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Original Article

Insecticidal effect of metabolites identified in edible mushrooms against *Rhyssomatus nigerrimus* Fahraeus

Efeito inseticida dos metabólitos identificados nos cogumelos comestíveis contra *Rhyssomatus nigerrimus* Fahraeus

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Abstract

Excessive use of insecticides has led to resistance of some pathogenic organisms (nematodes, bacteria and fungi), environmental contamination, and the presence of hazardous residues. Therefore, the aim of the present study was to evaluate synthetic metabolites derived from previous studies with edible mushrooms against the soybean weevil *Rhyssomatus nigerrimus* Fahraeus (Curculonidae) because of the relevance of pest control in an economically important crop. Furthermore, this is one of the first studies where edible fungal molecules are evaluated for the control of these insects. Initially, two *in vitro* tests (toxic effect and immersion) were evaluated against *R. nigerrimus*. In these tests, sensitivity and viability were determined in the 2% Tween control in water. For these two tests, the synthetic metabolites pentadecanoic acid (PNA), palmitic acid (PMA), stearic acid (STA), linoleic acid (LNA), β-sitosterol (βT) were evaluated individually as well as in combinations, "the fraction of standards (E1)". Based on the results obtained, the dip test was selected to evaluate the mixtures of two standards (1. PMA + βT, 2. PMA + PNA, 3. PMA + LNA, 4. PMA + STA, 5. STA + βT, 6. STA + PNA, 7. STA + LNA, 8. PNA + βT, 9. PNA + LNA, 10. LNA + βT), three (1. PNA + βT + LNA, 2. PNA + βT + STA, 3. STA + LNA + PNA and 4. STA + LNA + βT) and four (PNA, βT, LNA and STA). The results showed that the mixture of three standards caused a higher percentage of mortality relative to the control group: l. PNA + βT + LNA and 2. PNA + βT + STA with 54.44 and 48% mortality of *R. nigerrimus* insects exposed for 15 days. These results show the importance of evaluating mixtures of molecules against *R. nigerrimus*.

Keywords: synthetic molecules, mycochemistry, insecticidal activity, soybean weevil.

Resumo

O uso excessivo de inseticidas levou à resistência de alguns organismos patogênicos (nematódeos, bactérias e fungos), à contaminação ambiental e à presença de resíduos perigosos. Portanto, o objetivo do presente estudo foi avaliar a mortalidade de metabólitos sintéticos derivados de estudos anteriores com cogumelos comestíveis contra o gorgulho-da-soja *Rhyssomatus nigerrimus* Fahraeus (Curculonidae) por causa da relevância do controle de pragas em uma cultura economicamente importante. Além disso, este é um dos primeiros estudos em que as moléculas fúngicas comestíveis são avaliadas para o controle desses insetos. Inicialmente, dois testes in vitro (efeito tóxico e imersão) foram avaliados contra *R. nigerrimus*. Nesses testes, a sensibilidade e a viabilidade foram determinadas no controle de 2% de Tween na água. Para esses dois testes, os metabólitos sintéticos – ácido pentadecanoico (PNA), ácido palmítico (PMA), ácido esteárico (STA), ácido linoleico (LNA) e β-sitosterol (βT) – foram avaliados individualmente, bem como a combinação dos 5, "a fração de padrões (E1)". Com base nos resultados obtidos, o teste de imersão foi selecionado para avaliar as misturas de dois padrões (1. PMA + βT, 2. PMA + PNA, 3. PMA + LNA, 4. PMA + STA, 5. STA + βT, 6. STA + PNA, 7. STA + LNA, 8. PNA + βT, 9. PNA + LNA, 10. LNA + βT), três (1. PNA + βT + LNA, 2. PNA + βT + STA, 3. STA + LNA + PNA e 4. STA + LNA + βT) e quatro (PNA, βT, LNA e STA). Os resultados mostraram que a mistura de três padrões foi a com maior porcentagem de mortalidade em relação ao grupo controle, quais sejam, l. PNA + βT + LNA e 2. PNA + βT + STA, com 54,44% e 48% de mortalidade, respectivamente, em uma exposição de 15 dias contra *R. nigerrimus*. Estes resultados mostram a importância de avaliar as misturas entre moléculas contra *R. nigerrimus*.

