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Evaluation of the interaction of citral, geraniol and thymol on the poultry red mite *Dermanyssus gallinae* (DE GEER, 1778) under in vitro conditions

Avaliação da interação de citral, geraniol e timol no ácaro-vermelho-das-aves Dermanyssus gallinae (DE GEER, 1778) em condições in vitro

James Arbey Ávila Buitrago (ORCID: 0000-0002-2297-0927), Eneida Torres Cabra (ORCID: 0000-0003-2538-1666), Lizeth. A. Moreno (ORCID: 0000-0001-6913-7855), Luis A. Paez Guevara* (ORCID: 0000-0002-4135-3051)

Faculty of Agricultural and Environmental Sciences, Juan de Castellanos University Foundation, Tunja, Colombia. * Author for correspondence: lapaez@jdc.edu.co

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ABSTRACT

The control of *Dermanyssus gallinae* in small productions of laying hens is carried out by using chemical methods. However, its indiscriminate use has generated resistance and environmental pollution problems. This problem has encouraged the use of natural substances to control mites. Thus, the objective of this work was to evaluate the acaricidal activity of the bioactive citral (Ci), geraniol (Ge) and thymol (Thy) on *D. gallinae* under in vitro conditions using six concentrations (0.05, 1, 2, 3, 4 and 5g/mL). In addition, the interactions of the mixtures of the three bioactive were evaluated through binary (1:1) and tertiary (1:1:1) combinations. The interaction between of the combination of bioactive was performed by using CompuSyn software and calculating the combination index (CI). LC_{50}, LC_{90}, and LC_{99} with 95% confidence limits were estimated by Probit analysis. The bioactive Ci, Ge and Thy show acaricidal activity on the poultry red mite. The combination of Ge:Thy and Ci:Ge showed very strong synergism with CI of 0.084 and 0.052, whereas Ci:Thy showed strong synergism with CI of 0.122 at a concentration of 0.05g/mL. The tertiary combination in 1:1:1 showed a higher toxic effect and strong synergistic effects at low concentrations with 100% mortality at 1g/mL concentration with a CI of 0.147. The combination of natural bioactive could be an additional way to control *D. gallinae* without putting the welfare of the birds at risk and would be an environmentally friendly measure.

KEYWORDS: acaricide; additive; antagonism; poultry; synergism.

RESUMO

O controle de *Dermanyssus gallinae* em pequenas produções de poedeiras é realizado por métodos químicos. No entanto, seu uso indiscriminado tem gerado problemas de resistência e poluição ambiental. Este problema tem incentivado o uso de substâncias naturais para o controle de ácaros. Assim, o objetivo deste trabalho foi avaliar a atividade acaricida dos bioativos citral (Ci), geraniol (Ge) e timol (Thy) sobre *D. gallinae* em condições in vitro utilizando seis concentrações (0,05, 1, 2, 3, 4 e 5g/mL). Além disso, as interações das misturas dos três bioativos foram avaliadas por meio de combinações binárias (1:1) e terciárias (1:1:1). O sinergismo da combinação dos bioativos foi realizado utilizando o software CompuSyn e calculando o índice de combinação (CI). LC_{50}, LC_{90} e LC_{99} com limites de confiança de 95% foram estimados por análise Probit. Os bioativos Ci, Ge e Thy apresentam atividade acaricida sobre o ácaro-vermelho-da-aves. A combinação de Ge:Thy e Ci:Ge apresentou sinergismo muito forte com CI de 0,084 e 0,052, enquanto Ci:Thy apresentou forte sinergismo com CI de 0,122 na concentração de 0,05g/mL. A combinação terciária em 1:1:1 apresentou maior efeito tóxico e fortes efeitos sinérgicos em baixas concentrações com 100% de mortalidade em concentração de 1g/mL com CI de 0,147. A combinação de bioativos naturais poderia ser uma forma adicional de controle de *D. gallinae* sem colocar em risco o bem-estar das aves e seria uma medida ecologicamente correta.

PALAVRAS-CHAVE: acaricida; aditivo; antagonismo; aves; sinergismo.

