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Evaluation of the performance of synthetic acaricides and the essential oil of *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae) for the control of *Rhipicephalus* (*Boophilus*) *microplus*

[*Avaliação de acaricidas sintéticos e do oléo essencial de* Plectranthus amboinicus *(Lour.) Spreng (Lamiaceae) para o controle de* Rhipicephalus (Boophilus) microplus]

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ABSTRACT

Chemical acaricides are used as the primary method for controlling *Rhipicephalus* (*Boophilus*) *microplus*. However, the incorrect use of these compounds over the years has led to the emergence of resistant tick populations. This study aimed to evaluate the effectiveness of different combinations of synthetic acaricides and the essential oil of *Plectranthus amboinicus* on engorged females of *R.* (*B.*) *microplus.* Engorged females were obtained from infested bovines in dairy farms in the Agreste mesoregion of Pernambuco, Brazil. Five different combinations of commercial synthetic acaricides: I) Chlorpyrifos 30.0g + Cypermethrin 15.0g + Fenthion 15.0g; II) Chlorpyrifos 8.5g + Cypermethrin 15.0g + Ethion 16.0g; III) Deltamethrin 50.0g; IV) Amitraz 12.5g; V) Chlorpyrifos 15.0g + Cypermethrin 25.0g + Piperonyl butoxide 50.0g, and the 1% essential oil of *P. amboinicus* were used for the Adult Immersion Test. Only the combinations I and V of synthetic acaricides and essential oil showed efficacy over 95%. The findings of this study demonstrate that ticks' resistant populations are present in the study area, and the essential oil of *P. amboinicus* is a promising compound for developing products used for tick control. Finally, it is paramount to implement educational activities to improve farmers' awareness of the rational use of acaricides.
Keywords: commercial acaricides, resistance, Phytotherapeutic

RESUMO

Os acaricidas químicos são utilizados como o principal método para controlar Rhipicephalus (Boophilus) microplus. *No entanto, o uso incorreto desses compostos ao longo dos anos levou ao surgimento de populações de carrapatos resistentes. Este estudo teve como objetivo avaliar* in vitro *a eficácia de diferentes combinações de acaricidas sintéticos e do óleo essencial de* Plectranthus amboinicus *em fêmeas ingurgitadas de* R. (B.) microplus. *Fêmeas ingurgitadas foram obtidas de bovinos infestados, criados em fazendas leiteiras localizadas na mesorregião do Agreste do estado de Pernambuco, Brasil. Foram utilizadas cinco combinações diferentes de acaricidas sintéticos comerciais: I) Clorpirifós 30,0g + Cipermetrina 15,0g + Fention 15,0g; II) Clorpirifós 8,5g + Cipermetrina 15,0g + Etion 16,0g; III) Deltametrina 50,0g; IV) Amitraz 12,5g; V) Clorpirifós 15,0g + Cipermetrina 25,0g + Butóxido de piperonila 50,0g, e o óleo essencial de* P. amboinicus *a 1% para o teste de imersão de adultos. Apenas as combinações I e V de acaricidas sintéticos, juntamente com o óleo essencial, mostraram eficácia superior a 95%. Os resultados deste estudo demonstram que populações de carrapatos resistentes estão presentes na área de estudo, e o óleo essencial de* P. amboinicus *é uma substância promissora para o desenvolvimento de produtos utilizados no controle de carrapatos. Por fim, é fundamental a implementação de atividades educacionais para melhorar a conscientização dos produtores quanto ao uso racional de acaricidas.*

*Palavras-chave***:** *acaricidas comerciais, resistência, fitoterápico*

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INTRODUCTION

Rhipicephalus (*Boophilus*) *microplus* (Acari: Ixodidade) is the most important ectoparasite of cattle in tropical and subtropical areas worldwide. This tick species is responsible for economic losses in livestock animals due to reduced meat and milk production and transmission of pathogens (e.g., *Babesia* spp. and *Anaplasma* spp.) that may induce anemia and mortality (Jabbar *et al.*, 2015). In Brazil, it is estimated that an annual cost of around three billion dollars spent for the control of this ixodid (Grisi *et al*., 2014; Hurtado and Giraldo-Ríos, 2018; Rodriguez-Vivas *et al*., 2018).

