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*Full Length Research Paper*

# Effectiveness of phytogenic fly repellant product against dipteran flies

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**A study was conducted in the livestock dairy farm reported with the history of culicoides fly menace during summer season,the farm was treated with the herbal fly repellant product (AV/FRC/18) (*supplied by M/S Ayurvet Limited, Baddi, H.P., India*) recommended as 1 part with 20 parts of water for application on animal body and 1 part with 40 parts of water for application on drainage channels and animal premises ion the shed. The herbal fly repellant product was assessed for oviposition deterrent activity, ovicidal and larvicidal effect and for its efficacy to minimize the count of larval and adult culicoides in the drainage channel around cattle shed after application of AV/FRC/18. The product was found to be quite efficacious as a fly repellant for livestock dwellings, has a good larvicide potential in addition to ovicidal and oviposition deterrent activity. It has not been found to cause deleterious or adverse effects such as irritation, loss of production, mortality etc. on the experimental animals, rather it is safe for animal usage and for application in animal premises. The product is not having any residual effect, hence it is declared safe for usage.**

**Keyword:** Fly, larvicidal, deterrent, dipteran, repellant, herbal, ectoparasitic.

## INTRODUCTION

Flies and midges that affect livestock are not only a nuisance and irritation to the animal, they can also transmit diseases. Flies may be small but they are capable of causing untold damage, especially to animals such as cattle, swine and poultry which are traditionally farmed in intensive numbers. As the weather warms up, there are two main groups of flies which cause problems for cattle producers, flies which bite and feed on blood, and flies which feed on the secretions from the eyes, nose, udder and the sweat on the animal's coat and skin (Axtell and Arrends, 1990). Within the two groups there are a number of different types of flies, some of which transmit disease, and others which are just a plain nuisance, but still distract the cow from feeding. Among different flies, culicoides are one of the major domestic, medical and veterinary pest that cause, irritation, spoils food and acts as a vector for many pathogenic species (Blanton and Wirth, 1979). Milk quality also suffers from the presence of excessive numbers of culicoides flies and production can be downgraded in the absence of adequate fly control (Sacca, 1964). Culicoides is also generally termed as 'Biting Midges'. There are over 4,000 species of biting midges in the Ceratopogonidae family, and over 1,000 in just one genus, Culicoides. Mainly two species, Culicoides peregrinus and Culicoides schultzei, are common in India (Mullen and Durden, 2002). Culicoides are known to transmit diseases, exert extreme discomfort that ultimately results in reduced weight gain and lower milk yield in cattle (Smith and Rutz, 1991). Major animal disease causing pathogens transmitted by the bite of infected biting midges include African Horsesickness virus in equines that is confined primarily to Africa and Epizootic Hemorrhagic Disease virus in ruminants found in North America and principally having lethal effects on deer (Hoolbrook, 1996). For the control of culicoides, Fly treatment is important and has shown

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tremendous results. (Miller,1984). Integrated fly control means using a two-pronged attack on flies: larvicides to prevent fly larvae developing into adults, and adulticides to kill adult flies, is the most popular approach nowadays. Present study was done to assess the efficacy of plant based fly repellant product particularly against Culicoides flies.

Repellants have been suggested as a means to alleviate fly nuisance (Campbell, 1983). Development of pesticide resistance in dipteran flies (Mac donald et al., 1983) has prompted development of physical and biological control measures (Price and Chapman, 1987). The main focus of this experiment is on the biological control methods for house fly control that mainly comprise botanicals agents. Although several biocontrol agents are still in the nascent stage, some have shown reliable field performance and seem to be suitable candidates for commercialization. In this present investigation, a botanical based fly repellant product (AVFRC/18: supplied by M/S Ayurvet Ltd., Baddi, (H.P.), India) is scientifically screened for its efficacy against Culicoides peregrinus and Culicoides schultzei in livestock dwellings.