INTRODUCTION

Dermanyssus gallinae De Geer 1978 (Acari: Dermanyssidae) also called poultry red mite is a hematophagous ectoparasite widely distributed worldwide (SPARAGANO et al. 2014, TABARI et al. 2017). The *D. gallinae* infestations continue to be a threat to egg production systems, generating animal health and welfare problems, affecting production and public health (SIGOGNAULT et al. 2017). Additionally, *D. gallinae* is a vector of viral and bacterial pathogens such as paramyxoviruses (which causes the Newcastle disease), avian influenza (AIV) and equine encephalomyelitis (eastern, western, and Venezuelan), *Borrelia burgdorferi* (Lyme disease)*, Escherichia coli, Erysipelothrix rhusiopathiae, Pasteurella multocodia, Salmonella gallinarum y S. enteritidis, Shigella sp.* y *Sthapylococcus* (DE LUNA et al. 2008, VALIENTE et al. 2009, GEORGE et al. 2015, SOMMER et al. 2016).

The control of *D. gallinae* is carried out mainly with the use of chemical substances such as organochlorines, organophosphates, pyrethroids, carbamates and triazapentadiene derivatives, which have generated resistance and therefore loss of product efficacy, as mentioned by GEORGE et al. (2015) who found individuals resistant to carbamates and pyrethroids. In addition, the residuality of these pesticides can represent a great danger to humans and the environment. Considering the research has focused on the search for less polluting alternatives with fewer effects on the health of birds, among which are the use of inert powders, physical treatments, fungi, predators, essential oils and specific bioactive (TABARI et al. 2020).

The use of essential oils (EOs) has been proposed as one of the promising alternatives for the control of *D. gallinae* (GEORGE et al. 2009, 2010). However, one of the disadvantages is the difference in the proportions of the components of the EOs, generating variable acaricidal activity; even in oils from the same plant species (NECHITA et al. 2015). The foregoing is one of the main reasons why OEs have not been accepted in the markets, despite having less toxicity and low environmental persistence, compared to substances of synthetic origin (SPARAGANO et al. 2013). To solve this problem, research has focused on separating the bioactive components of plant essential oils (TABARI et al. 2020) and evaluating them separately to determine which of the bioactive has the greatest effect on pest control such as *D. gallinae*. Some bioactive explored as acaricides are citral (Ci), geraniol (Ge) and thymol (Thy) (SPARAGANO et al. 2013). Thy can be found in EOs from thyme (ARCHANA et al. 2011), Ge from palmarosa (GEORGE et al. 2009, SPARAGANO et al. 2013), and Ci from lemongrass (ELZEN et al. 2000).

Different authors such as ARAÚJO et al. (2016), LOPES et al. (2019), JYOTI et al. (2019) and GUEVARA & MOLINA (2019) have shown in other species that the use of combinations of metabolites could reduce the concentration of each substance and increase biological activity. The combination of bioactive and the difference in the mechanisms of action could generate synergism for the control of *D. gallinae*, thus reducing the possibilities of development of resistant populations. The combination of compounds can generate different interactions, presenting effects that may be synergistic, additive or antagonistic (BENAMAR-AISSA et al. 2022). Currently, these types of studies are focused on demonstrating the effect of the combination of drugs for the treatment of different types of diseases in humans (CHOU 2006, FU et al. 2016), supported by the use of software such as CompuSyn, which is based on the general dose: effect equation; that arises from the combination of the law of mass action with the mathematical principle of induction and deduction, allowing to determine the synergism between combinations of substances (CHOU 2006). In addition, this type of analysis has been implemented in the field of veterinary medicine to demonstrate with these models the synergistic effects of the combination of bioactive in pest control, such as tick control (ARAÚJO et al. 2016). It is noteworthy that this would be the first study reported on poultry red mites, where the CompuSyn software was used to verify the synergism with the binary and tertiary combination of bioactives. Thus, the objective of this work was to evaluate the interaction (synergistic, additive or antagonistic) of the combination of Ci, Ge and Thy on *D. gallinae* under in vitro conditions.

