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Efficacy and hair deposition of pyrethroid- and organophosphate-based acaricides applied at different working pressures for the control of *Rhipicephalus microplus* in dairy cattle

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A R T I C L E   I N F O

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A B S T R A C T

Rhipicephalus microplus significantly affects dairy cattle, with spray-applied acaricides commonly used for its control. However, increasing resistance and variations in application methods may compromise treatment efficacy. This study evaluated the efficacy of acaricide formulation containing pyrethroids and organophosphates, as well as its deposition in bovine hair when applied at different working pressures. Cattle were divided into three groups (n = 10 each): Group 1 received treatment at 50 psi using a backpack sprayer, Group 2 at 300 psi using a power sprayer, and Group 3 served as an untreated control. Female ticks (4.5–8 mm) were counted on days 3, 7, 14, 21, and 35 post-treatment (DPT). Hair samples were collected from six body regions (dewlap, belly, between the legs, loin, legs, and neck) at 0, 1, 2, and 3 DPT. Residual concentrations of cypermethrin, chlorpyrifos, and fenthion were measured using direct analysis in real-time mass spectrometry (DART-MS). A significant difference ($p \leq 0.05$) in tick reduction was observed between the treated groups only at 3 DPT. Peak efficacy occurred on day 21 in both treated groups, with efficacy ranging from 56.7% to 97.5% (50 psi) and 78.9–87.4% (300 psi). Cypermethrin levels were consistently higher at 50 psi across all days and most body regions. Chlorpyrifos and fenthion showed higher concentrations on day 0 at 300 psi, especially in the loin and neck. The pressure did not lead to significant differences in overall efficacy against *R. microplus*. However, it significantly affected the deposition of active ingredients in bovine hair.

1. Introduction

The cattle tick *Rhipicephalus microplus* is a major ectoparasite of cattle and a leading cause of economic losses in the global livestock industry. Its control relies primarily on chemical acaricides. However, the widespread and indiscriminate use of these compounds has contributed to the development of resistance within tick populations (Abbas et al., 2014; George et al., 2004; Klafke et al., 2024; Strydom et al., 2025). This growing resistance underscores the need for alternative strategies to enhance the efficacy of acaricides available on the market, mainly those used in dairy cows.

In dairy herds, acaricidal control consistently based on pyrethroid,

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organophosphate, and amidine, owing to their short withdrawal periods for milk. Currently, the most common commercial formulations are pyrethroid–organophosphate combinations, typically applied via spraying methods (Moraes et al., 2023). Multiple types of application equipment such as hand sprayers, backpack sprayers, power sprayers, spray dips, and spray races are used depending on operation scale and logistics (Rodrigues et al., 2015; Moraes et al., 2023). Despite the widespread use of these delivery methods, relatively few studies have systematically investigated whether spray pressure affects acaricide efficacy. Even fewer have examined how spray pressure influences the deposition of active ingredients in bovine hair (Drummond et al., 1968; Rodrigues et al., 2015; Roulston and Wilson, 1965; Wharton et al., 1970; Schnitzerling and Walker, 1985; Moraes et al., 2023; Batista Junior et al., 2024; Souza et al., 2025).

In studies evaluating combinations of pyrethroids and organophosphates and the influence of working pressure, two recent works stand out. However, neither assessed acaricide deposition in cattle hair. Moraes et al. (2023) compared different spraying devices, power sprayer, spray race, and backpack sprayer, operating at approximately 700 psi, 44 psi, and 31 psi, respectively. They reported no significant difference in tick counts between the treated groups after application, although acaricide efficacy tended to be higher at higher pressure. Similarly, Souza et al. (2025) evaluated the efficacy of commercial acaricide using the same equipment (power sprayer) under two different pressures (50 psi and 100 psi) and found no significant difference in tick counts between the treated groups. However, in both studies, efficacy values were consistently higher at higher application pressures. These findings suggest that, while tick counts may remain statistically similar across groups treated under varying pressures, acaricide efficacy tends to improve with increased pressure. This underscores the need for more controlled studies examining the role of application pressure in acaricide performance, particularly its impact on active ingredient deposition in bovine hair.

Given this context, the objectives of the present study were: (i) to evaluate the efficacy of a pyrethroid- and organophosphate-based acaricide applied at working pressures; and (ii) to assess the deposition of the active ingredients in cattle hair by analyzing samples collected from different body regions at multiple time points.

2. Materials and methods

2.1. Farm, herd and acaricide choice

The experiment was conducted at the dairy farm of the of the School of Veterinary and Animal Science of the Federal University of Goiás, located in the municipality of Goiânia, Goiás, Brazil (16∘ 35' 39.07" S, 49∘ 17' 1.22" W). The region has an "Aw" climate, tropical with two well-defined seasons, including a rainy summer (October to April), according to the Köppen–Geiger climate classification (Alvares et al., 2014). The herd consisted of 7/8 Holstein × 1/8 Gyr crossbred cows. The acaricide used was a commercial formulation containing chlorpyrifos (375 ppm), fenthion (185.5 ppm), and cypermethrin (185.5 ppm) (Colosso® FC30, Ourofino Saúde Animal).

