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Natural Plant Extracts Control Red Mite (*Dermanyssus gallinae*) on Laying Hen Farms

ABSTRACT

Numerous alternative methods have already been evaluated to avoid the side effects of chemical insecticides for pest control, including plant extracts with insecticidal potential. This research aimed to evaluate the insecticidal potential (using the degree of lethality (DL%) as the parameter) of different plant extracts: timbó root (*Derris urucu*), neem (*Azadirachta indica*), cinnamon (*Cinnamomum verum*), garlic (*Allium sativum*), *Persicaria lapathifolia*, pyroligneous extract, neem+andiroba, and azadirachtin concentrate. For comparison, negative control (water) and commercial positive control, Termidil 200 SC (imidacloprid) were used. Statistical analysis demonstrated the existence of three distinct groups in terms of their efficiency in controlling red mites in laying hens. The group with the highest efficiency (DL>80) includes the alcoholic extract of timbó (86.10%), neem essential oil (84.20%), neem+andiroba in combination (85.36%), and Azadirachtin concentrate (81.06%). The medium efficiency group (80<DL<60) comprised *Persicaria lapathifolia* (66.79%), and cinnamon (61.81%); while the low efficiency group (DL<50) included garlic (44.60%), pyroligneous extract (43.10%) and the positive control (38.50%). Based on these results, alcoholic extract of Timbó root, Neem essential oil, Neem + Andiroba in combination and Azadirachtin concentrated extract are recommended, due to their DL>80%. Garlic and pyroligneous extracts as well as chemical insecticides are not recommended due to their low efficiency. Moreover, it was demonstrated that there is the possibility of resistance against commercial chemical insecticide due to its continuous use on the farm where the insects were collected. *Persicaria lapathifolia* and cinnamon can be used, but their efficiency is between 60–80%.

INTRODUCTION

The red mite (*Dermanyssus gallinae*) is an ectoparasite found in layer farms, and is one of the main pests in poultry farming (Fujisawa *et al.*, 2023). This insect feeds on blood for a period of 30 to 60 minutes at night, remaining for the rest of the time in the aviary structure, normally in nests, dirt accumulations (feathers, cobwebs, etc.), cage wires, feeders, drinking fountains, and crevices, where they reproduce (Oliveira *et al.*, 2014). The mite induces severe stress, causing anemia and problems in the health and well-being of birds. Under favorable conditions, its life cycle is of about 7 days, consisting of 5 phases: egg, larva, protonymph, deutonymph, and adult (George *et al.*, 2015).

Mite infestations affect the technical performance of the flock, reducing egg production and weight, weight gain, and increasing feed conversion, generating a negative impact on economic performance; in addition to causing serious problems in the herd's health due to increased mortality, stress, weight loss, anemia, and flock immunity



(Kasburg *et al.*, 2016). Furthermore, they can act as a vector of viral and bacterial pathogens linked to production and public health; and contact between the parasite and humans can cause irritation, skin lesions, and dermatitis (Chirico *et al.*, 2003).

Several studies have been carried out to evaluate the impact of red mite infestations on the zootechnical indexes of laying bird flocks. Depending on the degree of infestation, an increase of 1 to 4% in mortality and a 10% reduction in egg production can be observed in birds housed in a cage system (Wójcik *et al.*, 2000). In the European Union, the estimated cost of infestation due to control and production losses is around €130 million per year (Sparagano *et al.*, 2009). In the Netherlands, the estimated cost resulting from infestations can reach €0.60/bird housed. Currently, the main treatments available are synthetic neurotoxic acaricides, such as organophosphates and pyrethroids (Abbas *et al.*, 2014).

Oral administration of systemic ectoparasiticides such as fluralaner has been approved in layers (Huyghe *et al.*, 2017). However, significant restrictions on their use have been imposed due to their negative impact on human health and the environment, and some neurotoxic substances can be carcinogenic or harm mental health (Ansari *et al.*, 2014). Research has sought alternatives that are less impactful on the sustainability of the production system, including the use of inert powders, physical treatments, fungi, predators, essential oils, and specific bioactives (Tabari *et al.*, 2017).