Palavras-chave: moléculas sintéticas, micoquímica, atividade inseticida, gorgulho-da-soja.

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1. Introduction

Soybean [*Glycine max* (L.) Merr.] is widely consumed. National soybean production in 2019 in Mexico was 114,527 tons (Mexico, 2019). However, in 2013 production was 239,248 tons of grain nationally, and the 2021/22 world soybean oil production is projected to be 627.6 million tons (USDA, 2021). This production and consumption of soybean is due to its nutritional value (Fresno, 2002; Vanegas-Pérez et al., 2009). However, there are factors that limit its production, such as insect pests.

The soybean weevil, *Rhyssomatus nigerrimus* Fahraeus (Coleoptera: Curculionidae), is currently considered the most economically important pest because of the direct and indirect damage it causes. Larvae and adults of this insect damage soybean seedlings, flowers, shoots, pods and beans (López-Guillén et al., 2012). Currently, *R. nigerrimus* is controlled with insecticides such as organophosphates and pyrethroids, as well as fipronil, aluminum phosphide or methyl bromide (Vargas and Guillén, 2014; RAPAM, 2017). Unfortunately, it has been observed that excessive use of these insecticides has led to resistance of some pathogenic organisms (nematodes, bacteria and fungi), environmental contamination and the presence of hazardous residues in food for human consumption (RAPAM, 2017). Due to these effects, alternative methods of control are being sought, one of which is the use of plant extracts, essential oils, the use microscopic or macroscopic fungi and their active compounds.

In the case of macroscopic fungi, there is evidence that these fungi have insecticidal activity (Cordier, 1876; Mier et al., 1996; Wang et al., 2002). One of the most reported genera with insecticidal activity is *Pleurotus* sp., some of whose metabolites have been reported to have possible biological activity (Cedillo, 2016; Pineda-Alegría et al., 2017; Cruz-Arévalo et al., 2020). In addition, this is one of the first studies that has evaluated molecules from edible fungi for the control of these insects. Therefore, the aim of the present study was to evaluate the mortality caused by synthetic metabolites derived from the edible mushroom *Pleurotus* sp. against the soybean weevil, *R. nigerrimus*.

2. Materials and Methods

2.1. Biological material: insects

The weevils were obtained from soybean crops in the municipality of Tapachula, Chiapas, Mexico (located at 14° 56” and 92° 10” W). Adult weevils were collected by hand and stored in 1 L plastic jars covered with organdy cloth. The insects were fed soybean pods following the methodology described by López-Guillén et al. (2016).

2.2. Molecules

Commercial molecules that have been identified in edible mushrooms were used (Pineda-Alegría et al., 2020). The fatty acids evaluated were 1) pentadecanoic acid (PNA) (CAS no.: 1002-84-2), 2) palmitic acid (PMA) (CAS no.: 57-10-3) 3) stearic acid (STA) (CAS no.: 57-11-4), 4) linoleic acid (LNA) (CAS no.: 60-33-3) and terpene β-sitosterol (βT) (CAS no.: 83-46-5). The fatty acids evaluated were in the form of their commercial standards purchased from SIGMA-ALDRICH® (Toluca de Lerdo, Estado de México, Mexico) (Pineda-Alegría et al., 2020).

2.3. Bioassays

Initially, two tests were used to determine the sensitivity of the technique, which were Toxic effect and Immersion.

2.3.1. Evaluation of insecticidal activity by toxic effect of commercial compounds

Plastic containers (3x2.5x3cm) were used and 3.0 cm diameter circles of Whatman paper No. 4 impregnated with PNA, PMA, STA, LNA, βT standard solutions were placed in each container. Five adult insects and food (*Ipomoea batatas* purple sweet potato squares 1cm x 0.5cm) were placed in each well. In addition, 7 to 10 replicates were used, and the jars were covered with organdy cloth. The treatments were the following: (A) PNA, (B) PMA, (C) STA, (D) LNA (20%), (E) βT, (F) “E1 standard fractions” (PNA, PMA, STA, LNA, βT) each at a concentration of 2.5% and (G) Negative control (Tween 2% in water). The containers with the weevils were observed and insect mortality was determined at 24, 48, 72 h and 144 h. These flasks were stored at 30 ± 2 °C temperature, 60 ± 5% relative humidity.