2.2. Groups, acaricidal bath and efficacy

For the experiment, 30 animals were selected and divided into three groups, with 10 animals per group, as recommended by previous studies and by Brazilian technical regulations for the licensing and/or renewal of licenses for veterinary antiparasitic products (Brasil, 1997; Moraes et al., 2023; Souza et al., 2025). The animals used had not received any acaricide treatment for at least 120 days. Block formation was performed based on mean counts of female *R. microplus* measuring 4.5–8.0 mm in length. Tick counts were performed on each animal three days prior to treatment (days −3, −2 and −1) on the left side of the body and not multiplied by two (Wharton and Utech, 1970).

The following groups were then formed: Group 1 - group treated using 50 psi (3.4 bar), Group 2 - group treated using 300 psi (20.7 bar) and Group 3 - control group (not treated). A power sprayer (HS-22, Yamaho®), equipped with a pressure gauge (0–400 psi), was used for acaricidal treatment. The choice of 50 psi is related to the operating pressure of the backpack spray, whereas the 300 psi value represents an average pressure of the equipment used by Moraes et al. (2023) and Souza et al. (2025). From a practical standpoint, this reflects the fact that there are at the types of acaricide spraying equipment for dairy cattle available on the market, operating at different pressure ranges.

On day 0, the acaricidal spray formulation was prepared according to the manufacturer's instructions. Each animal received a full-body application of 5 L (1 L/100 kg) of the solution, administered by the same operator to ensure consistency. To assess acaricidal efficacy, engorged female ticks measuring 4.5–8 mm in length were counted on days 3, 7, 14, 21, and 35 post-treatment, following the methodology described by Holdsworth et al. (2022). Acaricidal efficacy was calculated using the formula (Brasil, 1997):

$$\text{Efficacy percentage} = \left[1 - \frac{\text{Ta } x \text{ Cb}}{\text{Tb } x \text{ Ca}}\right] x100$$

Ta = average number of females between 4.5 and 8 mm in length counted on the animals in the treated group after the acaricide treatment; Tb = average number of females between 4.5 and 8 mm in length counted on the animals in the treated group from days −3 to −1; Ca = average number of females between 4.5 and 8 mm in length counted in the control group after the acaricide treatment; Cb = average number of females between 4.5 and 8 mm in length counted on the animals in the control group from days −3 to −1.

2.3. Cattle hair samples

Cattle hair was collected from the treated groups on different days after treatment (day 0 – six hours after treatment and daily until day 3) and regions of the body (dewlap, belly, between legs, loin, legs and neck). Approximately 1 g of hair was collected from each animal with the aid of scissors. The hair from each animal was stored individually in hermetic plastic bags, identified, and frozen (-20 °C) until the moment of evaluation. Cattle hair extracts were prepared by adding 300 μL of methanol to 10 mg of hair, followed by the addition of pyrilamine (1.0 μg mL^{-1}) as the internal standard (IS). The mixture was vortexed for 1 min, after which the supernatant was separated and filtered prior to analysis.

2.4. Direct analysis in real time mass spectrometry analysis (DART-MS)

The presence of fenthion, chlorpyrifos and cypermethrin in cattle hair was evaluated using direct analysis in real-time mass spectrometry (DART-MS) technique described by Batista-Junior et al. (2024). Methanol (>99.90 %) was acquired by Tedia (Ohio, USA). Fenthion (>99.90 %), chlorpyrifos (>99.90 %), and cypermethrin (>99.90 %) and pyrilamine (>99.90 %) were acquired from Sigma-Aldrich (Pennsylvania, USA).

The literature reports several methodologies for the analysis of these compounds based on MS approaches, many of which rely on hyphenation with chromatographic techniques. Although these methods yield reliable results, they are often time-consuming and involve high consumption of organic solvents. In contrast, DART-MS emerges as a faster and more sustainable MS-based technique, employing heated gas to desorb and ionize compounds directly from a sample. As demonstrated by Batista Junior et al. (2024), DART-MS enables both direct qualitative analyses, aimed at determining compound presence or absence, and quantitative analysis. Both strategies represent viable alternatives for assessing fenthion, chlorpyrifos, and cypermethrin; however, the present work focused on quantitative analysis to compare the levels of these compounds in relation to their application pressure on animals. Accordingly, calibration curves were constructed for each analyte, showing excellent linearity ($r^2 > 0.99$) and low limits of quantification, which enabled accurate quantification of the target compounds.

Five µL of the cattle hair extract were deposited onto a QuickStrip™ card, which was positioned between the DART ion source and the mass spectrometer inlet. Analyses were performed with a DART-VSP ion source (IonSense, Massachusetts, USA) coupled to a micrOTOF-Q III mass spectrometer (Bruker Daltonics, Bremen, Germany), operating in positive ion mode. The DART ion source employed helium (White Martins, 99.999 % purity) as the ionization gas, with the temperature of 200 °C, a grid voltage of 350 V, and a gas flow rate of 3.0 L min^{-1} . Mass spectrometric data were acquired over the *m/z* 200–500 range with an acquisition time of 30 s. Peak annotations for antiparasitic agents were based on exact mass measurements with a mass error below 10.0 ppm. Spectral data were processed using DataAnalysis software version 5.0 (Bruker Daltonics, Bremen, Germany).