The control of *R.* (*B.*) *microplus* is based on chemical acaricides belonging to different drug classes, including organophosphates, pyrethroids, amidines, macrocyclic lactones, phenylpyrazoles, and benzoylphenylureas (Koller *et al*., 2019). In recent years, only a few new products have been introduced, and many existing market options have become ineffective due to the emergence of resistance resulting from their incorrect and prolonged usage (Mendes *et al*., 2022). The resistance of *R*. (*B*.) *microplus* to synthetic acaricides is developed through the increase of gene expression and enzymatic activity or due to mutations in sodium channels of neuroreceptors, which reduces and impedes the penetration of chemical compounds (Ffrench *et al*., 2004; Dzemo *et al*., 2022).

Reports of multi-resistant tick populations are frequent in areas infested by *R.* (*B.*) *microplus*. Additionally, concerns have arisen among producers regarding environmental contamination and the presence of residues of these acaricides in meat and milk. In their haphazard attempts at control using conventional acaricides, producers inadvertently exacerbate the resistance problem (Mendes *et al*., 2022; Agwunobi *et al*., 2021). In Brazil, resistance of this tick to various chemical formulations has previously been reported in the northern, northeastern, central-western, southeastern, and southern regions (Santos and Vogel, 2012; Santana *et al*., 2016; Cruz, 2017; Silva *et al*., 2020; Valsoni *et al*., 2020).

Due to the necessity of finding alternative methods for controlling these ectoparasites, research involving plant derivatives has been encouraged and shows promising results. Remarkably, using phytomedicines rich in bioactive compounds with action against different life stages of ticks has gained attention (Banumathi *et al*., 2017; Vinturelle *et al*., 2021). For example, *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae), Cuban oregano, is a pantropically cultivated plant rich in bioactives such as carvacrol and thymol. It exhibits insecticidal, larvicidal, antioxidant, anti-inflammatory, antibacterial, and antifungal properties and acaricidal potential (Pinheiro *et al*., 2015; Arumugam *et al*., 2016; Leesombun *et al*., 2022).

This study evaluated the *in vitro* effectiveness of different combinations of synthetic acaricides and the essential oil of *P. amboinicus* on engorged females of *R*. (*B*.) *microplus.*

MATERIAL AND METHODS

Engorged females were obtained from animals reared in a dairy cattle farm in Bom Conselho (9° 10’ 6” S, 36° 41’ 8” W), Agreste region, state of Pernambuco, Brazil. The area experiences an average annual temperature of 21°C, with a mean precipitation ranging from 700 to 1150 mm, influenced by the region’s varied topography. This municipality stands as one of the major milk producers in the state, with its economy predominantly centered around agriculture and livestock. The prevalent bovine breed in this area is the Holstein, which exhibits low resistance to ectoparasites despite its high production capabilities (Hurtado and Giraldo-Ríos, 2018; Efetivo, 2021).

The study received approval from the Ethics Committee on Animal Use (CEUA) of the Federal Rural University of Pernambuco under protocol number 8717300420/ID 192/2020.

R. (*B*.) *microplus* engorged females were manually collected from infested cattle without ectoparasiticide treatment in the last 30 days. Specimens were stored in plastic vials and transported to the laboratory for further processing. They were morphologically identified, quantified, and weighted for the formation of groups.

The essential oil was obtained from *P. amboinicus* leaves using the steam distillation technique with helium gas, following Craveiro *et al.* (1976). This method involves suspending the botanical material in water, boiling it, and collecting and cooling the vapor. This process separates the condensed material into two phases: an aqueous phase and an organic phase, which corresponds to the essential oil. Subsequently, the chemical composition of the oil was evaluated using the Gas Chromatography-Mass Spectrometry (GC-MS) technique.

