

## INSECT REPELLENT ACTIVITY OF FLUFFYTAILS TICK REPELLENT IR3535 SPRAY ON HORSES.

Flies are winged insects that are usually just an annoyance. However, they can transmit disease and cause problems in animals. They belong to a large, complex order of insects called Diptera. Flies may feed on blood, saliva, tears, or mucus. They also spread bacteria, viruses, and parasites.

Biting flies feed on animal blood. This group includes mosquitoes, black flies, sand flies, biting midges, horse flies, and deer flies. The bites can be painful and may bring on allergic reactions. They can cause welts or skin irritation at bite sites, cause hoof damage from excessive stomping or can cause overall discomfort as horses try to avoid them. Biting flies are a nuisance if they are extremely numerous in the horse's environment or transmit a disease. Nonbiting flies include those that do not feed on blood and do not actually bite the host animal while feeding. Instead, these flies feed on bodily secretions. Both biting and nonbiting flies can transmit diseases to horses and other domestic animals.

Finally, some flies have larvae that may develop in the subcutaneous tissues of the skin or organs of animals, producing a condition known as myiasis (fly strike). The larvae, or maggots, may be free-living or may be parasites of the host animal. (<https://www.msddvetmanual.com/horse-owners/skin-disorders-of-horses/flies-and-mosquitoes-of-horses>)

In humans, mosquitoes are one of the most important disease vectors from a medical viewpoint in that they transmit several diseases such as malaria, filariasis, yellow and Dengue fever. Several synthetic repellents such as DEET, ethyl butyl-acetyl-aminopropionate (IR3535) and 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl) piperidine (Picaridin) have been widely used to prevent humans from receiving mosquito bites.

A study was conducted in horses where a formulation (a spray) containing IR3535 was observed for its effect on flies and mosquito repellency in horses.

### Materials & Methods:

#### Product/Formulation Details:

Fluffy tails Tick Repellent IR3535 Spray contains the repellent IR3535 along with Propylene glycol, Sodium citrate, Citric acid, PEG 40 hydrogenated Castor oil and Benzalkonium chloride. It is available in a spray form.

#### Method:

The study was conducted at 3 farms, Arohi Stud Farm, Shubh Stud Farm and K.K. Patel Stud Farm, all located in Bhadaj area of Gandhinagar, Gujarat. 12 horses of Kathiyawadi breed were used for the study. The study was performed from the 15th to the 19th of June 2023. The horses were of mixed breed, different sex and coat colour, aged 13–22 years and with a range of 450–650 kg body weight.

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The presence and number of all the insects (flies and mosquitoes) were recorded by three different operators throughout the entire study. One was positioned at the head and the other two at the right and left side of the animals, and they were appointed to count flies and mosquitoes that remained in contact with horses for at least 5 s. Flies and mosquitoes on horses' legs and bodies were counted from 0 to every hour for 10 hours. The counting time lasted 2 min and was calculated with the help of a stopwatch alarm.

#### Treatment Protocol:

Before treatment (day 1), horses were individually examined to evaluate pre-treatment insect counts. Two enumerations were carried out, in the morning (10:00 am) and in the afternoon (3:00 pm). Based on results, three groups were formed:

Group 1- Control Group (3 horses)

Group 2- The FluffyTails Tick Repellent IR3535 Spray (20%) treated group (6 horses)

Group 3- Neem-based treatment group (3 horses)

Before treatment, horses were carefully brushed to remove dirt and debris from the coat. On day 0, the product to be tested was applied using the spray bottle. The product was directly sprayed all over the horse's body once a day. All parts of the horse were sprayed, including mane and tail. After administration of the product, horses were adequately spaced from each other to facilitate insect counting. The repellency was studied for 10 hours.

Neem oil (20%) was applied to horses belonging to Group 3. It was applied uniformly all over the body.

Insect counts were performed on day 0, before administration of the product (at 10:00 am) and subsequently at 10, 20 and 30 min and at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h (8pm) after the administration.

#### Statistical Analyses:

The counts for each group were represented as Mean ± SEM. The insect counts obtained during complete treatment duration from 10mins to 10 h after administration was compared with the pretreatment values (0 min.) using ANOVA.

The counts across different treatments at different time intervals were also compared with the values corresponding to that of Control using unpaired Students t-test.

The percentage of effectiveness for the different time points after treatment was calculated using the average number of insects counted for each horse belonging to the two groups, control and treated, according to the following formula (Abbott 1987):

$$\% \text{ efficacy} = \frac{\text{average no. of insects in the control group} - \text{average no. of insects in treated group}}{\text{average number of insects in the control group}} \times 100$$

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## Results:

The insect count in the Control Group (Group 1) was almost similar for 7 hrs ie. upto 5 pm. During 6pm to 8pm (8-10h) the count of insects increased significantly ( $p < 0.001$ ), probably due to their natural increased prevalence in the evenings (Table 1).

In the Group 2 which received the Fluffy Tails Tick Repellent IR3535 Spray, there was a significant reduction ( $p < 0.001$ ) in the insect count from 10min after treatment to 7h post treatment, as compared to the pretreatment count (0 min). The count was also significantly reduced during the 8<sup>th</sup> hour ( $p < 0.01$ ) and the 9<sup>th</sup> hour ( $p < 0.05$ ). During the last observation ie. the 10<sup>th</sup> hour (8pm) the effect was found to be non-significant (Table 1).