Cattle hair obtained from animals with no antiparasitic treatment was used as the control matrix. Aliquots of 10 mg of this control hair were spiked with chlorpyrifos at concentrations ranging from 0.025 to 5.0 ng mL^{-1} for fenthion and cypermethrin and 0.050–10.0 ng mL^{-1} for chlorpyrifos, adding IS at 1.0 µg mL^{-1}. All experiments were performed in quintuplicate ($n = 5$). The analytical response was expressed as the ratio of the analyte peak intensity to that of the IS, and linearity across the investigated concentration ranges was assessed using calibration curves. Figures of merit of the DART-MS method for determination of fenthion, chlorpyrifos, and cypermethrin was evaluated according to the validation of analytical procedure guidelines prescribed by Brazilian guidelines based on National Health Surveillance Agency (ANVISA, 2017).

2.5. Acaricide deposition analysis

For quantification of fenthion, chlorpyrifos and cypermethrin in real samples, five µL of the cattle hair extract were deposited onto a QuickStrip™ card, which was positioned between the DART ion source and the mass spectrometer inlet. The ratio between the analyte and IS signal intensities was used as the analytical response, and calibration curves were employed for analyte quantification.

2.6. Statistical analysis

The data obtained from *R. microplus* females counts were transformed using ln (x + 1). The logarithmic transformation was chosen as a standard approach for ectoparasite count data and, in this study, effectively normalized the residuals and homogenized the variances, allowing the appropriate use of the linear mixed-effects model. Specifically, after applying the ln (x + 1) transformation, the data met the assumptions of normality (Shapiro–Wilk test, $p > 0.05$) and homogeneity of variances (Levene's test, $p > 0.05$), thereby validating the use of the linear mixed-effects model. This approach is well established in the field of veterinary parasitology and provides a robust fit to the data, being preferable to count-based models when model assumptions are satisfied. Post-transformation, the data met assumptions of normality, homogeneity of variances, and residuals. The transformed data were analyzed using a linear mixed model for repeated measures, which included fixed effects for treatments, study day, and their interactions.

In the linear mixed-effects model, the experimental unit (animal) was included as a random effect to account for inherent variability among subjects (replicates) and for the dependence of repeated measurements over time. A first-order autoregressive covariance structure (AR(1)) was assumed, as tick counts measured at closer time points are expected to be more highly correlated than those measured at more distant time points. Tick counts between treatments were compared using Tukey's test, which adjusts p-values for multiple comparisons, with the significance level set at $p \leq 0.05$.

3. Results

3.1. Tick counts and acaricide efficacy

The tick counts and efficacy are shown in Table 1. The mean tick counts for the group treated with 50 psi and 300 psi ranged from 27.94 to 5.63 and 20.25–6.88, respectively, on days 3–35 after treatment. In contrast, the control group exhibited mean counts ranging from 70.56 to 33.16 over the same period. No significant differences ($p > 0.05$) were observed in mean tick counts among the treatments and control groups on day 0. A significant difference between the treated groups was observed only on day 3 post-treatment ($p \leq 0.05$). Both treated groups demonstrated peak efficacy on day 21, with the 50 psi group showing efficacy ranging from 56.7 % to 97.5 %, and the 300 psi group ranging from 78.9 to 87.4 %.

3.2. DART-MS method evaluation

The calibration curves exhibited good linearity within these ranges ($r^2 > 0.99$). Figures of merit were evaluated, including the limit of quantification, precision, accuracy, and matrix effect are shown in Table 2. Precision and accuracy values were in accordance with analytical method validation guidelines, remaining below 15 % even at the lowest level of quantification. The absence of significant matrix effects was observed, with values between 80 % and 120 %.

3.3. Acaricide deposition in cattle hair

The mean concentration of the analytes cypermethrin, chlorpyrifos, and fenthion in the hair of cattle treated with acaricide, without considering specific body regions, in each evaluation day at different working pressures are shown in Table 3. Fig. 1 illustrates these results, considering only six hours post-treatment. At a working pressure of 50 psi the three analytes showed similar concentrations on days 0 and 1, and on days 2 and 3. From day 2 onwards, a decrease in concentration was observed. The same trend was seen with the 300 psi treatment. Evaluating within the same analyte, comparing the working pressures,

Table 1
Mean counts of *Rhipicephalus microplus* females (4.5–8 mm) from cattle treated or not with acaricides using different working pressures and efficacy.

