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Insecticidal properties of *Pinus taeda* essential oil

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ABSTRACT

This study aimed to evaluate the yield of essential oil extracted from different sample and pH compositions, as well as the insecticidal potential of *Pinus taeda* essential oil. For this, samples composed only of needle and composed of needle and branches were kept in solvent water at different pH (3, 4, 5 and 6). Subsequently, the oils extracted in the different pH treatments were added in a proportion of 25% to Tween® (20%) and sunflower oil (55%) forming a concentrated bioinsecticide solutions at each pH. Solutions were diluted in deionized water to final concentrations of 12.5, 6.2, 3.1 and 0% oil. The samples containing needles and branches, as well as those extracted at pH between 4 and 6, provided a higher yield of essential oil. The bioinsecticide formulations containing the highest concentrations of essential oil extracted at pH 3 showed the best results in the control of *S. zeamais*. The essential oil of *P. taeda* has potential for the preparation of bioinsecticides and can be extracted from samples containing needles and twigs in a solvent with pH 3. Bioinsecticide solutions of higher concentrations are more efficient in controlling *S. zeamais* after 24 hours of exposure.

Keywords: bioinsecticides, extraction, pH, phytotoxicity, corn weevils.

INTRODUCTION

Modern agriculture must incorporate scientific innovations to maximize productivity while ensuring environmental sustainability. The latter is increasingly demanded by the new sustainability standards required by society and desired by farmers.[1] Among the advances in science and technology, we highlight the advances in the field of genetic improvement, crop nutrition and pest management.

Regarding pest management, although Brazil practices a high technological standard by producers, agriculture in the country has suffered considerable economic losses in recent years due to pest attacks.[2] According to Vieira Filho et al.,[3] pests, along with adverse weather conditions are the main reasons for the failure of grain harvesting and storage. It should be noted that grain production grew 182% between 1995 and 2017 and presented a new record in the 2019 harvest, with 240 million tons produced in an area of 63 million hectares, only 7% of the national territory.[3]

The insects cause the deterioration of the grain using the nutrients for its growth and reproduction, and is in this group the species *Sitophilus zeamais* (Coleoptera - Curculionidae) which causes direct action, ie, has the ability to damage the still healthy grains.[4] In addition, *S. zeamais* is considered of greater importance for grains stored in Brazil because it has cross-infestation, is a deep pest, has high biotic potential both in the larval and adult phases.[5] Overcoat, it is considered one of the most economically worrying pests, which justifies most of the chemical control practiced.[6]

The recommendation of chemical control currently involves many aspects, ranging from the resistance of pests to insecticides that affect agribusiness, to a social demand for the quality of the environment and for food free from pesticides. Due to these problems, research is being carried out with the aim of employing botanical products in pest management.[7,8]

The search for more sustainable alternatives that are less harmful to health and the environment has led to the replacement of synthetic pesticides with biopesticides and other organic solutions. In this sense, essential oils stand out as powerful natural insecticide agents, in addition to their antibacterial and antifungal properties.[9]

Several plants have been studied as potential tools in the new management and control strategies for pests of economic importance,[10,11] with many plants still needed to be discovered and introduced in order to be a more sustainable alternative in agriculture.[12] This is due to the fact that plants are rich in bioactive substances[13] that make up the essential oils.[14] Such substances are mainly terpenic in nature and present as volatile molecules of low molecular weight, accumulating in all plant organs and resulting in inhibition of growth and toxic effect, as well as inducing repellent action, antimycotic, analgesic, acaricide[15] and antimicrobial.[16]

Given the above, it is evident the importance of studies aimed at the prospection of natural compounds for the preparation of bioinsecticides, especially considering the great diversity of plants and environmental responsibility. Thus, this work aimed to evaluate the yield of essential oil extracted from different sample compositions and pH of the solvent, as well as the insecticide potential of solutions containing different concentrations of essential oil of *Pinus taeda*, extracted at different pH.

