Research Note

IN VITRO EVALUATION OF A PERMETHRIN-BASED PESTICIDE FOR THE CONTROL OF HAEMATOBIA IRRITANS[1,2]

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Dairy production in tropical regions faces several challenges. These include low quality forages, environmental conditions that hinder milk production, and elevated populations of arthropods (Berman, 2011). Among arthropods, biting flies are characterized by a piercing-sucking mouthpart. These types of flies are attracted to their prey by movement and the carbon dioxide they exhale. When attracted to mammal hosts, they land on their surfaces to pierce the skin and insert their mouthpart to feed on blood (Fernandes et al., 2020). The constant piercing effect may cause pain, irritation, and discomfort to the host. The cows respond with behavioral changes such as repetitive tail switching and head tossing that hinder weight gain and milk yield in dairy cattle (Boland et al., 2008). Some biting flies' species include the horn fly, *Haematobia irritans* (Linnaeus) (Diptera: Muscidae), and the stable fly, *Stomoxys calitrans* (Linnaeus) (Diptera: Muscidae), both reported in Puerto Rico.

The horn fly is one of the most economically important obligate blood-feeding ectoparasites that affect livestock (Byford et al., 1992). Losses due to horn fly infestations include weight losses ranging from 8.9 g and 28.0 g per day, based on studies done in Brazil (Bianchin and Alves, 2002) and Argentina (Guglielmone et al., 2001). In the United States, the annual economic losses due to horn fly infestations reached approximately $2.3 billion in 2020 (Brewer et al., 2021).

Chemicals are the most used method to control the flies on dairy farms due to their low cost and fast reductive action on fly populations. However, their indiscriminate use promotes the development of chemical-resistant fly populations, ultimately reducing their efficacy. Among the chemicals used for controlling insects are pyrethroids, such as permethrin. This chemical has a neurotoxic effect, generating paralysis and subsequently, death (Casida and Durkin, 2013). A consequence of using this control method is the proliferation of resistant populations of insects (Soderlund, 2012) and toxicity in humans (Bradberry et al., 2005). The development of chemical resistance has been correlated with indiscriminate use over time (Scott et al., 2000). The objective of our study

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was to evaluate the efficacy of a commercial pyrethroid insecticide on horn flies within an in vitro setting.

To evaluate the efficacy of the permethrin-based insecticide (KattleGuard III,1.0% permethrin, 1.0% Piperonyl Butoxide Technical and 98.0% of other ingredients)[6] on *H. irritans*, the impregnated paper bioassay method was adapted from Sheppard and Hinkle (1987). The insecticide was evaluated undiluted (1% of permethrin) or diluted with acetone in four concentrations (0.50%, 0.25%, 0.12% and 0.06% of permethrin). To impregnate the filter paper (Whatman® Qualitative Filter Paper, Grade 4, Circles; Buckinghamshire, United Kingdom), 1 ml of each dilution was dispersed evenly over the individual papers. To determine whether the filter impregnation time could influence the mortality rate of flies, each impregnated filter paper was refrigerated inside aluminum foil for 24, 48, 72, and 96 hours to allow the acetone to evaporate.

Horn flies were collected from the dairy farm at the Lajas Agricultural Experiment Substation of the University of Puerto Rico. The experimental dairy farm is located at latitude 18°01'27.4"N, longitude 67°04'33.3"W. The routine use of chemicals for fly control at the dairy farm was interrupted for two weeks prior to the collections. The flies were collected directly from the cows by the Cow Vac ® (Spalding Labs, Reno, NV). Flies were transferred to a plastic bag using a Heavy-Duty Hand-Held Vac/Aspirator (Bio-Quip, Rancho Dominguez, CA).