Days	Experimental groups/Average number of *R. microplus* (4.5 – 8.0 mm) in cattle			P value	CV	Efficacy (%)	
	T01: 50 psi	T02: 300 psi	Control			T01	T02
0*	66.44 ^{A}	65.06 ^{A}	64.81 ^{A}	0.9523	20.45	-	-
3	27.94^{B}	20.25 ^{C}	70.56 ^{A}	0.0001	35.96	61.4	71.4
7	5.06^{B}	4.25^{B}	63.25 ^{A}	< 0.0001	47.04	92.2	93.3
14	1.31^{B}	1.38^{B}	61.63 ^{A}	< 0.0001	53.45	97.9	97.8
21	0.75^{B}	1.31^{B}	56.56 ^{A}	< 0.0001	57.93	98.7	97.7
28	3.81^{B}	6.00^{B}	47.56 ^{A}	< 0.0001	53.74	92.2	87.4
35	5.63^{B}	6.88^{B}	33.19 ^{A}	< 0.0001	63.21	83.5	79.4

CV: coefficient of variation
Values followed by the same uppercase letter in the row do not differ from each other ($p > 0.05$)

Table 2
Limits of quantification, precision, accuracy, and matrix effect values for the analysis of acaricides by DART-MS.

Analyte	LOQ (ng mL^{-1})	Concentration (ng mL^{-1})	Precision (%)	Accuracy (%)	Matrix effect %
Fenthion	0.025	0.25	5.01	4.67	98.00
		0.5	9.99	14.8	88.40
		2.5	6.58	2.66	102.80
Chlorpyrifos	0.05	0.50	8.54	12.67	85.00
		1	14.31	7.89	106.30
		5	4.61	5.39	115.80
Cypermethrin	0.025	0.05	10.39	12.38	90.70
		0.5	6.68	6.52	94.70
		2.5	9.94	13.47	81.55

LOQ: limits of quantificationd

Table 3
Mean concentrations of each analyte in the hair of cattle treated with an acaricide formulation containing cypermethrin, chlorpyrifos, and fenthion, applied using a power sprayer at working pressures of 50 and 300 psi, over a three-day period.

Analyte	Day	Working pressure / Mean and standard deviation of the analyte (ng/mL)	
		50 psi	300 psi
Cypermethrin	0*	0.02946 ± 0.04832^{Aa}	0.00268 ± 0.00285^{Ba}
	1	0.02916 ± 0.03331^{Aa}	0.00248 ± 0.00205^{Ba}
	2	0.01311 ± 0.02631^{Ab}	0.00069 ± 0.00075^{Bb}
	3	0.00701 ± 0.00885^{Ab}	0.00117 ± 0.00198^{Bb}
Chlorpyrifos	0*	0.01479 ± 0.02678^{Ba}	0.05860 ± 0.07667^{Aa}
	1	0.01894 ± 0.03257^{Aa}	0.06694 ± 0.14906^{Aa}
	2	0.00564 ± 0.00747^{Ab}	0.01229 ± 0.02022^{Ab}
	3	$0.01157 \pm 0.02319^{Aab}$	0.00828 ± 0.01361^{Ab}
Fenthion	0*	$0.00464 \pm 0.00507^{Bab}$	0.05177 ± 0.07235^{Aa}
	1	0.01467 ± 0.04576^{Aa}	0.03907 ± 0.06801^{Aa}
	2	0.00276 ± 0.00569^{Ab}	0.01377 ± 0.02481^{Ab}
	3	0.00237 ± 0.00503^{Ab}	0.00671 ± 0.01026^{Ab}

Values followed by the same letter, uppercase in the row and lowercase in the column, do not differ from each other ($p \geq 0.05$)
* Six-hour post-treatment

cypermethrin consistently showed higher concentration ($p \geq 0.05$) in the hair at 50 psi across all evaluation days than at 300 psi. In contrast, the highest concentrations ($p \geq 0.05$) of chlorpyrifos and fenthion were observed on day 0 at 300 psi than at 50 psi. On days 1, 2, and 3, the concentrations of chlorpyrifos and fenthion in the hair remained similar regardless of whether 50 or 300 psi was used.

When each mean concentration of each analyte was analyzed by body region and working pressure (Table 4 and Fig. 2), cypermethrin levels did not differ significantly ($p \geq 0.05$) among body regions when animals were treated at working pressures of 50 and 300 psi, except in the dewlap region, where lower concentrations were observed at 50 psi. For chlorpyrifos, no significant differences were observed among body regions at either pressure ($p \geq 0.05$), except in the loin, where significantly higher concentrations were detected at 300 psi. A similar pattern was observed for fenthion, with significantly higher concentrations ($p < 0.05$) in the loin and neck regions when animals were treated at a working pressure of 300 psi compared with 50 psi.

4. Discussion

This is the first study to evaluate whether variations in application pressure affect acaricide deposition in cattle hair. In this study, varying the working pressures did not significantly affect the tick count and mean efficacy of the acaricide against *R. microplus* across treatment groups. A difference in tick counts was observed only on day 3 post-