MATERIAL AND METHODS

Collection and storage of plant material

The experiments was carried out at the Laboratory of Entomology of the School of Agricultural Sciences and Veterinary Medicine of the Pontifical Catholic University of Parana (PUCPR), in the city of Curitiba, Paraná (Brazil). The plant material used for the analyses were branches and needles of *Pinus taeda* (approximately 10 years old), collected in the city of São José dos Pinhais (Paraná, Brazil) with the aid of telescopic pruning shears (Figure 1A). After collection, the plant material was stored in plastic packaging and taken to the laboratory, where it was separated into branches and needles (Figure 1B).

Extraction of essential oil

For the extraction of the essential oils were used 250g of sample composed exclusively of approximately two centimeters of needles and another sample containing needles and branches. Analytical balance was used for sample preparation (Figure 1C).

Evaluation of the plant origin of the essential oil and the extraction pH

The extraction was performed by steam dragging using a Clevenger apparatus (Figure 1D), where the samples remained for two hours with water at different pH (3, 4, 5 and 6) as solvent. This procedure was repeated three times for each type of sample and pH of the water. The extracted

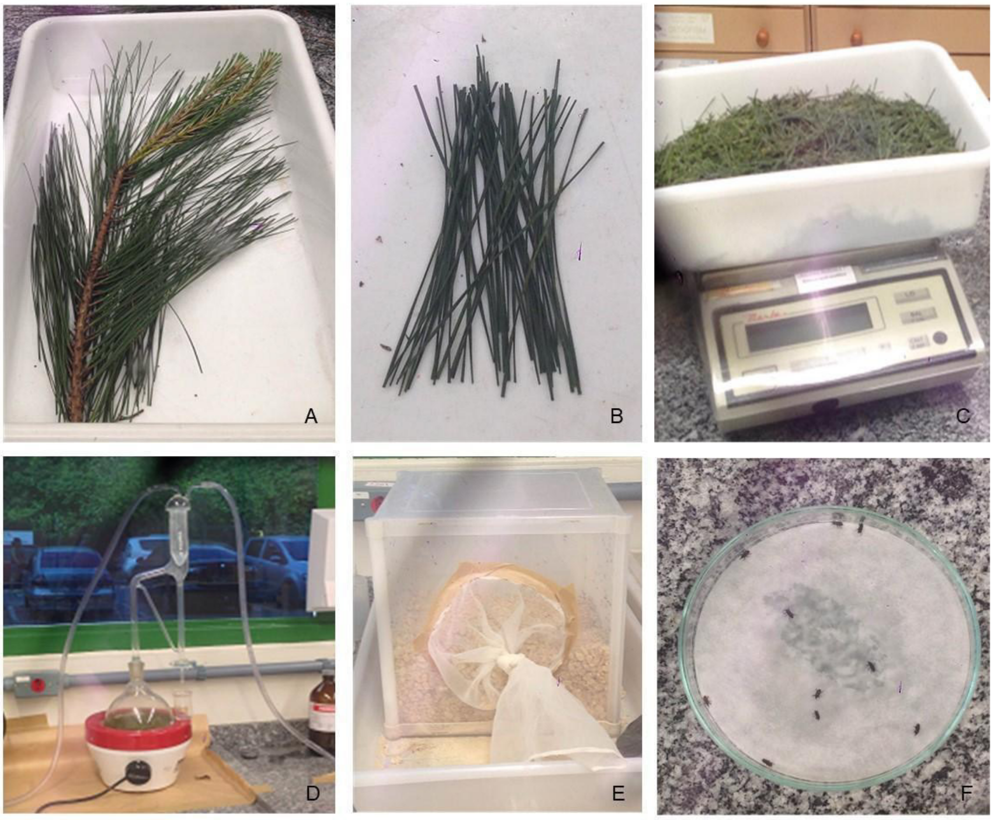

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Figure 1. Branches and needles of *Pinus* sp. (A). *Pinus* sp. (B). Sample with 250g weighed in an analytical balance (C). Clevenger apparatus extraction (D). Plastic box containing *Sitophilus zeamais* (E). Petri dish with *Sitophilus zeamais* after insecticide treatment (F).

oil was quantified in terms of volume in mL for calculation of yield and later stored in plastic containers with lid identified by treatment. The yield of the essential oil was calculated by the ratio between the volume of essential oil (mL) and the mass of plant material (g), according to the Brazilian Pharmacopoeia.[17]

The experiment was a 2x4 factorial (plant material and water pH) in a completely randomized experimental design with three repetitions.