2.3.2. Immersion test

The next test was the immersion test, which consisted of submerging the insects for 20 seconds in the treatments at a concentration of 2.5%: (A) PNA, (B) PMA, (C) STA, (D) LNA (20%), (E) βT and (F) “Fraction of E1 standards” (PNA, PMA, STA, LNA, βT) and a negative control (Tween 2% in water). These insects were treated and placed in plastic containers, and insect mortality was determined at 24, 48, 72 and 144 hrs.

2.4. Assessment of combinations of standards

To evaluate the standards, the immersion bioassay against *R. nigerrimus* described above was used. The number of deaths observed every day for a period of 10 to 20 days was counted. Initially, combinations of two compounds were numbered from 1 to 10 (1. PMA + βT, 2. PMA + PNA, 3. PMA + LNA, 4. PMA + STA, 5. STA + βT, 6. STA + PNA, 7. STA + LNA, 8. PNA + βT, 9. PNA + LNA, 10. LNA + βT) and evaluated at a concentration of 2.5%.

Subsequently, the combination of three compounds (1. PNA + βT + LNA, 2. PNA + βT + STA, 3. STA + LNA + PNA and 4. STA + LNA + βT) were also evaluated. Finally, in the evaluation of the combinations, the mixture of 4 compounds (PNA, βT, LNA and STA) was also evaluated.

2.5. Data analysis

The formula used to determine insecticidal effect was (Equation 1):

$$\%Mortality = \frac{(\text{Number of dead insects}) \times (100)}{(\text{Number of dead insects} + \text{Total number of live insects})} \quad (1)$$

The results obtained from the mortality tests of the different treatments (Toxic effect and Immersion) at different hours of exposure were analyzed using a generalized linear model (GLM) to evaluate differences with respect to the control (Tween 2% in water). In addition, a comparison of means of the percentages obtained relative to the control was performed. The analyses were performed with Statgraphics Centurion XV (Statpoint Technologies Inc., 2005).

3. Results

3.1. Evaluation of the insecticidal activity by toxic effect and immersion of the commercial compounds

The percentages of the *in vitro* insecticidal effect of the toxic effect and immersion tests of the commercial compounds can be observed in Table 1. For the case of the toxic effect test, 24, 48 and 72 h exposure to the evaluated treatments had no effect nor were significant differences observed between groups or controls; these groups were (A) PNA, (B) PMA, (C) STA, (D) LNA (20%), (E) βT, (F) "E1 standard fractions" (PNA, PMA, STA, LNA, βT) and (G) Negative control (2% Tween in water). In general, an increase in mortality was observed as time elapsed (significant differences (P<0.05)).

On the other hand, for the immersion test of the standards against *R. nigerrimus*, compounds derived from edible fungi (PNA, PMA, STA, LNA, βT) individually did not show a significant effect on *R. nigerrimus* mortality, while the 2% Tween control showed low *R. nigerrimus* mortality during the days the test was performed.

3.2. Assessment of the combinations of the standards

The results of mortality caused by the standard mixtures against *R. nigerrimus* can be seen in Table 2. The mixture containing PNA + βT caused 35% mortality of the insects, significantly different from the control. On the other hand, with the mixtures of three standards, significant mortality relative to the control was observed for two mixtures: l. PNA + βT + LNA and mixture 2. PNA + βT + STA with 54.44 and 48.00% mortality of *R. nigerrimus* insects in 15-days of exposure. The results of the mixture with four standards (PNA, βT, LNA and STA) showed no significant difference relative to the control group.

4. Discussion

The aim of the present study was to evaluate the effect of synthetic metabolites derived from edible mushroom on the mortality of the soybean weevil, *R. nigerrimus*. This is the first study to evaluate this type of synthetic molecules against weevils (*R. nigerrimus*). When evaluated individually, the effect of the molecules was not significantly different from the control (Tween 2% in water). However, these molecules had been previously evaluated against *Tyrophagus putrescentiae* and biological activity was observed. Particularly, linoleic acid at a concentration of 50% caused 100% mortality of mites after 3 hours of exposure (González-Juárez, 2020). These differences may be because a mite such as *T. putrescentiae* has a soft body, while the soybean weevil has a hard exoskeleton, "theoretically" making penetration of the synthetic compounds more difficult.