MATERIAL AND METHODS

The *D. gallinae* mites used in the trials were collected from *Gallus gallus* Linnaeus, 1758 (Galliformes: Phasianidae) from backyard productions, in the municipality of Duitama located in the department of Boyacá-Colombia. The mites were placed in a sealable container, transported to the Clinical Laboratory of the Juan de Castellanos University Foundation and stored at an average temperature of 18 ± 1 °C and a relative humidity of 80±10%, with cycles of 16 hours of light and 8 of darkness two days before being used. For the identification, a Motic® SMZ-168l stereoscope and the taxonomic keys of DI PALMA et al. (2012). Fed adults were used, assuming that the darker colored individuals had fed more recently than the lighter individuals. Products Ci, Ge and Thy were purchased from Sigma (Sigma-Aldrich, Germany). The solutions were stored in a sealed brown container until the mite tests were carried out. Trichlorfon was used as a positive control (commercially available). The ethanol used was analytical grade.

Contact toxicity bioassay

The toxicity of bioactive Ci, Ge, and Thy against *D. gallinae* was performed through contact bioassays following the methodology described by GEORGE et al. (2009) and TABARI et al. (2020). The concentrations of the treatments were: 0.05g/mL, 1g/mL, 2g/mL, 3g/mL, 4g/mL and 5g/mL. 1 mL was sprayed onto 4.25 cm diameter filter paper (Whatman No. 2) and allowed to dry for 5 minutes at room temperature. Then, the paper was placed on the Petri dish and immediately 10 adult mites per dish and a piece of cotton (5mm x 5mm). Impregnated with 100 µl of distilled water were placed. Finally, each Petri dish was covered and sealed with Parafilm to be checked 24 hours after application. Binary pooling (Ci:Ge, Ci:Thy, Ge:Thy) was performed through a diagonal constant ratio pooling design (1:1) maintaining the concentrations listed above. The tertiary pool (Ci:Ge:Thy) was performed from a diagonal constant ratio pool design (1:1:1). The control group was ethanol (solvent used with the bioactives) and the positive control group (chemical acaricide - trichlorfon). For all tests, three replicates were performed.

Statistical analysis

An analysis of variance was performed to find statistically significant differences among treatments by bioactive and the Shapiro-Wilk test is performed for the normality of the data. A Tukey test to identify differences between pairs of means using the statistical program R 4.1.2 environment R-Studio. The percentage of mortality was determined according to the formula of HENDERSON & TILTON (1955) and the lethal activity was classified according to KIM et al. (2007). Differences between trials of combinations were performed by ANOVA with a p-value < 0.05. The estimated marginal means post hoc analysis was performed by using the “emmeans” function of the emmeans R package. The lethal concentration 50, 90 and 99 (LC_{50}, LC_{90}, LC_{99}) was calculated through Probit analysis using R 4.1.2 environment R-Studio. The Combination Index (CI) was calculated according to CHOU (2006), the values allow quantitatively observing the additive effects (CI > 1.1), synergistic (0.1 > CI < 0.9) and antagonistic effects. (1.1 > CI < 10) as reported by CHOU (2010) in drug combinations. This index allows evaluating the effect of the interactions among bioactive through the CompuSyn software version 1.0.1 (free software). It was only determined in the combination trials.

RESULTS

Individual bioactive assays

Mortality results for *D. gallinae* using the bioactive Ci, Ge and Thy separately are shown in Figure 1. For Ge at the lowest concentration (0.05g/mL) mortality was less than 5%, Thy 30% and Ci 0%; whereas at a concentration of 1g/mL, a mortality of 100% was observed for Ge, 75% for Thy and 3% for Ci. The data revealed that the three evaluated bioactive have a strong concentration-dependent acaricidal activity, observing that Ge is the bioactive with the highest acaricidal activity from concentration at 1g/mL, followed by Thy at concentration of 3g/mL and Ci at concentration of 4g/mL. The control treatment did not show mortality and the commercial acaricide showed 100% mortality from 0.05g/mL. Finally, at 0.05g/mL, the evaluated bioactive little acaricidal activity (Figure 1), because of the low levels of mortality (<30%). This as reported by KIM et al. (2007) which indicates that lethal activity strong, when mortality >80%; moderate, when mortality 80–61%; weak, when mortality 60–40%; little or no activity, when mortality <40%.