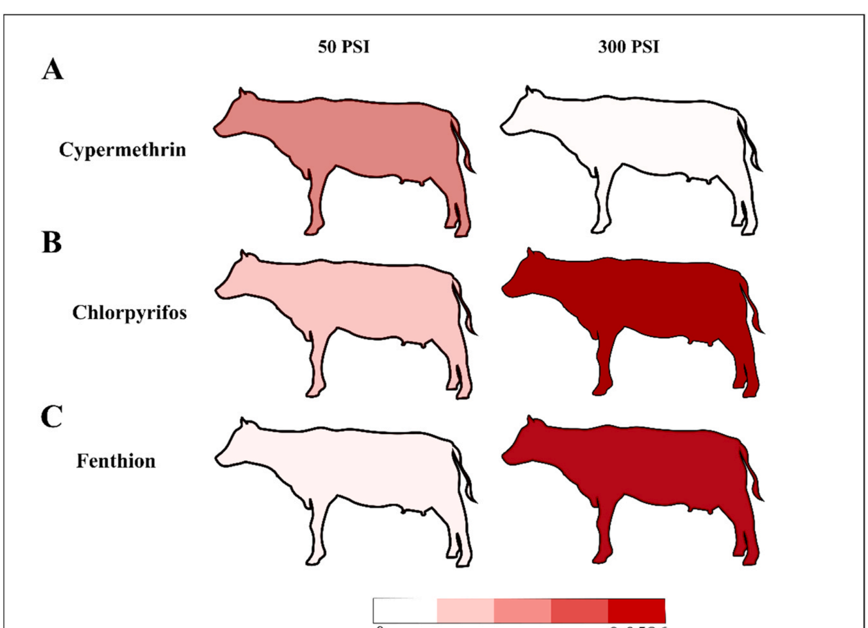

Fig. 1. Illustrative figure demonstrating the mean concentrations of each analyte in hair samples on the animals', treated with an acaricide formulation containing cypermethrin, chlorpyrifos, and fenthion, applied using a power sprayer at working pressures of 50 and 300 psi, six hours post-treatment.

Table 4
Mean concentrations of each analyte in hair samples collected from different body regions of cattle treated with an acaricide formulation containing cypermethrin, chlorpyrifos, and fenthion, applied using a power sprayer at working pressures of 50 and 300 psi, across all evaluation days.

Analyte	Body region	Working pressure / Mean and standard deviation of the analyte (ng/mL)	
		50 psi	300 psi
Cypermethrin	Dewlap	0.0025 ± 0.0050 A	0.0009 ± 0.0009 A
	Belly	0.0351 ± 0.0598 A	0.0008 ± 0.0006B
	Between legs	0.0257 ± 0.0445 A	0.0015 ± 0.0017B
	Loin	0.0145 ± 0.0114 A	0.0041 ± 0.0037B
	Legs	0.0173 ± 0.0179 A	0.0017 ± 0.0017B
	Neck	0.0224 ± 0.0218 A	0.0013 ± 0.0013B
Chlorpyrifos	Dewlap	0.0141 ± 0.0339 A	0.0048 ± 0.0049 A
	Belly	0.0193 ± 0.0329 A	0.0081 ± 0.0096 A
	Between legs	0.0047 ± 0.0067 A	0.0224 ± 0.0359 A
	Loin	0.0095 ± 0.0098B	0.0786 ± 0.0761 A
	Legs	0.0187 ± 0.0354 A	0.0829 ± 0.1783 A
	Neck	0.0092 ± 0.0133 A	0.0167 ± 0.0337 A
Fenthion	Dewlap	0.0268 ± 0.0541 A	0.0055 ± 0.0051 A
	Belly	0.0018 ± 0.0034 A	0.0075 ± 0.0111 A
	Between legs	0.0010 ± 0.0013 A	0.0193 ± 0.0295 A
	Loin	0.0012 ± 0.0011B	0.0713 ± 0.0747 A
	Legs	0.0027 ± 0.0041 A	0.0460 ± 0.0870 A
	Neck	0.0025 ± 0.0024B	0.0141 ± 0.0181 A

Values followed by the same uppercase letter in the row do not differ from each other ($p > 0.05$)

treatment, with the group treated at 300 psi exhibiting the lowest number of ticks. To date, one previous study has directly compared tick counts and acaricide efficacy across different working pressures using the same spraying equipment. Souza et al. (2025), employing the same power spray, full-cone spray pattern, medium droplet size, and working pressures of 50 and 100 psi, reported higher efficacy for the combination of chlorpyrifos + high-cis cypermethrin with higher pressure 100 psi. However, they did not observe any differences in tick counts at any time point after treatment, which aligns with our findings. Moraes et al. (2023), using different equipment, found that treatment using a power sprayer (~700 psi) resulted in greater acaricide efficacy (alpha-cypermethrin + ethion + chlorpyrifos) compared to a spray race (~44 psi) or a backpack sprayer (~31 psi). Notably, only on day 3 post-treatment the animals treated with the power sprayer have significantly fewer ticks than those treated by other methods. Taken together, these findings suggest that although higher working pressure may enhance acaricide performance under certain conditions, such effects appear to be transient and strongly influenced by management and environmental factors, reinforcing the need for standardized and comparative studies.