## MATERIALS AND METHODS

A study was conducted in the livestock dairy farm, MAFSU, Parbhani, Maharashtra, that was reported with the history of culicoides fly menace during summer season, April, 2011. The livestock farm was identified with excessive numbers of Culicoides peregrinus and Culicoides schultzei. The farm premises were treated with the herbal fly repellant product (AV/FRC/18) (supplied by M/S Ayurvet Limited, Baddi, H.P., India). The product comprises of oil of herbs viz. Eucalypta globulus, Cedrus deodara, Pinus longifolia & many others in a fixed concentration and is recommended as 1 part with 20 parts of water for application on animal body and 1 part with 40 parts of water for application on drainage channels and animal premises in the shed, that were taken into account throughout experiment. For current study, two species of Culicoides flies (Culicoides peregrinus and Culicoides schultzei), their larval and adult stages were under consideration. Trial was conducted by using following standard methods of insecticide/Pesticide evaluation recommended by (World Health Organization WHO) and as per the methods recommended by (Busvine, 1971) for evaluating the efficacy of test product. In the experiment, larvae/immature stages /instars were collected from natural breeding sites of Culicoides by employing standard entomological techniques.

### Oviposition deterrent and Ovicidal activity test

herbal fly repellant product was assessed for oviposition deterrent activity. To ascertain the oviposition deterrent activity, AV/FRC/18 was diluted to test concentration of 1:40. The filter paper discs to be used in the breeding chamber were soaked in these dilutions and then added in the breeding chamber. For the test, 5 female Culicoides were placed for oviposition. Observations on egg laying were recorded after 48, 72 and 96 hrs. For each dilution six replications were kept with a control, in which Whatman filter paper disc soaked in water was added. To determine the Ovicidal effect of AV/FRC/18, the product was diluted to test concentration and then filter paper discs on which the female have laid the eggs were placed in the diluted test product. Observations on hatching were recorded after every 12 hrs, and for each dilution six replications with a control was followed.LC50 and other values were determined by graph and formula methods of Finneys probit analysis as described by (Ragupathy, 2002).

### Field Tests

Trials against Culicoides peregrinus and Culicoides schultzei breeding in drainage channels were the site selected for application of herbal fly repellant product at recommended concentration of 1:20. Each drainage channel was considered individually. Drains were divided into sectors. Assessment of the larval population was done by collecting mud samples and processing it by sedimentation technique. Level of effectiveness was from the number of larvae found after treatment compared with pre-treatment levels. Tests were further conducted with a graduated series of dosages of herbal fly repellant product applied to filter paper (Whatman no.1 or equivalent) 12 x 15 cm size. The papers were air dried for 24 hours and then inserted into testing tubes (Prepared locally as per the dimensions and design recommended by WHO to test the susceptibility of adult Culicoides. Groups of 25 non blood fed/ fed female Culicoides, 2 to 5 days old, were placed in each tube, exposed to the treated paper for 1 hour and returned to holding tubes for the determination of the 24 hours dosage / mortality relationship. From the results the probit mortality / log dose regression and hence the LD50 were computed or plotted on appropriate graph paper. Criterion: LD 50 < 0.16 mg / cm2 at 24 h.

### Larvicidal Lab tests

Screening of herbal fly repellant product were performed by exposing larvae in de-ionized or distilled water treated with a series of at least 5 concentrations of the herbal fly repellant product. Twenty late 3rd or young 4th instar larvae were used in the petri dish (water temperature 25±1°C). Tests were replicated 3 times, each time from separately reared batches of larvae. Mortality was

**Table 1.** Oviposition deterrent and ovicidal effect of Ayurvet AV/FRC/18 against *Culicoides peregrinus* and *C. schultzei* species