Group 3 horses which were applied Neem oil (20%) showed significant reduction ( $p < 0.001$ ) in insect count from 10 mins of application to 1 h post treatment as compared to pretreatment (0 min). A significant reduction was also observed at 2h ( $p < 0.01$ ) after which there was no significant reduction till the end of the study ie. 10<sup>th</sup> h post application (Table 1).

**Table 1: Effect of Fluffy Tails Tick Repellent IR3535 Spray on Insect Counts.**

Time	Group 1 (Control Group)	Group 2 (Fluffy Tails Tick Repellent IR3535 Spray)	Group 3 (20% Neem Oil)
0 min	23.33 ± 0.88	24.00 ± 0.58	24.67 ± 0.67
10 mins	23.00 ± 1.73	17.67 ± 1.33 <sup>a***</sup>	15.00 ± 0.58 <sup>a***, b*</sup>
20 mins	21.67 ± 0.33	12.00 ± 0.68 <sup>a***, b***</sup>	13.33 ± 0.88 <sup>a***, b***</sup>
30 mins	23.67 ± 1.86	9.33 ± 0.49 <sup>a***, b***</sup>	10.67 ± 0.33 <sup>a***, b**</sup>
1 h	21.33 ± 0.33	4.67 ± 0.71 <sup>a***, b***</sup>	14.67 ± 0.88 <sup>a***, b**</sup>
2h	20.67 ± 0.67	2.50 ± 0.34 <sup>a***, b***</sup>	20.33 ± 0.88 <sup>a**</sup>
3 h	19.67 ± 0.88	1.67 ± 0.33 <sup>a***, b***</sup>	21.67 ± 0.88
4 h	19.33 ± 0.33	1.50 ± 0.43 <sup>a***, b***</sup>	23.00 ± 0.58 <sup>b**</sup>
5 h	22.67 ± 0.67	4.50 ± 0.72 <sup>a***, b***</sup>	22.67 ± 0.88
6 h	22.67 ± 0.88	10.00 ± 0.58 <sup>a***, b***</sup>	23.67 ± 0.88
7 h	25.67 ± 2.85	16.67 ± 1.20 <sup>a***, b**</sup>	24.00 ± 0.58
8 h	32.00 ± 1.00 <sup>a***</sup>	19.67 ± 0.95 <sup>a**, b***</sup>	26.00 ± 0.58 <sup>b**</sup>
9 h	32.00 ± 0.58 <sup>a***</sup>	20.83 ± 0.91 <sup>a*, b***</sup>	26.67 ± 0.33 <sup>b**</sup>
10 h	31.67 ± 1.20 <sup>a***</sup>	25.83 ± 0.60 <sup>b**</sup>	26.67 ± 0.67 <sup>b*</sup>

Values are represented as Mean ± SEM. <sup>a\*</sup> $p < 0.05$ ; <sup>a\*\*</sup> $p < 0.01$ ; <sup>a\*\*\*</sup> $p < 0.001$ - Counts from 10 mins to 10h were compared with 0h or pretreatment counts within the Group.

<sup>b\*</sup> $p < 0.05$ ; <sup>b\*\*</sup> $p < 0.01$ ; <sup>b\*\*\*</sup> $p < 0.001$ - Groups 2 and 3 were compared with Group 1.

The counts at different time intervals post Fluffy Tails Tick Repellent IR3535 Spray when compared to the counts at corresponding time intervals of the Control Group was found to be significant after 20mins post treatment to the end of treatment ie. 10<sup>th</sup> h (Table 1).

The Neem oil treated horses (Group 3) also showed significant reduction in insect count from 10mins to 1h as compared to the Control Group. After 2 h to 7h it showed no significant effect (except at the 4<sup>th</sup> h) as compared to the corresponding time intervals of Control Group. The rise in insect count from the 6 pm to 8pm in the Control Group was significantly reduced in Group 3 due to Neem oil (Table 1).



The % efficacy of Fluffy Tails Tick Repellent IR3535 Spray increased from 23% after 10mins of treatment to 92.24% after 4 hours and then reduced gradually to 18.42% after 10hrs of treatment. Similarly, the % efficacy of Neem oil was found to be 34.78% post 10mins which increased to 54.93% at 30mins and then reduced to 31.25% after 1 h of application. After 2 h the efficacy was only 1.61% and then showed no efficacy after that till the 7<sup>th</sup> h after which it showed slight variable efficacy ranging from 6.49% to 18.75% during the last 4 hours (5 to 8pm) of observation period (Table 2).

**Table 2: % Efficacy of Repellency of FluffyTails Tick Repellent IR3535 Spray on Insect Counts.**

Time	Group 2 (Fluffy Tails Tick Repellent IR3535 Spray)	Group 3 (20% Neem Oil)
0 min	--	--
10 mins	23.19	34.78
20 mins	44.62	38.46
30 mins	60.56	54.93
1 h	78.13	31.25
2h	87.90	1.61
3 h	91.53	-10.17
4 h	92.24	-18.97
5 h	80.15	0.00
6 h	55.88	-4.41
7 h	35.06	6.49
8 h	38.54	18.75
9 h	34.90	16.67
10 h	18.42	15.79

**Conclusion:**

Application of IR3535 containing spray effectively repelled flies and mosquitoes for 9 hours. Traditionally the farm/stable owners used Neem oil or fogging by burning Neem leaves which helped to keep away the flies and mosquitoes from the horses only for a span of maximum 1-2 hrs. Thus, spraying the horses only once a day helps to keep them away and protected from the flies and mosquitoes, and proves to be a better option. Spraying Fluffy Tails Tick Repellent IR3535 Spray on the horses twice a day can offer protection from these insects for almost 18 hours.

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