larvicidal efficacy (count of larvae in droppings/unit area 3x3x3” under the Shed)

The larvicidal action of different concentrations is presented in Table 4. The larval population of the *Musca* spp. in the droppings was 21.50+1.57 and 24.33+2.16 before application of the test compound which was significantly ($P < 0.01$) reduced to 2.0+0.37 and 8.33+0.92 at 24 hrs after

treatment indicating 90.69 per cent and 65.76 per cent reduction in the population of larvae in the group-I and group-II respectively. However, in the control group, there is 15.7 % increase in the larval count after 24 hrs from 21.00±1.65 to 24.30±1.45.

Table 4: Larval count of *Musca* spp. in the droppings under the egg layer poultry shed after application of Keetguard liquid.

Conc. of compound	Mean±S.E	Count of <i>Musca</i> spp. larvae in droppings/unit area (3x3x3" area) the under the Shed		Percent reduction in population of larvae
		Before treatment	After treatment	
Keetguard liquid (1:20)	Mean±S.E	21.57±1.57	2.0±0.37	90.69
Keetguard liquid (1:40)	Mean±S.E	24.33±2.16	8.33±0.92	65.76
Untreated	Mean±S.E	21.00±1.65	24.30±1.45	+ 15.7

These results indicate that keetguard liquid has got larvicidal action at both concentrations but the effect is more pronounced in Group I at the concentration of 1:20. The larvicidal effect of herbal fly repellents were also reported by Khater *et al.*, (2009), Oyedokun *et al.* (2011) and Jesika (2012). In some studies also, it was confirmed that some essential oils, such as that extracted from cedarwood (Adams, 1991; Grace et al., 1994), Litsea cubeba (Lin and Yin 1995), and cinnamo- mum spp. (Lin and Yin 1995), were repellents to termites. (Eisner et al., 1986) also confirmed that the all the known botanical/herb based fly repellants/ feed deterrents occur in varying proportion in wide range of herb extracts volatile or essential oils. (Campbell, 1983) also established the fly repellency or feeding deterrence properties of terpenoids.

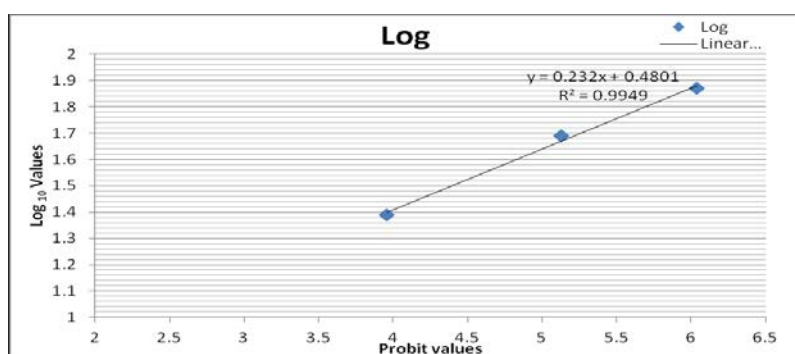


Figure 5. Effective concentration (EC 50) of keetguard liquid was calculated by employing probit analysis on the basis of graph method

CONCLUSION

From the study conducted it was concluded that the compound Keetguard liquid has got potential fly repellent and larvicidal activity, which is efficacious at both the concentrations of 1:20 and 1:40. On the basis of results so obtained in present experimental trial, the natural or biological ectoparasitocidal & fly repellent product Keetguard Liquid is found to be efficacious as cypermethrin.

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