Evaluation of the extraction pH and the concentration of essential oil

For the second experiment only the extracts obtained from the samples containing needles and branches were used. Thus, for each pH of the extraction water, a concentrated emulsifiable solution was formulated composed of 25% essential oil of *P. taeda*, 20% surfactant Tween and sunflower oil in sufficient quantity for the final volume of the formulation (approximately 55%). This concentrated solution was then diluted in deionized water, resulting in oil concentrations of 12.5, 6.2, 3.1 and 0%. The 0% concentration (control treatment), which consisted of using only deionized water in the filter paper.

For the toxicity test of the insecticide formulation, maize weevils (*Sitophilus zeamais*) were collected at PUCPR and kept in a plastic box with constant temperature and ambient humidity conditions for mass production (Figure 1E). To carry out the experiment, individuals of similar sizes were selected.

Petri dishes containing filter paper impregnated with 1000 µL of the formulated solutions were used. Subsequently, 10 insects of the same age were placed in confinement for 24h at room temperature to assess mortality (Figure 1F). The experiment was a factorial 4x5 (water pH and solution concentration) in a completely casualized experimental design with four repetitions of 10 experimental units each (insects).

Statistical analysis

The data were evaluated for normality by the Shapiro-Wilk test at 5% probability and subsequently underwent variance analysis to verify differences between treatments.

For treatments with significant differences, Tukey's test was performed to compare the means to 5% probability of error. The statistical analyses were performed using the R Core Team software.[18] The lethal concentration (LC50) was also calculated for the different pHs of essential oil extraction by the Trimmed Spearmankarber method[19] and the Dunnett procedure.[20]

RESULTS AND DISCUSSION

The analysis of variance did not detect significant interaction between treatments tested for the extraction of bioactive compounds of *Pinus taeda*. However, there was significant influence for the two isolated treatments (plant material and pH) (Figure 2). Regarding the pH of the extraction water (p = 0.00373), the treatment with pH 6 was responsible for the higher yield of extracted essential oil (0.32% or 0.8 mL/250g), without differing statistically from the treatments with pH 4 and 5 (0.25% or 0.63 mL/250g and 0.29% or 0.72 mL/250g, respectively). The more acidic pH of the solvent resulted in the extraction of the lower yield of essential oil (0.22% or 0.52 mL/250g) (Figure 2A). In studies with species of the genus *Pinus*, yields values ranging from 0.13 to 0.48%[21] and 0.33% of essential oil yield were found in *P. pinaster.*[22] Studies with other genera also found similar results as for *Baccharis dracunculifolia*, in which the yield was 0.8 mL/100g[23] and *Baccharis articulata* obtained 0.55 mL/100g[24] both using the hydrodistillation technique for extraction.

The pH of the solvent used (3, 4, 5 and 6) was an important parameter in the yield of essential oil of *Pinus taeda* by hydrodistillation, where the closer to the neutral pH of the water, the higher the yield of extracted oil. With regard to the type of plant material (p = 7.5-07), it was observed that the samples composed only of needles result in lower yield of extracted oil (0.18%), significantly lower than the sample containing a mixture of branches and needles (0.36%) (Figure 2B). These results are comparable to those found by Tomazoni et al.[25] (in a study with *Pinus taeda* (0.209%) and *Pinus elliotti* (0.12%) using hydrodistillation of needles.

The choice of the method, as well as the extraction conditions such as time, solvent, pH, type of plant material, among others, are factors that influence the yield of essential oil,[26] which signals the importance of a prior assessment of these factors for success in extracting essential oils. Thus, the result of this work is innovative, since there are no works on the variation of the solvent pH as well as the best type of vegetable material in the hydrodistillation of *Pinus taeda* oil.