The analyte quantification was consistent with Batista Junior et al. (2024) and confirming the efficiency of the DART-MS method for the quantification of these three acaricides in bovine hair samples. Regarding acaricide deposition in cattle hair, treatments applied at 50 psi resulted in a higher concentration of cypermethrin compared to chlorpyrifos and fenthion. In contrast, at 300 psi, chlorpyrifos and fenthion exhibited the highest levels of deposition. Previous studies have suggested that spray application parameters are closely linked to treatment efficacy, which in turn may be associated with the amount of

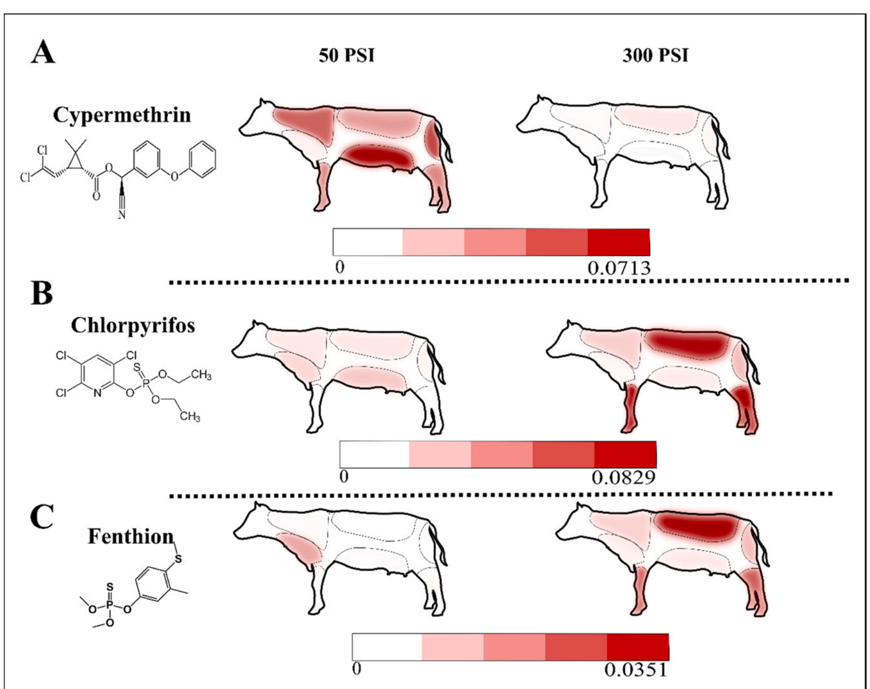

Fig. 2. Illustrative figure demonstrating the mean concentrations of each analyte in hair from different body regions of cattle treated with an acaricide formulation containing cypermethrin, chlorpyrifos, and fenthion, applied using a power sprayer at working pressures of 50 and 300 psi, across all evaluation days.

active ingredient deposited on the animal's hair (Nolan et al., 1979; Roulston and Wilson, 1965; Schnitzerling and Walker, 1985). For example, Wharton et al. (1970) found that the highest coumaphos deposition on bovine hair (698 μg/g) was achieved with a power spray, which also had the highest efficacy (99.5 %) when compared to plunge dip (443 μg/g and 97.5 % efficacy). The physicochemical differences between the chemical compounds could explain the observed variation in the amount of each compound in animal hair. Pyrethroids are highly lipophilic, whereas organophosphates have moderately hydrophilic characteristics, but with some lipophilicity (Aznar-Alemany, Eljarrat, 2020; Ragnarsdottir, 2000). These characteristics likely influence how each compound interacts with the lipid-rich environment of bovine hair under different application pressures.

Studies suggest that pressure is a determinant factor in acaricide treatment efficacy, because low-pressure spray tends to produce droplets that do not penetrate easily into the animal's skin, remaining mainly on the hair layer (Wharton et al., 1970; Moraes et al., 2023; Souza et al., 2025). For organophosphates, these compounds undergo hydrolysis easily in aqueous media, and without adequate penetration of the solution into the animal's hair layer, they tend to concentrate in droplets on the hair, gradually dissipating. Moreover, the solution that remains on the hair surface is subject to environmental factors, such as sunlight exposure, which can accelerate processes like photooxidation and photoisomerization (Kang et al., 1995; Ragnarsdottir, 2000). In contrast, due to cypermethrin high lipophilicity and strong affinity for organic surfaces, cypermethrin tends to rapidly distribute and adhere to the hair upon application. This behavior likely contributes to the higher concentrations of cypermethrin observed in samples from animals treated at lower spray pressures.

When the pressure increased to 300 psi (6x), other factors began to influence treatment outcome. In addition to enhancing droplet penetration into the hair due to increased impact, the high pressure also reduced droplet size during spraying (Nuyttens et al., 2007; Souza et al., 2025). For lipophilic compounds in particular, this reduction in droplet size may lead to partial separation of the active ingredient from the aqueous phase, compromising solution homogeneity and potentially affecting its adhesion to the hair. Additionally, electrostatic attraction plays a key role in the persistence of the product on the hair. Since bovine hair carries a negative charge, it attracts positively charged molecules present in the acaricide solution (Roulston and Schnitzerling, 1965). Cypermethrin, coumaphos, and fenthion differ in their ionic properties, resulting in varied interactions with hair, which are influenced by their specific charges. During spraying, the animal's hair acts as a filter, promoting product accumulation as pressure increases (Roulston and Schnitzerling, 1965; Schnitzerling and Walker, 1985). Combined with molecular ionization, this factor may be decisive for the distribution and persistence of the product in the animal's hair. In this context, organophosphates exhibit favorable conditions for higher retention, which likely explains the greater deposition of this molecule observed in this study under 300 psi.