| Conc of AV/FRC/18 | Oviposition deterrent Number of females (out of the five) released for laying the eggs | | Ovicidal effect Number of eggs hatched (out of fifty) | |
|---|---|---|---|---|
| | *C. peregrinus* (mean ± SE) | *C. schultzei* (mean ± SE) | *C. peregrinus* (mean ± SE) | *C. schultzei* (mean ± SE) |
| 0.0625 | $4.50^{a} \pm 0.19$ | $4.33^{ab} \pm 0.29$ | $30.50^{b} \pm 1.00$ | $45.50^{a} \pm 0.65$ |
| 0.125 | $2.83^{b} \pm 0.12$ | $2.17^{c} \pm 0.29$ | $20.33^{c} \pm 0.72$ | $37.83^{b} \pm 0.65$ |
| 0.250 | $2.50^{b} \pm 0.10$ | $2.83^{c} \pm 0.22$ | $14.66^{d} \pm 0.74$ | $21.83^{c} \pm 0.30$ |
| 0.50 | $1.00^{b} \pm 0.00$ | $0.00^{d} \pm 0.00$ | $5.00^{e} \pm 0.00$ | $5.00^{d} \pm 0.00$ |
| Control | $4.67^{a} \pm 0.21$ | $4.83^{a} \pm 0.30$ | $46.67^{a} \pm 1.04$ | $46.6^{a} \pm 0.72$ |
| CD | 0.55 | 0.64 | 2.9 | 2.14 |
| Statistics | HS | HS | HS | HS |

Means bearing at least one common superscripts within a coloum do not differ significantly.
HS- Highly significant ($P < 0.05$)
CD- Critical Difference

observed after 24 hours and the probit mortality / log dose regression were computed by applying the formula (Raghupaty, 2002)

## RESULTS AND DISCUSSION

Oviposition detterent activity of AV/FRC/18 against Culicoides peregrinus spp. and C. schultzei spp

To ascertain the efficacy of AV/FRC/18 in ovipostion deterrent activity, the numbers of females out of total five under observation released for laying eggs were enumerated for both the species of culicoides at different concentrations of test product. At highest 0.5% concentration of AV/FRC/18 the number of females released for laying eggs were significantly lower 1 out of 5 (20%) for C. peregrinus in comparision to untreated control 4.67 out of 5. Similarly for C. schultzei, AVFRC/18 showed 100% oviposition deterrent activity at 0.5% concentration in comparison to untreated control exhibiting 4.83 out of 5 females releasing eggs. Even the lower concentration of product 0.125%, the oviposition deterrent acitivity is as high as more than 50% (Table I).

### Ovicidal effect of AV/FRC/18 against Culicoides peregrinus spp. and C. schultzei spp.

To ascertain the ovicidal efficacy of AV/FRC/18, the total number of eggs hatched out of total fifty was enumerated for both the species of culicoides at different concentrations of test product. At highest 0.5% concentration of AV/FRC/18 the number of hatched eggs were significantly lower, 5 out of 50 (10%) for C. peregrinus in comparision to untreated control 46.7 out of 50 (93.4%), this suggests 90% ovicidal effect of test product. Similarly for C. schultzei, AVFRC/18 showed 90% ovicidal activity at 0.5% concentration in comparison to untreated control exhibiting 46.6 out of 50 hatched eggs (93 % approximately). Even the lower concentration of product of 0.125%, the ovicidal acitivity is as high as 60% for C. peregrinus spp. Results are. given in Table 1

### Larvicidal Effect

In the lab test conducted with the aim of screening of AC/FRC/18 performed by exposing larvae in de-ionized or distilled water treated with a series of at least 5 concentrations of the AC/FRC/18 on twenty late 3rd or young 4th instar larvae of Culicoides peregrinus spp., the highest mortality of 100% was observed at the 0.1% concentration followed by 50% mortality at 0.05% concentration, 25% mortality at 0.025% concentration of test Product, as observed after 24 hours. The probit mortality / log dose regression were computed by applying the formula (Raghupaty, 2002) and given in (Table 2 Below).

### Culicoides population in the cattle shed after application of AV/FRC/18

Herbal fly repellant product (AV/FRC/18) was applied in animal shed and premises in dilution of 1: 40 using spray technique. The count of culicoides midges in 1×1 sq. feet area in the cattle shed in untreated square area was 30.33±3.23. However, it was reduced to 3.00±0.01 in 1×1 sq. feet area after application of AV/FRC/18. The % reduction in population of flies was 90.1%. Results are summarised in (Table 3 Below).