In the second experiment, significant interaction was observed between the treatments tested (p = 0.00014) (Table 1). The highest percentages of mortality were observed in insecticide formulations with higher concentration of *P. taeda* oil and extracted at more acid pH (25 and 12.5% at pH 3). These results indicate that, although the most basic pH provides the highest volume of essential oil by extraction of steam dragging using a Clevenger apparatus, the insecticide solutions prepared with oils extracted in more acidic solvent have the best control responses of *S. zeamais*. Thus, the concentration above 12.5%, which is equivalent to 125 µL/plate, is sufficient to obtain a high efficiency in the control of *S. zeamais* under experimental conditions (Table 1). According to the Ministry of Agriculture,[27] an insecticide is considered effective if it results in at least 80% pest mortality, a threshold that was reached at concentrations above 12.5%.

These values are intermediate when compared to the results found by Rodrigues et al.[23] that require a dose of 450 µL/*Baccharis dracunculifolia* oil plate for the control of *S. zeamais*, and by Vedovatto et al.[28] in which 100% mortality was obtained with the application of 115 µL/essential oil plate of *Cinamodendron dinisii*. On the other hand, the results observed in this study were higher than those observed when compared to Jung et al.,[29] who concluded that *Eugenia uniflora* L. essential oil in concentrations of 1.25, 2.5 and 5% and the alcohol extract of *Melia azedarach* L. at 10% showed potential insecticide on soldiers of *Atta laevigatta* Smith.

Another aspect observed is that in the lower concentration of bioinsecticide solution (3.1%) there was no insect mortality, a result similar to the control treatment, which was composed only by deionized water (Table 1).

Essential oil solutions of *P. taeda* resulted in a median lethal concentration (LC50) in *S. zeamais* ranging from 11.24 to 12.47% in the different pH of the extraction water. These results are high when compared to studies carried out for oils extracted from other plant species such as basil (*Ocimum basilicum*) where LC50 in bean woodworm adults (*Zabrotes subfasciatus*) ranged between 0.937, 1.482 and 0.714%.[30] In another study, analyzing the oil of the *Carapa guianensis* seed (Aubl.) was observed LC50 was 4.332, 4.850 and 4.903% on the oviposition of *Rhipicephalus* (*Boophilus*) *microplus*, *Anocentor nitens* and *Rhipicephalus sanguineus,* respectively.[31] Furthermore, it should be noted that the concentration of 12.5% is equivalent to 125 mL/L, which is also considered high when compared to the data of Pinto Junior et al.[32] who evaluated the bioactivity of essential oils of *Ocotea odorifera* and *Eucalyptus viminalis* in the control of larvae and adults of mealworms (*Alphitobius diaperinus*). The authors found that larvae and adults were more susceptible to essential oil of sassafras (adult LC50 0.26 mL/L and larva 0.12 mL/L) than to eucalyptus oil (adult LC50 1.37 mL/L and larva 1.72 mL/L).

The insecticide action can be explained due to the chemical composition of the essential oil of *P. taeda*. For Tomazoni et al.[25] and Pagula and Baeckström,[33] the major compounds found for this species were α-pinene (33.94%) and β-pinene (16.73%), in addition to Δ-cadinene (8.31%) and bicyclo-germacrene (6.87%). α-pinene is a terpene with antimicrobial property.[34] As for β-felandreno, it has already been reported to have repellent activity on *Bemisia tabaci*, reducing its infestation in tomato plantations.[35] Campbell et al.[36] also pointed out the influence of this compound on the food-seeking capacity of *Aedes aegypti*.

In this study, it was observed that samples containing both needles and branches result in higher yield of essential oil extracted by hydrodistillation. As for the extraction conditions, the solvent with more acidic pH results in lower oil yield, but with greater effectiveness in the combat of *S. zeamais*. Therefore, it can be affirmed that the essential oil of *P. taeda* has an insecticidal action on *S. zeamais*, being more evident the activity in higher dosages (12.5 and 25%) after 24 hours of exposure.