Table 1 shows the different toxicity data (LC_{50}, LC_{90} y LC_{99}) of the three bioactives. The LC_{50} data indicate that Ge is the most active compound for the control of *D. gallinae*, followed by Thy. The least toxic was Ci. On the other hand, when comparing LC_{90} and LC_{99}, Ge and Thy presented the highest mortality values. Finally, no statistically significant differences were observed with toxicity for Ge and Ci.

Interaction of binary and tertiary combinations of Ci, Ge, and Thy

The evaluation of the binary combinations Ci:Ge, Ci:Thy and Ge:Thy on *D. gallinae*; together with the tertiary combination Ci:Ge:Thy are shown in Table 2. The Ge:Thy combination presented the highest mortality (90%) with the lowest concentration, followed by Ci:Ge (77%) and Ci:Thy (67%), respectively. The 100% mortality fluctuated for each of the combinations, presenting the following order: Ge:Thy (2g/mL) > Ci:Thy (3g/mL) > Ci:Ge (4g/mL). Additionally, the tertiary combination presented the lowest mortality value (63%) with the lowest concentration (0.05g/mL) compared to the binary combinations. However, 100% mortality was obtained by increasing the concentration to 1g/mL. Finally, the results showed a significant difference (p < 0,05) when the bioactive were evaluated at concentrations of 0.05g/mL and 1g/mL. At higher concentrations, the data did not show statistically significant differences among treatments, presenting 100% mortality.