Count of larval culicoides in the drainage channel around cattle shed after application of AV/FRC/18

Herbal fly repellant product was applied in drainage channels around cattle shed in dilution of 1: 40. The

**Table 2.** Probit chart of AV/FRC/18 against the larvae of *Culicoides peregrinus spp.*

| Conc % | Long Conc. (X) | No of larvae expose (n) | No of larvae dead (r) | % mort-ality | Correc-ted % mortalit | Empiri cal probit | Expec-ted probit (Y) | Working probit (y) | Weigh -ing coeffic. (W) | nw | nwx | $nwx^2$ | nwy | $nwy^2$ | Nwxy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.10 | 1.000 | 20 | 20 | 100 | 100 | 8.71 | 8.40 | 8.7 | 0.005 | 0.1 | 0.1 | 0.1 | 0.87 | 7.569 | 0.87 |
| 0.05 | 0.699 | 20 | 10 | 50 | 50 | 5.00 | 6.11 | 4.425 | 0.405 | 8.1 | 5.66 | 3.95 | 35.84 | 158.60 | 25.05 |
| 0.025 | 0.397 | 20 | 05 | 25 | 25 | 4.32 | 3.99 | 4.427 | 0.405 | 8.1 | 3.22 | 1.28 | 5.66 | 158.78 | 14.26 |
| 0.0125 | 0.096 | 20 | nil | 00 | 00 | 0.00 | 1.33 | 1.33 | 0.005 | 0.1 | 0.0096 | 0.0009 | 0.133 | 0.176 | 0.012 |
| ∑ | | | | | | | | | | 16.4 | 8.998 | 5.330 | 42.503 | 325.125 | 40.20 |

**Table 3.** *Culicoides* population in the cattle shed after application of AV/FRC/18

| Conc of AV/FRC/18 | Count of *Culicoides* midges in 1x1 sq feet area in the cattle shed in untreated square area | Count of *Culicoides* midges in 1x1 sq feet area in the cattle shed in treated square area | Percent reduction in population of flies |
|---|---|---|---|
| 1 in 40 parts of water ( 10%) | 30.33±3.23 | 3.00±0.00 | 90.10% |

**Table 4.** Count of larval *Culicoides* in the drainage channel around cattle shed after application of AV/FRC/18

| Conc of AV/FRC/18 | Count of *Culicoides* larvae in 10 gms of mud the drainage channel around cattle shed before treatment | Count of *Culicoides* larvae in 10 gms of mud the drainage channel around cattle shed after treatment channel | Percent reduction in population of larvae |
|---|---|---|---|
| 1 in 40 parts of water ( 10%) | 69.83±2.67 | 6.66±0.33 | 90.46% |

count of culicoides larvae in 10gm of mud of the drainage channel around cattle shed before treatment in the untreated 1×1 sq. feet area was 69.83±0.67. However, it was reduced to 6.66±0.33 in 1×1 sq. feet area after application of AV/FRC/18. The % reduction in population of flies was 90 %. LC50= 0.078% and LC95= 0.3153% values were obtained against Culicoides larvae for AV/FRC/18 .Results are summarised in Table 4.

The results in the present study are in concommitance with those reported by (Ahmad et al., 1995; Appel et al., 2001; Ngoh et al., 1998), whose results showed that certain essential oils derived from herbs possess potent ectoparasiticidal and repellant activity against cockroaches. Similar results were found against mosquitoes & flies (Watanabe et al., 1993), livestock ticks (Lwande et al., 1999), house flies (Singh et al., 1991) and termites (Zhu et al., 2001a,b). 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 1995a), and cinnamomum spp. (Lin and Yin 1995b), 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 repellancy or feeding deterrancy properties of terpentenoids. Based on the above data (Tables 1-4), it can be said that control strategies involving spraying of AV/FRC/18 on drainage channel and in the cattle shed will help substantially to reduce Culicoides ( fly pest population).

## CONCLUSION

It can be concluded that the product plant based herbal ectoparasiticidal product is quite efficacious as a fly repellant in livestock dwellings, has a good larvicide potential in addition to ovicidal & oviposition deterrent activity for the two species of Culicoides flies.

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