Due to the scarcity of information covering studies of acaricidal plants, researches such as the present are necessary in order to evaluate insecticidal potential for the control of red mites. Our study evaluated plant extracts from Timbó (*Derris urucu*) root, Neem (*Azadirachta indica*), Cinnamon (*Cinnamomum verum*), Garlic (*Allium sativum*), *Persicaria lapathifolia*, Pyroligneous extract, Neem + Andiroba and an Azadirachtin concentrate in terms of lethality degree.

MATERIALS AND METHODS

The study was carried out at the Embrapa Swine and Poultry Laboratory, in Concórdia, Santa Catarina, Brazil. The red mites (*Dermanyssus gallinae*) were collected at the facilities of the Technical Reference Unit of Embrapa Swine and Poultry, located in Ouro, Santa Catarina, Brazil, and then transported to the laboratory in order to perform the bioassays.

The plants used to obtain the tested extracts were chosen due to previous reports of their insecticidal action against other insects in the literature. The natural plants evaluated for their insecticidal potential to control red mites in layers were extracted from: Timbó root (*Derris urucu*), Neem (*Azadirachta indica*), Cinnamon (*Cinnamomum verum*), Garlic (*Allium sativum*), *Persicaria lapathifolia*, Pyroligneous Extract, Neem + Andiroba and concentrated Azadirachtin. The extracts were produced and stabilized in the Laboratory of EPP Ophicina Orgânica e Fertilizantes, located in Atibaia, São Paulo, Brazil. Natural water and the commercial chemical insecticide Termidil 200/SC (imidacloprid) were used as negative and positive controls, respectively. They are routinely used to control mites on farms.

The insects collected at the Technical Reference Unit were placed in plastic pots (500 mL) and taken to the Embrapa Swine and Poultry Laboratory. After 24 hours, the mites, without initial counting, were placed in petry dishes lined with paper towel. The plant extracts were diluted in water, and a solution of each compound was prepared at a concentration of 2%. They were sprayed on the petry plates 60 minutes after the insects were placed on them. The air psychrometric variables were kept constant at 35 °C and 70% of relative humidity in an attempt to maintain conditions as close as possible to the field situation. After 48 hours of contact between the mites and the compounds, the number of live and dead insects was counted using magnifying glasses to determine the degree of lethality (DL) of each plant extract.

A completely randomized design was used, with 10 treatments, 8 plant extracts and negative and positive controls, with 10 replications per treatment. Mortality data were corrected using the Schneider-Orelli formula (Alves *et al.*, 2005) and transformed according to Eq (1) before statistical analysis. The data were evaluated for variance using the F test and the means were compared using the Tukey test at a 5% significance level using the SAS version 9.1 software (Sas Institute Inc 2012).

$$y = \text{Arcsen} \sqrt{\frac{x}{100}} \quad (1)$$

RESULTS

The comparison of the acaricidal activity of the evaluated extracts (diluted in 2% distilled water) on red mites, including the average degree of lethality



($DL_1 \pm SD$) and the corrected degree ($DL_2 \pm SD$) in relation to the negative control (water) obtained for all treatments, are presented in Table 1. Of the total plant extracts, four showed $DL > 80\%$, namely: Timbó root (86.10 ± 0.06), Neem + Andiroba (85.36 ± 0.08), Neem (84.20 ± 0.07), and concentrated Azadirachtin (81.06 ± 0.05).

Table 1 – Effect of the evaluated extracts and control treatments on the average degree of lethality (DL^1) and corrected by control treatment ($DL^2 \pm SD$).

Natural plants and Control	DL^1 (%)	DL^2 (%)
Timbó Root (<i>Derris urucu</i>)	86.84	86.10 ± 0.06^a
Neem + Andiroba	86.10	85.36 ± 0.08^a
Neem (<i>Azadirachta indica</i>)	84.94	84.20 ± 0.07^{ab}
Concentrated Azadirachtin	81.80	81.06 ± 0.05^b
<i>Persicaria lapathifolia</i>	67.53	66.79 ± 0.07^c
Cinnamon (<i>Cinnamomum verum</i>)	62.55	61.81 ± 0.04^c
Garlic (<i>Allium sativum</i>)	44.60	43.86 ± 0.03^d
Pyroligneous Extract	43.10	42.36 ± 0.06^d
Positive Control (Termidil 200 SC)	38.50	37.76 ± 0.06^{de}
Negative Control (water)		0.74 ± 0.01^f