Another factor that may have influenced the concentration of the molecule in the animal's hair is the methodology used for chemical analysis and compound identification. During quantification by DART-MS, optimal analytical responses for organophosphates were observed at ionization temperatures between 150 and 200 °C, whereas cypermethrin exhibited better responses at temperatures around 200 °C (Batista Junior et al., 2024). Considering the analysis of the three compounds, it is important to consider the thermal stability of each one, since the analytical method employs heated gas. Therefore, the use of temperatures that allow the simultaneous analysis of these three molecules without inducing degradation or signal instability is essential. For standardization, a temperature of 200 °C was selected, which may have favored the recovery of organophosphates while potentially underestimating cypermethrin levels, as pyrethroids are less volatile and more thermally stable. Nevertheless, the temperature employed enabled the determination of these three compounds with limits of detection around 0.01 ng mL^{-1}, which is satisfactory for the analyses proposed herein. Regarding the observed differences in analyte deposition across body regions such as the dewlap, loin, and neck, these variations may be random, the lack of a clear trend in analyte concentrations could be primarily attributed to external factors and intrinsic biological variability among animals (hair density, grooming behavior, exposure to sunlight, friction, and local contact with substrates) once the analytical method employed has been previously validated, with satisfactory performance demonstrated through robust repeatability, accuracy, and precision values. The present experiment alone cannot explain these regional discrepancies, suggesting the need for further investigation.

Finally, these findings highlight the complexity of interactions between spray parameters, acaricide formulation, and compound-specific behavior in field conditions. Moreover, they emphasize the importance of analytical methodology in accurately quantifying compound retention in hair. Although efficacy remained acceptable under both pressures, understanding how spray pressure alters active ingredient distribution may help optimize treatment protocols, especially for formulations with borderline efficacy. Further studies are warranted to explore the role of application variables, such as nozzle type, droplet size, environmental conditions, and acaricide volatility, product performance and persistence in different anatomical regions of the animal. And works that evaluate phenotypic and molecular evaluations of acaricide resistance.

5. Conclusion

Differences in mean counts of *R. microplus* only during the first hour post-treatment (up to day 3 post-treatment at 300 psi compared to 50 psi). In practice the higher pressure used can reduce the tick burden only in the first three days after treatment. After this period, no differences in tick counts were observed, and consequently, no differences in efficacy against this tick were detected. Furthermore, variations in working pressure significantly influenced the deposition of active ingredients on bovine hair. Cypermethrin showed greater accumulation at 50 psi, whereas higher deposition of chlorpyrifos and fenthion was observed at 300 psi.

CRediT authorship contribution statement

Batista-Junior Almir Custodio: Writing – review & editing, Methodology, Investigation. **Maciel Lanaia Italla Louzeiro:** Investigation. **Botelho Ana Flavia Machado:** Writing – review & editing. **Vanessa Ferreira Salvador:** Investigation. **Lorena Lopes Ferreira:** Writing – review & editing, Writing – original draft. **Luccas Lourenzzo Lima Lins Leal:** Investigation. **Vando Edesio Soares:** Formal analysis. **Rafael Moreira do Nascimento:** Investigation. **Marcus Antonio Martins Buso:** Investigation. **Gabriel Resende Souza:** Writing – original draft. **Monteiro Caio Marcio de Oliveira:** Writing – review & editing. **Lidia Mendes de Aquino:** Investigation. **Welber Daniel Zanetti Lopes:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology. **de Moraes Igor Maciel Lopes:** Investigation. **Andrea Rodrigues Chaves:** Writing – review & editing, Methodology.

Ethics approval

The project was approved by the Ethics Committee on the Use of Animals (CEUA) of Federal University of Goiás (UFG) (Protocol 234/2022) and followed the ethical principles of animal experimentation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applied.

Data availability

The data supporting the results presented in the article can be found in the tables presented in the manuscript.

References

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z., 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Vet. Parasitol. 203, 6–20. https://doi.org/10.1016/j.vetpar.2014.03.006.

Alvares, C.A., Stape, J., Sentelhas, P.C., Gonçalves, J.L.M., Sparovek, G., 2014. Koppen's climate classification map for Brazil. Meter Zeit. https://doi.org/10.1127/0941-2948/2013/0507.

ANVISA – Agência Nacional de Vigilância Sanitária (National Health Surveillance Agency, Brazil). Resolução da Diretoria Colegiada – RDC Nº 166, de 24 de julho de 2017. Dispõe sobre a validação de métodos analíticos e dá outras providências. Avaiable: 〈https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2017/rdc0166_24_07_2017.pdf〉.

Aznar-Alemany, Ò., Eljarrat, E.J.P.I., 2020. Introduction to pyrethroid insecticides: chemical structures, properties, mode of action and use. Pyrethroid Insectic. 1–16.

Batista Junior, A.C., Maciel, L.Í.L., Rocha, Y.A., Souza, G.G., Vaz, B.G., Lopes, W.D.Z., Botelho, A.F.M., Chalom, M.Y., Chaves, A.R., 2024. Assessing antiparasitic compounds persistence in cattle hair by DART-MS. J. Am. Soc. Mass Spectrom. v. 36 (n. 1), 201–208. https://doi.org/10.1021/jasms.4c00422.