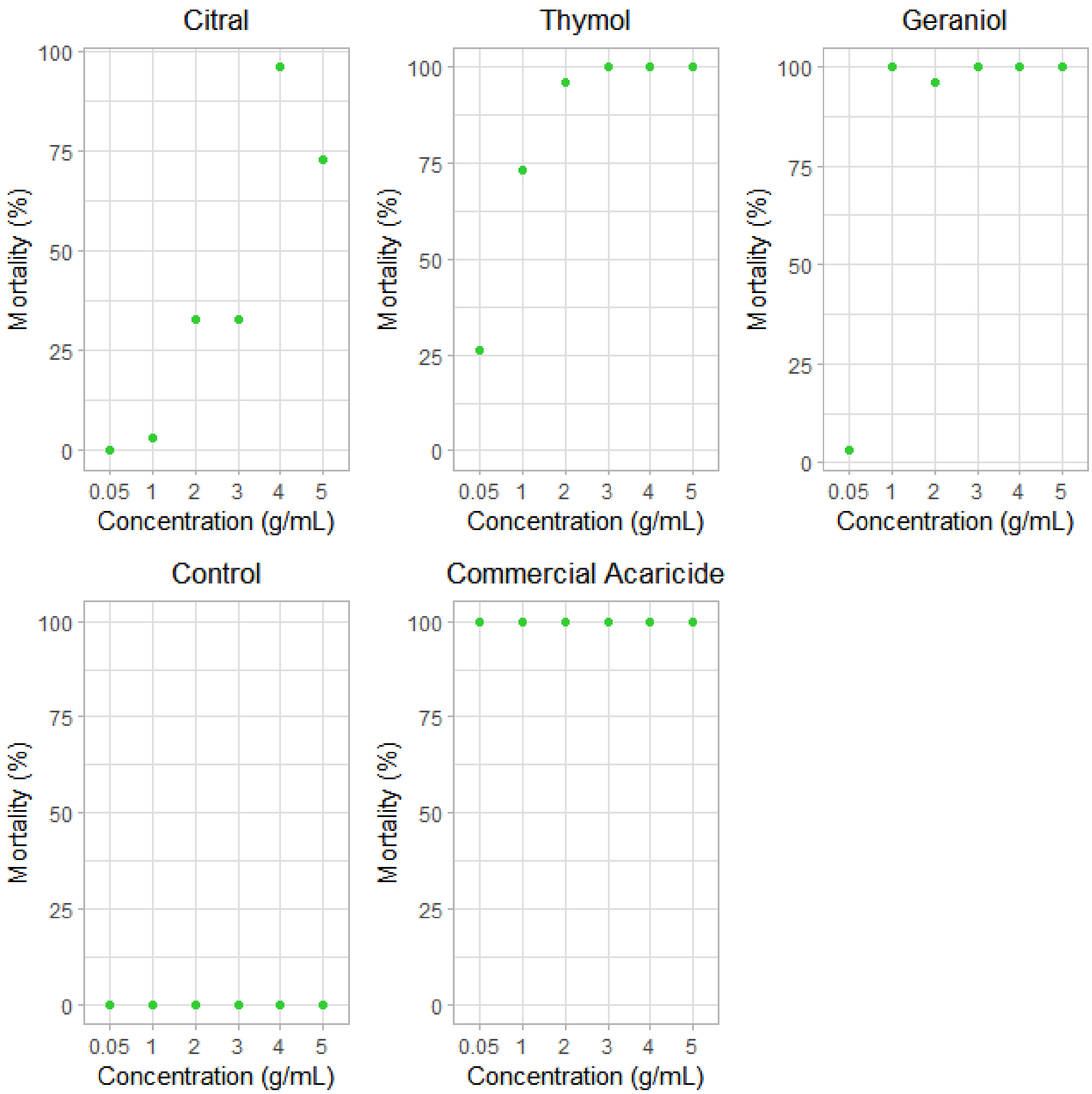

Figure 1. Mortality percentage of *Dermanyssus gallinae* treated individually with citral, thymol, geraniol, ethanol (control) and trichlorfon.

Table 1. Lethal concentration 50, 90 and 99 of Citral, Geraniol and Thymol on *Dermanyssus. Gallinae.*

Bioactive	Concentration	LC_{50}	LC_{90}	LC_{99}
Ci	g/mL	3.16 $\pm$ 0.79	4.59 $\pm$ 1.08	5.75 $\pm$ 1.54
Ge	g/mL	0.37 $\pm$ 0.93	1.80 $\pm$ 1.00	2.96 $\pm$ 1.36
Thy	g/mL	0.43 $\pm$ 0.92	1.86 $\pm$ 1.00	3.02 $\pm$ 1.36

95% confidence limits.

The data revealed that all binary 1:1 and tertiary 1:1:1 combination of bioactive at a concentration of 0.05g/mL showed synergistic effects (Table 3). At a concentration of g/mL Ci:Ge and Ge:Thy showed additive effect and the combination of Ci:Thy antagonistic. When increasing the concentration to 2g/mL Ci:Ge and Ci:Thy, the behavior was additive, but Ge:Thy was synergistic. At a concentration of 3g/mL, a similar behavior (synergistic) was observed for Ci:Thy and Ge:Thy and for Ci:Ge antagonist. On the other hand, both Ci:Thy, Ge:Thy and Ci:Ge presented a synergistic effect at concentrations of 4 g/mL and 5 g/mL, except Ge:Thy which at 5g/mL was additive. Finally, the combination of the three bioactive showed synergistic effects in all treatments.

Table 2. Mortality (%) of *Dermanyssus gallinae* adults treated with binary and tertiary combinations of Citral, Geraniol and Thymol at different concentrations.