^{a-e} Different superscript letters in the same column indicate significant differences ($p < 0.05$)

The statistical analysis of DL corrected for the negative control effect showed a significant effect ($p < 0.01$) for all treatments. As shown in Table 1, the analysis of variance identified three distinct groups in terms of efficiency in controlling the red mite: Group 1, formed by Timbó root, Neem + Andiroba, Neem and concentrated Azadirachtina, presented $DL > 80\%$. Group 2 formed by *Persicaria lapathifolia* and Cinnamon, had $80 < DL < 60$; and Group 3, formed by Garlic, Pyroligneous Extract and Positive Control, had $DL < 50$.

DISCUSSION

Lundh *et al.*, (2005) found substantial reductions (92%) in mite population during the experimental period when studying the effect of different formulations of Azadirachtin. Camarda *et al.*, (2018) perceived a huge reduction (99%) in mite population when using neem oil in enriched cage poultry systems. Due to the reclusive life cycle of *D. gallinae*, Locher *et al.* (2010) recommend up to three repeated acaricide applications within 1 week to ensure that the emerging generation does not flee after the initial treatment, along with any existing treatment against nymphs and adults (George *et al.*, 2010b). Chirico *et al.* (2003) raised the hypothesis that the incorporation of any compound harmful to *D. gallinae* in traps could be a

very promising concept to control the mite, concluding that traps impregnated with neem oil significantly reduced a field population of *D. gallinae* (Lundh *et al.*, 2005).

Cinnamon contains many substances and groups of substances, including essential oils, diterpenes, catechins, proanthocyanidins, tannins, dyes, phenolic carboxylic acids, lignans and mucins, which can act on octopaminergic and GABA receptors (Na *et al.*, 2011). In the present study, the cinnamon extract presented an intermediate DL (%) (61.81 ± 0.04) when compared with the other extracts; a result lower than those obtained by Radsetoulalova *et al.* (2017), who found mortality $> 99\%$ when using doses diluted in distilled water of 0.25 and 0.5 $\mu\text{L}/\text{cm}_2$, but higher when using doses between 0.12 (30.5%) and 0.03 (23.3%) $\mu\text{L}/\text{cm}_2$. Regarding the use of methanol as a diluent, mortality rates were higher in relation to ethanol, being 99.7; 99.5, 75.6 and 56.5%, for doses of respectively 0.5; 0.25; 0.12 and 0.06 $\mu\text{L}/\text{cm}_2$. According to Kim *et al.* (2004), cinnamon essential oil has a potent acaricidal activity against adults of *D. gallinae*. However, cinnamon bark oil is highly effective on eggs, and less effective on young and adult mites of *D. gallinae* (George *et al.*, 2010a).

Despite presenting $DL < 70\%$, *Persicaria lapathifolia* ($66.79 \pm 0.07\%$) and cinnamon ($61.81 \pm 0.04\%$) presented bioinsecticide potential for controlling red mites. In the specific case of *Persicaria lapathifolia*, little is known about its insecticidal potential in pest control, although traditional producers (tacit knowledge) report its efficiency as a repellent for various insects.

The alcoholic extract of Timbó root ($86.10 \pm 0.06\%$), the essential oils of Neem/Andiroba ($85.36 \pm 0.08\%$) and Neem ($84.20 \pm 0.07\%$), corrected for the control negative ($0.74 \pm 0.01\%$), presented the highest DL at laboratory level in relation to the other compounds used in the bioassay. Concentrated Azadirachtin, the active ingredient present in Neem extract, also showed promising results ($81.06 \pm 0.05\%$).

The bioactive components of Neem produce a cholinergic effect, inhibiting acetylcholinesterase, interfering with the polymerization of tubulin during mitosis (action of Azadirachtin), or interrupting the cell cycle, while also acting on receptors such as GABA, tyramine and octopamine. These mechanisms of action result in the interruption of feeding, growth and egg laying, while also causing repellency, decreased survival, and sterility of mites (Ogbuwu *et al.*, 2011). These factors probably defined the lethality of Neem essential oil (84.20%) and the compound Neem +



Andiroba (85.36%) observed in the present study. This confirms different results present in the literature, such as those obtained by Camarda *et al.* (2018), who obtained a drop in the degree of infestation in the field from 94.65 to 99.80% when nebulization of neem oil was used at a 20% dilution. Similar results were obtained elsewhere where an antiparasitic effect of neem oil to control the red spider mite was reported (Mattos *et al.*, 2009).