The tests of combinations of the standards showed that only some combinations, mainly the one containing three standards, had significant effect on *R. nigerrimus* mortality. Of these mixtures, mixture l. PNA + βT + LNA and mixture 2. PNA + βT + STA caused 54.44 and 48% mortality, respectively, after 15 days of exposure. In this regard, there are few studies that evaluate only synthetic compounds.

Insecticidal activity of LNA in mixtures with other compounds has been reported in vegetable oils (Salas, 1985; Merino et al., 2012). In one of these studies, six different vegetable oils (olive, sesame, peanut, soybean, coconut and castor) were evaluated against *Sitophilus oryzae*. Mortality rates were 100% for adults after 3 hours of contact (Salas, 1985). Subsequently, in 2012, 13 oils of vegetable and mineral origin (*Prunus amygdalus*, *Corylus avellana*, *Cucurbita pepo L., Zea mays L., Arachis hypogaea*

Table 1. Mortality percentages of two *in vitro* insecticidal tests of commercial compounds derived from edible mushrooms against *Rhyssomatus nigerrimus* at different exposure times.

Test	Toxic effect				Immersion			
Treatments	Hours of exposure							
	24	48	72	144	24	48	72	144
PNA	10.0±5.1 a	29.0±7.0 a	40.0±7.9 a	94.0±7.3 a	0.0 a	0.0±0.8 a	0.0±1.4 a	10.0±4.5 a
PMA	10.0±5.1 a	24.0±7.0 a	44.0±7.9 a	90.0±7.3 a	0.0 a	0.0±0.8 a	0.0±1.4 a	4.0±4.5 a
STA	16.0±5.1 a	31.3±7.0 a	42.0±7.9 a	76.0±7.3 a	0.0 a	0.0±0.8 a	2.5±1.4 a	7.5±4.5 a
LNA	16.1±5.1 a	42.3±7.0 a	54.1±7.9 a	97.7±7.3 a	0.0 a	0.0±0.8 a	0.0±1.4 a	2.0±4.5 a
βT	13.3±5.1 a	29.0±7.0 a	41.7±7.9 a	88.0±7.3 a	0.0 a	2.0±0.8 a	4.0±1.4 a	12.0±4.5 a
"E1"	21.5±5.1 a	26.3±7.0 a	29.2±7.9 a	54.2±7.3 b	0.0 a	2.0±0.8 a	2.0±1.4 a	10.0±4.5 a
Tween 2%	5.7±5.1 a	31.4±7.0 a	60.0±7.9 a	92.2±7.3 a	0.0 a	0.0±0.8 a	0.0±1.4 a	9.6±4.5 a

a, b: different letters indicate significant differences between columns (P<0.05). PNA: pentadecanoic acid; PMA: palmitic acid; STA: stearic acid; LNA: linoleic acid; βT: β-sitosterol; E1: fraction of the 5 standards.

Table 2. Dip test mortality percentages of combinations of standards against *R. nigerrimus* at 15 days of exposure.

Mixtures	Treatment	Percentage of mortality
Bioassay of the mixture of two standards	PMA + β T	10.8±6.6 a
	PMA + PNA	12.5±6.6a
	PMA + LNA	18.0±6.6ab
	PMA + STA	24.5±6.6ab
	STA + βT	13.9±6.6a
	STA + PNA	24.8±6.6ab
	STA + LNA	24.5±6.6ab
	PNA + βT	35.0±6.6b
	PNA + ALN	30.0±6.6ab
	LNA + βt	12.0±6.6a
	Tween 2%	15.8±6.6 a
Mix of three standards	PNA + βT+ LNA	54.4±9.3 b
	PNA + βT + STA	48.0±9.3 b
	STA + LNA + PNA	40.5±9.3 ab
	STA + LNA + βT	45.5±9.3 ab
	Tween 2%	17.3±9.3 a
Mix of the four standards	PNA, βT, LNA y STA	14.9±7.0 a
	Tween 2%	27±7.0 a

a, b: different letters indicate significant differences between Mixed sections (P<0.05). PNA: pentadecanoic acid; PMA: palmitic acid; STA: stearic acid; LNA: linoleic acid; βT: β-sitosterol.