Seven homogeneous groups containing ten females (~ 2 g) each were formed and separated as follows: I) Chlorpyrifos 30.0g + Cypermethrin 15.0g + Fenthion 15.0g; II) Chlorpyrifos 8.5g + Cypermethrin 15.0g + Ethion 16.0g; III) Deltamethrin 50.0g; IV) Amitraz 12.5g; V) Chlorpyrifos 15.0g + Cypermethrin 25.0g + Piperonyl butoxide 50.0g; 1% *P. amboinicus* essential oil, and a control group (distilled water). All treatments were conducted in triplicate, and the result was obtained by calculating the averages of the treatments. Synthetic acaricides were used according to the manufacturer's instructions.

Engorged females were subjected to the Adult Immersion Test (AIT) for *in vitro* detection of acaricide effectiveness (Drummond *et al*., 1973). After immersion for five minutes, specimens were placed in an incubator for oviposition under controlled conditions (26 ± 1°C, 80 ± 5% relative humidity) for 15 days. Subsequently, the egg mass was weighed, stored in sealed plastic vials, and incubated under controlled conditions (see above) for 25 days. Upon egg hatching, the hatching percentage was visually determined, and the product's efficacy was calculated according to Drummond *et al*. (1973). The product was considered adequate if the efficacy value was ≥ 95%.

RESULTS

The chemical characterization of the *P. amboinicus* essential oil is presented in Table 1. The compound Carvacrol (54.81%) predominated, followed by various terpenes.

Table 1. Proportion of compounds present in the essential oil of *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae)

Compound	Relative Area Quantification (%)
1,2,3,4,5-Pentamethyl-cyclopentane	0.50
Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl)	0.27
β-Myrcene	0.99
o-Cymene	0.19
3-Carene	8.92
γ-Terpinene	0.40
5-Isopropyl-2-methylbicyclo [3.1.0] hexan-2-ol	10.66
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α.,2β.,5α.)	1.08
Terpinen-4-ol	0.31
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)	0.60
Thymol	0.39
Carvacrol	54.81
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	12.51
α-Caryophyllene	3.15
Caryophyllene oxide	3.63
Ledol	1.03
Deconexent	0.56

Groups I, V, and *P. amboinicus* essential oil were effectiveness against *R.* (*B.*) *microplus* (Table 2).

Table 2. Efficacy of synthetic acaricides and *P. amboinicus* oil against *R.* (*B.*) *microplus*.

Treatment	Egg Weight (g)	Hatch Rate (%)	Reproductive Efficiency (%)	Treatment Efficacy (%)
Control	0.90	100.0	100.0	-
I	0.27	10.0	3.0	97.0
II	0.40	24.0	10.67	89.33
III	0.90	92.70	92.70	7.30
IV	0.87	95.0	91.83	8.70
V	0.20	5.33	1.18	98.86
Essential oil	0.13	7.66	1.11	98.89

Among the synthetic products, the treatments that exhibited efficacy involved combinations of different active ingredients or compound synergies, such as treatments I and V, with respective efficacies of 97.0% and 98.86%. Notably, there was a reduction in egg laying of 70.0% in treatment I and 77.78% in treatment V when compared to the control group, accompanied by a decrease in the egg laying rate of 90.0% and 94.67%, respectively. All females of all groups remained alive during the whole study period, but the egg production and hatchability were altered.

DISCUSSION

This study demonstrates that tick-resistant populations are present in the study area, and the essential oil of *P. amboinicus* was effective against *R.* (*B.*) *microplus*. Despite no mortality of engorged females, synthetic compounds used for the treatment I and V inhibited egg laying and hatchability. Likely, higher concentrations of organophosphates with pyrethroids in treatment I and the potentiation of the insecticidal effect of organophosphates by piperonyl butoxide synergism in treatment V were responsible for the efficacy of these treatments (Koller *et al.*, 2019). None of the synthetic compounds or the essential oil achieved a complete efficacy (100%) as previously reported in the study area (Santana *et al.*, 2013). Actually, resistant populations of *R.* (*B.*) *microplus* to combinations of cypermethrin with chlorpyrifos and cypermethrin, chlorpyrifos, and citronellol were previously reported in the Garanhuns microregion, Pernambuco (Santana *et al.*, 2013).