The adoption of good production practice measures associated with biosecurity strategies are essential for the success of the activity and the maintenance of laying hens' health. Usually, the presence of mites and lice is related with the low adoption of biosecurity criteria, such as dirt and dust in the production environment, and debris stored on the farm or the production system.

Several authors have demonstrated that the use of combinations of metabolites could reduce the concentration of each substance and increase biological activity in different species (Tabari *et al.*, 2017). The combination of bioactives and the difference in mechanisms of action could generate synergistic and additive effects for the control of *D. gallinae*, thus reducing the possibilities of developing resistant populations. In this context, the results obtained for the associated compound Neem + Andiroba (85.36%), compared to the use of Neem (84.20%), had an increase of 1.16% in DL, which may be an indication of synergistic or additive effect. However, to prove this effect, new tests must be carried out to evaluate the individual and associated effects.

The toxicity of Timbó root is mainly attributed to rotenone, which is a crystalline, odorless and tasteless isoflavonoid, biosynthesized by secondary metabolism and which always appears accompanied by other flavonoid compounds (rotenoids) such as deguelin, tephrosine and toxicorol (Lima, 1987). Rotenone causes death in animals by inhibiting the mitochondrial respiratory chain, blocking the phosphorylation of ADP to ATP, with fish and insects being highly sensitive (Mascaro *et al.*, 1998). This is the likely reason why rotenone from Timbó root has presented the highest efficiency value (86.10%) in lethality when compared with the other extracts. This result corroborates the findings of Mattos *et al.*, (2009), who observed a high degree of lethality using alcoholic extract of Timbó root (86.10%). They also verified a significant insecticidal effect for the control of mites in laying hens in the field, indicating that Timbó extract has an antiparasitic effect.

According to Ranjbar-Bahadori *et al.* (2014), garlic extract is efficient in controlling infestations of blood-sucking mites. The results obtained by the authors showed that on days 1 and 7 after administration of the extract, the efficacy rate was 92.05% and 74.62%, respectively, concluding that its use in controlling *D. gallinae* is more effective in a short period of time. The results obtained in the present work demonstrated a low efficiency of the garlic extract ($43.86 \pm 0.03\%$) when compared with the other evaluated compounds, presenting an effect close to that obtained by the pyroligneous extract ($42.36 \pm 0.06\%$), and higher than the positive control ($37.76 \pm 0.06\%$).

The commercial product (Termidil 200/SC) used as a positive control has imidacloprid in its composition and is a first generation neonicotinoid which acts as an agonist of insect nicotinic receptors. It was used to control mite infestations on the farm where insects were collected to carry out the bioassay. Although the experimental design was not carried out with the objective of evaluating possible resistance acquired by insects to the continuous use of the insecticide, the result obtained for DL ($37.76 \pm 0.06\%$) is an indication of resistance occurrence, which has made it difficult to control the mite infestation on the farm.

CONCLUSION

Based on the results obtained in this work, the alcoholic extract of Timbó root, Neem essential oil, the association of Neem + Andiroba, and the concentrated extract of Azadirachtin are recommended for the control of red mites, as they showed efficiencies higher than 80%. *Persicaria lapathifolia* and cinnamon can be used, but with a lower efficiency (60–80%). Garlic and pyroligneous extracts, as well as chemical insecticides, are not recommended due to their low efficiency. The results presented by the commercial chemical insecticide demonstrated the possibility of resistance occurring due to its continuous use on the farm where the insects were collected.

AUTHOR CONTRIBUTIONS

Conceptualization, GSS; Funding acquisition, GSS; Investigation, GSS and PGA; methodology, GSS, PGA and ACJr; Project administration, GSS; Resources, GSS; Supervision, GSS; Validation, GSS and ACJr; visualization, GSS and ACJr; formal analysis, PGA; data curation, PGA; writing—original draft preparation, GSS, PGA, ACJr and VF; writing—review and editing,



PGA and VF. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data will be available upon request.

CONFLICT OF INTEREST

The authors declare no conflict of interest of any kind.

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