L., Helianthus annuus L., Juglans regia L., Olea europea L., Vitis vinifera L., Ricinus communis L., Sesamum indicum L. and *Glycine max L.*) were used against the corn weevil *Sitophilus zeamais* Motschulsk. Mortality rates ranged from 50% to 100% (Merino et al., 2012). One of these evaluations of linoleic acid was a study that evaluated olive oil against *Rhyzopertha dominica* and determined the fatty acid composition (oleic, palmitic and linoleic acids), which caused a mortality rate of 72.5 to 95% (0.4 mL/25g) (Kerbel et al., 2021).

Few studies have evaluated isolated compounds or standards, and their results only report effects on mortality. However, more studies are needed to determine the effect of the standards (pentadecanoic acid, palmitic acid, stearic acid, linoleic acid, β-sitosterol) against this family of insects.

There are studies on the fungus *Pleurotus* spp against insect pests, but not against *R. nigerrimus*. The reported studies of *Pleurotus* against insects have shown insecticidal activity. In 1996, powdered *Pleurotus ostreatus* basidiomes was evaluated and insecticidal activity was observed against *Drosophila melanogaster* and *Spodoptera littoralis* (Mier et al., 1996). Subsequently, a 2011 study investigated the residual effect of extracts of *P. ostreatus* basidiomes against *Tribolium castaneum* adults. Different extracts and extract fractions (petroleum ether fraction, methanol-chloroform extract, and hot water) were evaluated. The range of the results obtained from the fractions (LD_{50}) was 1.54 mg/cm^2 to 0.20 mg/cm^2 after 30 hours of exposure (Rahman et al., 2011). The Faridur study was one of the first reports of activity against the order Coleoptera, which includes *R. nigerrimus*. This is congruent with the results of insecticidal activity obtained from the combinations of standards, possibly indicating that in the case of the order Coleoptera the highest activity might be found in the whole extract of *Pleurotus* sp.

However, another study recently evaluated *in vitro* extracts of the fungus *P. ostreatus* for the control of the corn weevil (*Sitophilus zeamais* Motschulsky) where the tests used were toxicity by contact and fumigation, repellency and antixenosis. The highest toxicity was obtained by fumigation with ethyl acetate extract, which caused 100% mortality at a concentration of 30 μL in 0.15 L^{-1} air and a LC_{50} of 18.3 μL in 0.15 L^{-1} air. The extracts in ethyl acetate and distilled water showed repellent activity, and all recorded an antixenosis effect (Pino et al., 2019). This was the first study of *Pleurotus* spp fungi against a member of the Curculionidae family, to which *R. nigerrimus* belongs, and the results highlight the importance of evaluating the whole extract and combinations of standards.

More studies and tests are still needed to determine the activity of fungal extracts and their chemical standards, as well as to understand the mechanisms of action of the standards and to know more about the volatiles that interact with *R. nigerrimus* (Espadas-Pinacho et al., 2021). Currently, only insecticides and their mechanisms of action are described, for example, control of *R. nigerrimus* with Fipronil, an insecticide that acts on nerve functions by blocking GAPA (gamma aminobutyric acid) barrier channels (Cole et al., 1993). The results of the present study can contribute to exploration of new molecules for inclusion in the control of the insect *R. nigerrimus*.

5. Conclusions

The PNA, PMA, STA, LNA and βT standards when evaluated individually were not significantly different from the control group in their effect against *R. nigerrimus*. The combination PNA + βT showed 35% mortality, which was significantly different from the 15.83% caused by the control group against *R. nigerrimus*. For the case of the combination of three standards, significant mortality of *R. nigerrimus* insects, relative to the control, was observed for two mixtures; these were l. PNA + βT + LNA and mixture 2. PNA + βT + STA with 54.44 and 48% mortality after 15 days of exposure, while the evaluations of the other mixtures showed no effect relative to the control group.

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