Concentration (g/mL	Mortality percentage			
	Ci:Ge	Ci:Thy	Ge:Thy	Ci:Ge:Thy
0.05	77 (16,66)	67 (8,81)	90 (10,01)	63 (6,66)
1	83 (12,01)	73 (12,01)	97 (3,33)	100 (0,00)
2	97 (3,33)	97 (3,33)	100 (0,00)	100 (0,00)
3	97 (3,33)	100 (0,00)	100 (0,00)	100 (0,00)
4	100 (0,00)	100 (0,00)	100 (0,00)	100 (0,00)
5	100 (0,00)	100 (0,00)	100 (0,00)	100 (0,00)

***** Standard error of mean (S.E.M).

In sum, in this study a total of four combinations among Ci, Ge and Thy with six different concentrations (0.05g/mL, 1 g/mL, 2 g/mL, 3 g/mL, 4 g/mL and 5 g/mL) were evaluated, resulting in: two treatments with very strong synergism, five with strong synergism, seven with synergism, two with moderate synergism, one with slight synergism, five with additive effects, one with moderate antagonism and finally one with antagonism.

Table 3. Effect of binary and tertiary bioactive combinations on adult *Dermanyssus gallinae* under in vitro conditions.

Bioactive compound	Treatment/ Concentration (g/mL)	CI	Effect
Ci:Ge	.05	0,052	Very strong synergism
	1.0	0,915	Nearly additive
	2.0	0,950	Nearly additive
	3.0	1,425	Moderate antagonism
	4.0	0,589	Synergism
	5.0	0,736	Moderate synergism
Ci:Thy	.05	0,122	Strong synergism
	1.0	2,084	Antagonism
	2.0	1,060	Nearly additive
	3.0	0,239	Strong synergism
	4.0	0,319	Synergism
	5.0	0,399	Synergism
Ge:Thy	.05	0,084	Very strong synergism
	1.0	0,941	Nearly additive
	2.0	0,433	Synergism
	3.0	0,650	Synergism
	4.0	0,866	Slight synergism
	5.0	1,083	Nearly additive
Ci:Ge:Thy	0.05	0,128	Strong synergism
	1.0	0,147	Strong synergism
	2.0	0,295	Strong synergism
	3.0	0,443	Synergism
	4.0	0,591	Synergism
	5.0	0,739	Moderate synergism

DISCUSSION

Compounds of natural origin have been proposed as an alternative for pest control, due to their low toxicity, repellency and disincentive to feeding and oviposition in different types of pests (MIRESMAILLI & ISMAN 2014). The effects of these substances have been attributed to neurotoxic actions affecting: the central nervous system due to suppression of acetylcholinesterase activity, nicotinic acetylcholine receptors (nAChRs), octopamine (OA) and tyramine (TA) receptors γ-aminobutyric acid (GABA)-dependent sodium and chloride channels (JANKOWSKA et al. 2017).

Also, Ci and Thy have shown inhibitory effects on the enzyme acetylcholinesterase, responsible for controlling the concentration of the excitatory neurotransmitter acetylcholine (ACh) in the synaptic cleft (BLENAU et al. 2011); enzyme that allows the neuroneuronal and neuromuscular connection (CAMILO et al. 2017). Furthermore, ENAN (2005) indicates that Thy also affects the OA and the TA receptors. On the other hand, it has been reported that the activity of Ge is essentially due to damage to the membrane and ion channels, generating changes in the activity of the membrane-bound protein and intracellular signaling pathways (WARBER 1998, KAUR et al. 2011). Additionally, this bioactive could have a similar effect to Ci, considering that at the structural level they differ by the presence of a carbonyl and hydroxyl functional group, respectively (ELGENDY & KHAYYAT 2008, SEO et al. 2008).

The high percentages of mortality at low concentrations and the lower values of LC_{50}, LC_{90} and LC_{99} presented by Thy and Ge, may be related to the mechanisms of action that each one presents and the presence of OH in its structure (ENAN 2005). Additionally, the low toxicity of Ci with respect to Thy and Ge would be related to the absence of the hydroxyl functional group, reducing the possible interactions with the receptors. This was observed by ENAN (2005) who determined that the OH group in the structure of the bioactive affected the binding activity of the receptor. For the bioactive with the absence of the OH group, the binding activity to the TA receptor presented an inhibition of only 7% with p-cymene (it does not present OH) and for Thy, an inhibition of approximately 30% was observed.