Brasil, 1997. Portaria Nº 48, de 12 de Maio de 1997, do Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Regulamento Técnico para Licenciamento e/ou Renovação de Licença de Produtos Antiparasitários de Uso Veterinário. 〈https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-pecuarios/produtos-veterinarios/legislacao-1/portaria/portaria-sda-mapa-no-48-de-12-05-1997.pdf〉.

Drummond, R.O., Ernst, S.E., Trevino, J.L., Graham, O.H., 1968. Insecticides for control of the cattle tick and the southern cattle tick on cattle. J. Econ. Entomol. 61, 467–470. https://doi.org/10.1093/jee/61.2.467.

George, J.E., Pound, J.M., Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitol 129, 353–366.

Holdsworth, P., Rehbein, S., Jonsson, N.N., Peter, R., Vercruysse, J., Fourie, J., 2022. World Association for the Advancement of Veterinary Parasitology (WAAVP) second edition: guideline for evaluating the efficacy of parasiticides against ectoparasites of ruminants. Vet. Parasitol. 302, 109613. https://doi.org/10.1016/j.vetpar.2021.109613.

Kang, J., Zettel, V.H., Ward, N.I., 1995. The organophosphate pesticides. J. Nutr. Environ. Med. v. 5 (n. 4), 325–339.

Klafke, G.M., Golo, P.S., Monteiro, C.M.O., Costa-Júnior, L.M., Reck, J., 2024. Brazil's battle against *Rhipicephalus (Boophilus) microplus* ticks: current strategies and future directions. Braz. J. Vet. Parasitol. https://doi.org/10.1590/S1984-29612024026.

Moraes, N., Nicaretta, J.E., Rodrigues, D.C., Gonzaga, B.C.F., Barrozo, M.M., Vale, F.L., Sousa, L.J.P., Coutinho, A.L., Gomes, G.W., Teixeira, W.F.P., Lopes, W.D.Z., Monteiro, C., 2023. Comparison of the efficacy of different methods to apply acaricides for control of *Rhipicephalus (Boophilus) microplus*. Ticks Tick. borne Dis. 14, 102190. https://doi.org/10.1016/j.ttbdis.2023.102190.

Nolan, J., Roulston, W.J., Schnitzerling, H.J., 1979. The potential of some synthetic pyrethroids for control of the cattle tick. Aust. Vet. J. 55, 463–466.

Nuyttens, D., Baetens, K., De Schampheleire, M., Sonck, B., 2007. Effect of nozzle type, size and pressure on spray droplet characteristics. Biosyst. Eng. 97, 333–345. https://doi.org/10.1016/j.biosystemseng.2007.03.001.

Ragnarsdottir, K.V., 2000. Environmental fate and toxicology of organophosphate pesticides. J. Geol. Soc. v. 157 (n. 4), 859–876.

Rodrigues, D.S., Muller, R.P.B.W., Leite, R.C., 2015. Aplicação de carrapaticidas em bovinos. Resistência e Controle do Carrapato-do-boi. Instituto de Zootecnia, Nova Odessa.

Roulston, W.J., Schnitzerling, H.J., 1965. Relation of the formulation of cattle sprays to the deposition and loss of DDT. J. Sci. Food Agric. v. 16 (n. 4), 179–185.

Roulston, W.J., Wilson, J.T., 1965. Chemical control of the cattle tick *Boophilus microplus* (Can.). Bull. Entomol. Res 55, 617–635. https://doi.org/10.1017/S0007485300049737.

Schnitzerling, H.J., Walker, T.B., 1985. Factors affecting the performance of acaricides used for control of the cattle tick, *Boophilus microplus*. Trop. Pest Manag. 31, 199–203. https://doi.org/10.1080/09670878509370982.

Souza, G.R., de Araújo, B.R.G., Coelho, S.G., Araújo, R.N., Lopes, W.D.Z., Rodrigues, D.S., Ferreira, L.L., 2025. First evaluation of the efficacy and operational cost of acaricidal bath combining different droplet sizes, pressures and spray patterns in dairy cows naturally infested with *Rhipicephalus microplus*. Trop. Anim. Health Prod. 57 (3), 138. https://doi.org/10.1007/s11250-025-04378-8.

Strydom, T., Lavan, R.P., Torres, S., Pinilla, J.C., 2025. The economic impact of endo-and ectoparasites in dairy cattle. Parasit. Vectors 18 (1), 495. https://doi.org/10.1186/s13071-025-07064-8.

Wharton, R.H., Roulston, W.J., Utech, K.B.W., Kerr, J.D., 1970. Assessment of the efficiency of acaricides and their mode of application against the cattle tick *Boophilus microplus*. Aust. J. Agric. Res. 21, 985–1006. https://doi.org/10.1071/AR9700985.

Wharton, R.H., Utech, K.B.W., 1970. The relation between engorgement and dropping of *Boophilus microplus* (Canestrini) (Ixodidae) to the assessment of the tick numbers on cattle. J. Aust. Entomol. Soc. 171–182. https://doi.org/10.1111/j.1440-6055.1970.tb00788.x.