The low efficacy of deltamethrin and amitraz (treatments III and IV) underscores the exacerbation of the tick resistance to two different chemical groups simultaneously observed in the same region almost a decade ago (Santana *et al.*, 2016). Resistance by ticks to more than one group of compounds simultaneously has been reported in various regions of Brazil by different studies (Santos and Vogel, 2012; Santana *et al.*, 2016; Cruz, 2017; Silva *et al.*, 2020; Valsoni *et al.*, 2020).

The worsening of parasitic resistance arises from the need to implement combinations of different classes of compounds within the same product, as seen in treatments I, II, and V, replacing acaricides that lack such class associations (treatments III and IV). However, these non-associated acaricides lose their efficacy more rapidly due to intrinsic and extrinsic factors related to ticks, such as mutations and the artificial selection pressure imposed by improper management of commercial acaricides (Koller *et al.*, 2019; Dzemo *et al.*, 2022). Nevertheless, even acaricide combinations lose efficacy over time, as observed in treatment II. Therefore, it is necessary to introduce compounds that synergize with other products, like piperonyl butoxide used in treatment V, which, while not lethal to ticks exposed to acaricides, hindered their reproduction due to its cytotoxicity (Banumathi *et al.*, 2017; Rodriguez-Vivas *et al.*, 2018).

The tick resistance to synthetic acaricides is exacerbated by the incorrect use of these chemical compounds, which are sold everywhere without any control. In Brazil, most veterinary use acaricides are sold without needing a prescription as long as registered and approved by the Ministry of Agriculture, Livestock, and Supply. The artificial selection pressure on these parasites generates resistant populations (Koller *et al.*, 2019). This insensitivity of a portion of the *R.* (*B.*) *microplus* population to treatments contributes to the difficulty in establishing effective control alternatives worldwide (Agwunobi *et al.*, 2021).

As a result, research on plant extracts and essential oils with insecticidal and acaricidal bioactive compounds has intensified in recent decades. Also, due to the reduced efficacy of synthetic acaricides and the lower environmental and product residue left by these phytotherapies compared to synthetic acaricides (Hurtado and Giraldo-Ríos, 2018). Particularly the use of phytotherapy based on the exploitation of bioactive present in plants, especially those with known insecticidal action, such as carvacrol, which is the main component of *P. amboinicus* essential oil (Araujo *et al*., 2016; Leesombun *et al*., 2022).

The higher efficacy of the essential oil herein observed can be attributed to carvacrol and thymol. The combination of these terpenes demonstrates toxicity against larvae of *R*. (*B*.) *microplus* and *R. sanguineus*, enhancing the larvicidal effect through the synergism of these bioactives (Araujo *et al*., 2016). Furthermore, carvacrol has a cytotoxic effect on semi-engorged *R. sanguineus* females, leading to morphological alterations in oocytes and germinal vesicles and cytoplasmic vacuolization. Although it may not be lethal to all ticks, the survivors have their reproduction altered, resulting in effective long-term control (Souza *et al*., 2019).

A wide range of bioactives derived from the secondary metabolism of *P. amboinicus*, especially terpenes, serve as defense compounds for the plant. However, they also promise arthropod control by enhancing, through synergy, the effect of the primary compounds in the essential oil, such as carvacrol (Camilo *et al*., 2017). The use of terpenes against arthropod control has been reported as an alternative measure of control to be used integrated with chemical compounds.

CONCLUSION

The resistance of *R.* (*B.*) *microplus* to synthetic acaricide formulations and their combinations has led to the increased use of products with higher concentrations by rural producers. The lack of efficacy in treatments II, III, and IV follows the same pattern observed in other regions of Brazil, emphasizing the need for educational efforts to promote the rational use of acaricides. Additionally, phytotherapies, such as *P. amboinicus* essential oil, represent an alternative tick control source. Further *in vitro* and *in vivo* studies with different concentrations are required to better characterize this natural compound's role in the control of ectoparasites.

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