The flies were transported to the laboratory at UPRM in a foam container with ice packs. At the laboratory, each impregnated filter paper was placed inside a 150 ml glass jar (one jar per insecticide concentration). Horn flies (n=20) were knocked down with CO_2 and assigned to each jar (20 flies per jar). The control consisted of an acetone impregnated filter with no insecticide. Fly mortality was evaluated at 20, 40, 60, and 120 minutes after assigning flies to a jar containing the impregnated filters. Mortality was determined by the fly's inability to walk, fly, or move (Holderman et al., 2018). Each treatment was replicated three times; groups were arranged in a randomized complete block design. The experiment was conducted in February and duplicated in March of 2019. The data was analyzed using a generalized linear model (PROC GLIMMIX) and two or three-way analysis of variance (ANOVA) using the SAS software (version 9.4; SAS Institute, Cary, NC). A Fisher's Least Significant Difference (LSD) analysis was used to identify significant differences in mortality rates between groups. A probability value of $P < 0.05$ was used to indicate statistical significance.

Within the family of Pyrethroids, Permethrin is one of the insecticides most used to control flies in dairy cattle (Holderman et al., 2018). The effectiveness of this insecticide against specimens of *H. irritans* was evaluated in vitro. The results corresponding to the effect of the chemical on the mortality rate of *H. irritans* (Table 1) show no significant difference between filtered paper impregnation times (24, 48, 72, and 96 hours). For this reason, filtered papers were impregnated with different insecticide concentrations of permethrin (1%, 0.50%, 0.25%, 0.12% and 0.6%) for 24 hours. Results of fly mortality rates after being exposed to the chemical dilutions (Table 1) show significant differences between treatments ($p<0.0001$) and evaluation times ($p<0.0001$). However, there was no significant difference in fly mortality ($p>0.1645$) between the evaluated periods (February vs. March, data not shown). When the commercial insecticide was used as recommended by the manufacturer (1% permethrin), a 100% mortality rate was observed at all evaluated times (Table 2). We hypothesize that the reason for 100% fly mortality is due

[6]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

TABLE 1.—*Three-way ANOVA results showing the individual effect of impregnation time, treatment, evaluation time or their interactions over horn fly mortality rates (in vitro).*

		February-19		March-19	
Model	DF	F-Value	P-Value	F-Value	P-Value
Impregnation Time (I)	3	2.93	0.1217	0.6	0.6361
Treatment (T)	4	595.73	**< 0.0001**	389.98	**< 0.0001**
Evaluation Time (E)	3	36.32	**< 0.0001**	44.79	**< 0.0001**
I*T	12	8.50	**< 0.0001**	3.48	**0.0001**
I*E	9	0.86	0.5590	1.45	0.1712
T*E	12	10.72	**< 0.0001**	12.06	**< 0.0001**
I*T*E	36	0.8	0.7783	0.74	0.8587

to the direct, continuous, and enclosed interaction of the flies with the insecticide. However, the interaction between flies and the commercial permethrin in an in vivo setting is completely different, by being exposed to wind, water, and solar radiation.

The literature characterizing the effect of this insecticide (or technical-grade permethrin) on flies under field conditions is limited. For example, permethrin has been used as fly control in research focused on bunching behavior against flies (El Ashmawy et al., 2020) and in another study that estimated the fly's activity during different seasons and pest control managements such as the use of pyrethroid insecticides (El Ashmawy et al., 2021). Other studies reported no significant difference in fly numbers when using automatic insecticide dispensers (sprayers; 1% permethrin and 1% piperonyl butoxide) as a control method for stable flies (Gerry and Abdelfattah, 2021). Neither of the published literature characterized the effect of permethrin on the control of horn flies under field conditions.

Additional factors should also be considered. Variables such as insecticide resistance must be taken into consideration, genetic or behavioral in nature. Behavioral modifications include mechanisms by which flies avoid contact with the insecticide by moving away from areas with high insecticide concentrations (i.e., repellency), subsequently returning when the concentration in the host declines (Guglielmone et al., 2001). In addition, flies tend to move to the body parts of the host where insecticide concentrations are low (Foil and Hogsette, 1994). Genetic resistance of *H. irritans* populations to permethrin has been previously reported (Holderman et al., 2018). Specifically, the knockdown resistance genotype L150F, which confers resistance to pyrethroids, has been identified in *H. irritans* collected from fields in the United States (Holderman et al., 2018).