Authors such as SPARAGANO et al. (2013) demonstrated acaricidal activity using undiluted Ge and Ci, obtaining 100% mortality of *D. gallinae*. However, when used at a concentration of 10%, mortality decreased to 34.6% for Ci and 20.7% for Ge, respectively. This behavior was associated with a higher concentration of the bioactive (eugenol, geraniol and citral) exerting a greater acaricidal effect. These results are different from those obtained in this study, since a higher activity was observed for Ge with 100% mortality at a concentration of 1% and for Ci a mortality higher than 70% was obtained with concentrations of 4% and 5%, respectively.

The lowest LC_{50} to kill *D. gallinae* was for Ge followed by Thy and Ci (0.37%, 0.43% and 3.16%), respectively. Different authors have evaluated the use of pure bioactive and EO for the control of *D. gallinae* showing promising results, such as the work published by TABARI et al. (2015) where they evaluated the contact toxicity of Thy on *D. gallinae* obtaining a LD_{50} of 0.00351 mg/mL. On the other hand, GEORGE et al. (2009) evaluated different essential oils, registering values of 0.039 mg/mL for DL_{50} using thyme oil where its main compound is Thy (80%). The works of TABARI and GEORGE show a much lower LC_{50} than the results found in this work. Furthermore, SPARAGANO et al. (2013) observed an LC_{50} of 40% for Ge and 30% for Ci on *D. gallinae*, which are much higher than those shown in this study. Finally, the results in this work show us that it is possible to use lower concentrations and re-evaluate the synergism of the bioactive.

The results show a difference in mortality values, LC_{50}, LC_{90} and LC_{99} in the previously related works and those obtained in this investigation; where less amount of bioactive was required than reported by SPARAGANO et al. (2013). This difference could be related to several factors: a) the concentrations used in this work were higher, b) the use of essential oils implies synergistic effects of the compounds present in the oil, c) and methodological changes depending on the amount of solution used in the tests.

Interaction of binary and tertiary combinations of Ci, Ge and Thy on *D. gallinae.*

The combination of bioactive implies synergistic effects, less probability of generating resistance, shorter action times and less residuality (DI PALMA et al. 2012, MA et al. 2014, NOVATO et al. 2019). In this work, a higher acaricidal activity was observed in all the combinations, compared to the individual tests. All combinations had mortality rates >60% to 0.05g/mL which were much higher than the individual trials. The binary combination with the greatest toxic effect was Ge:Thy, and this is possibly due to the different mechanisms of action of Ge and Thy, which cause synergistic effects on the nervous system of red mites and the presence of OH groups that increase the possible interactions with the receptors, favoring their death at low concentrations. This result agrees with the individual results, where Ge and Thy presented the highest toxicity against *D. gallinae*. The combination of bioactive and essential oils to increase biological efficacy has been mentioned in several works. For example, KOUL et al. (2013) evaluated the toxicity against larvae of *Helicoverpa armigera* Hubner 1808 (Lepidoptera: Noctuidae), *Spodoptera litura* Fabricius 1775 (Lepidoptera: Noctuidae) and *Chilo partellus* Swinhoe 1885 (Lepidoptera: Crambidae) using binary mixtures of essential oils. The authors found that the combination of thymol:linalool or thymol:1,8-cineole synergized toxicity. MA et al. (2014) reported that the binary mixture of carvacrol and thymol (1:1) showed synergistic fumigant activity against adult *Culex pipiens pallens* (Diptera: Culicidae). In addition, works such as those by NTALLI et al. (2011) found that mixtures of thymol:eugenol and thymol:transanethol showed a strong synergistic effect against the nematode *Meloidogyne incognita*, MASOUMI et al. (2016) presenting a significant increase of approximately 40% in the acaricidal activity against *D. gallinae* with the combination of carvacrol:thymol in proportions 4:1. The authors concluded that the combination had synergistic effects. ARAÚJO et al. (2016) determined that the combination of thymol:carvacrol on *Rhipicephalus microplus* Canestrini 1888 (Acari: Ixodidae) and *R. sanguineus* (Acari: Ixodidae) larvae had a synergistic effect against these ectoparasites, with combination indices between 0.095 and 0.890, values obtained with the CompuSyn software. NOVATO et al. (2019) evaluated the acaricidal activity of thymol, carvacrol and eugenol against *R. microplus*. The binary combinations (1:1) between carvacrol:thymol presented the highest percentage of control (96.1%) and a synergistic effect with a CI of 0.68 at a concentration of 3.125 mg/mL, while carvacrol:eugenol and thymol:eugenol presented additive effects with mortalities below 70% with CI of 0.95 and 0.94 at the same concentration.