We hypothesize that insecticide effectiveness in controlling flies will be lower under in vivo conditions. According to the manufacturer, the commercial insecticide has a 15-day effectiveness span from the day of application. However, we propose that its effectiveness over horn flies is less. This could be explained by other resistant mechanisms such as target site mutations, detoxification, cattle breed, insecticide misuse, or geographic conditions. For a thorough assessment of the effectiveness of permethrin based KattleGuard III, we propose a study describing horn fly control effectiveness of the commercial insecticide under field study conditions over a period of 15 days after insecticide application.

Moreover, the commercial insecticide has different components (e.g., piperonyl butoxide, and petroleum distillate) which by themselves, or in combination with permethrin, could play a role in horn fly mortality. Therefore, the effect of environmental inter-

TABLE 2.—*Two-way Anova results showing the effect of commercial permethrin-based insecticide treatments and exposure times over horn fly mortality rates (in-vitro).*

	February- 2019				March- 2019			
	Evaluation Time (min)				Evaluation Time (min)			
	20	40	60	120	20	40	60	120
Treatment	% of Mortality[2]				% of Mortality[2]			
As recommended	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
Dilutions[1]								
0.50%	51.67 ± 14.53 b	68.33 ± 12.02 b	91.67 ± 6.01 a	100 ± 0 a	18.33 ± 10.93 b	88.33 ± 9.28 a	100 ± 0 a	100 ± 0 a
0.25%	5.00 ± 2.89 c	5.00 ± 2.89 c	11.67 ± 1.67 b	13.33 ± 1.67 b	6.67 ± 1.67 b	33.33 ± 12.02 b	56.66 ± 23.15 b	68.33 ± 22.42a
0.12%	3.33 ± 1.67 c	5.00 ± 0 c	5.00 ± 0 c	6.67 ± 1.67 c	3.33 ± 1.67 b	5.00 ± 0 c	6.67 ±1.67 c	10.00 ± 5.00 b
0.06%	5.00 ± 2.89 c	5.00 ± 2.89 c	5.00 ± 0 c	5.00 ± 2.89 c	0 ± 0 b	5.00 ± 5.00 c	8.33 ± 3.33 c	8.33 ± 3.33 b
Acetone (Control)	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 b	0 ± 0 c	0 ± 0 c	0 ± 0 b

[1]Commercial permethrin-based insecticide (KattleGuard III) has 1% A.I. in its proportion; this was diluted in the acetone.

[2]% of mortality (represented as mean± standard error of the mean) within a column followed by the same letter are not significantly different at $p< 0.05$ using least significant difference (LSD).

actions and the individual effect of permethrin (or any of the other components) as the causative agent of fly mortality should be evaluated.

CONCLUSIONS

Our in vitro assessment revealed promising results, with the 1% permethrin concentration, KatleGuard III exhibiting remarkable efficacy by achieving 100% fly mortality within 20 minutes of exposure. This finding indicates the rapid action of this pyrethroid-based insecticide against horn flies under controlled conditions.

In vitro assays offer controlled environments that do not fully emulate the dynamic conditions present in the field. In an uncontrolled environment, insecticide bioassays encounter various environmental and management factors, which can influence the effectiveness of an insecticide differently than under laboratory conditions. Also, it's important to acknowledge the potential challenges associated with resistance, both in terms of genetic mutations and behavioral adaptations of horn fly populations. The development of resistance mechanisms, such as avoiding areas with high insecticide concentrations (repellency) or seeking body parts with lower concentrations, is important now for finding effective fly control. Thus, our study highlights the importance of continuously monitoring and assessing the evolution of resistance patterns in horn fly populations.

To provide dairy farmers with evidence-based recommendations, we propose rigorous field studies that assess the effectiveness of insecticides over a 15-day period following application. Such studies should consider the diverse range of variables that can impact fly control under field conditions.

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