On the other hand, the treatments with the presence of Ci presented lower mite mortality than the combination of Ge:Thy, even the combination of Ci:Thy at a concentration of 1g/mL resulted in a decrease in the acaricidal activity presenting an antagonistic effect and at increasing the concentration to 2g/mL its effect was additive. However, at higher concentrations the effects were synergistic (Table 3). This behavior can be attributed to the fact that the phenomenon of synergistic or antagonistic action is given not only by the molecular structure, the type and position of the functional groups, but also by the mutual proportions of individual substances in the mixture (PAVELA 2014). On the other hand, the difference in the mortality of Ci:Ge and Ci:Thy may be associated with a greater toxicity generated by Ge with respect to Thy, which is corroborated by the individual trials where Ge was the bioactive with the highest mortality.

The tertiary combination of Ci:Ge:Thy showed 100% mortality at a concentration of 1g/mL, the acaricidal activity being stronger in relation to that presented by the binary combinations. Presumably, combining the three bioactive would increase the percentage of mortality of *D. gallinae*, considering the differences in the mechanisms of action. This result was confirmed with the CI value, where a synergistic effect was observed in all concentrations, relating it once again to what was mentioned by PAVELA (2014). Tertiary combination trials are intended to increase acaricidal activity to ensure that resistant populations do not arise at low concentrations, as demonstrated by JYOTI et al. (2019) who combined essential oils of clove (*Syzygium aromaticum* (L.) Merr. & Perry 1939 Myrtales: Myrtaceae), cinnamon bark (*Cinnamomum zeylanicum* Blume 1826 Laurales: Lauraceae) and lemongrass leaves (*Cymbopogon citratus* Stapf. (Poales: Poaceae) in proportions 1:1:1 for the control of *R. microplus* and observed a decrease in LC_{50}, LC_{90} and LC_{99} compared to binary mixtures. On the other hand, VAN SAUERS (2009) evaluated the acaricidal effect of the mixture of Thy, tobacco essential oil and Ge on *D. gallinae*, obtaining an average mortality rate of 100% with a 0.5% suspension, higher than that presented by binary mixtures. This shows that the greater the presence of chemical components in the mixtures, the acaricidal effect can be enhanced.

Finally, the behavior of the CI values obtained in this study do not present simple linear relationships or exponential behaviors, according to the findings found by CHOU (2006), when evaluating drugs for the treatment of diseases in humans. In this type of study where the dose-response of a drug is evaluated, the response variable changes with the dose. The mentioned before, could explain the difference between each of the CI values calculated for each of the concentrations evaluated in this study. The identification of the synergistic effects of the binary and tertiary combinations of the bioactive Ci, Ge and Thy are significant results for the control of the poultry mite. The search for synergistic mixtures is a practice that aims to reduce the concentrations needed with biological activity against the target organism (ASSOUGUEM et al. 2022).

CONCLUSION

The bioactive Ci, Ge and Thy present acaricidal activity on the poultry red mite (*D. gallinae*) after 24 hours of application under in vitro conditions. The binary combinations significantly increased the acaricidal activity against *D. gallinae*, thus providing synergistic and additive effects according to CI values. Furthermore, the tertiary combination Ci:Ge:Thy in 1:1:1 showed strong synergism at low concentrations. The combination of natural bioactive could be an additional way to control *D. gallinae* without putting the welfare of the birds at risk and would be an environmentally friendly measure. Finally, the synergism of the bioactive combination was verified with the CI values obtained by using the CompuSyn software.

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