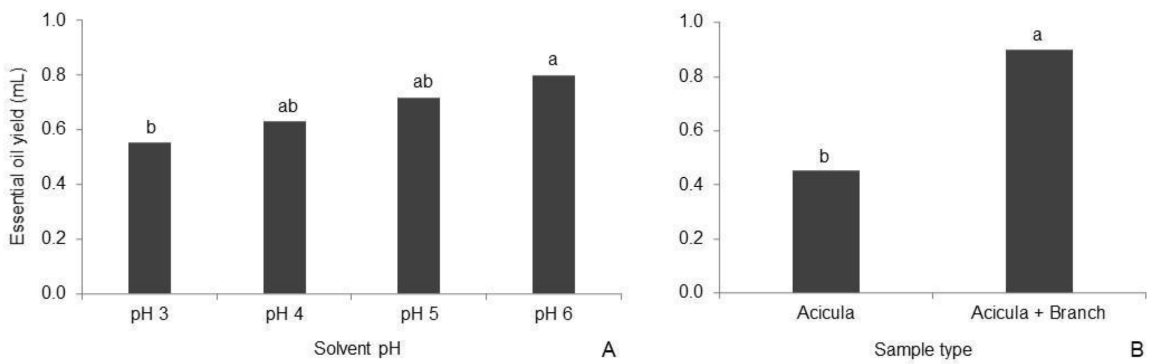

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Figure 2. Yield of essential oil extracted by steam drag using water in different pH as solvent (A) and different types of plant sample (B) of *Pinus taeda*.

Table 1. Percentage of mortality of maize weevils (*Sitophilus zeamais*) kept in insecticide solution with different concentrations of essential oil of *Pinus taeda* extracted at different pH and lethal concentration (LC50) after 24 hours

Treatments	pH 3	pH 4	pH 5	pH 6
25.0	100.0 aA*	85.0 aAB	70.0 aB	70.0 aB
12.5	65.0 bA	55.0 bA	50.0 bA	50.0 bA
6.2	0.0 cB	5.0 cB	35.0 bA	30.0 cA
3.1	0.0 cA	0.0 cA	0.0 cA	0.0 dA
0	0.0 cA	0.0 cA	0.0 cA	0.0 dA
Mean	33.0	29.0	31.0	30.0
CV (%)		21.2		
LC_{50} Value (%)	11.24	12.35	11.87	12.47
Limits 95% confidence	10.11-12.48	10.41-14.65	8.40-16.77	8.98-17.32
TSK Trim value (%)	0	15.0	30.0	30.0

Thus, the results of this work corroborate the studies that aim to analyze the efficiency of medicinal and aromatic plants for pest control and open possibilities for the development of products based on essential oils of *P. taeda*. It should be noted that the studies of natural insecticide prospecting produced by techniques that are easy to obtain, with adequate efficiency and low toxicity to the environment and to humans are extremely relevant and provide a very promising market. This is due to the fact that the use of natural insecticides is desirable in pest management programs, as they are not persistent in the environment, are generally selective for non-target insects, and have low toxicity to humans. Furthermore, it is relevant to mention that using this type of insecticide leads to reduced pest control costs, enhancing integrated management in both commercial plantings and organic cultivation.

CONCLUSIONS

The essential oil of *P. taeda* has the potential to prepare bionseticides and can be extracted from samples containing needles and branches in solvent with pH 3. The insecticide action on *S. zeamais* is more evident in higher dosages after 24 hours of exposure.

AUTHOR CONTRIBUTIONS

Conceptualization: Airton Rodrigues Pinto Junior, André Luís Lopes da Silva.

Data curation: Airton Rodrigues Pinto Junior.

Formal analysis: Kelen Haygert Lencina.

Funding acquisition: Airton Rodrigues Pinto Junior, André Luís Lopes da Silva.

Investigation: Airton Rodrigues Pinto Junior, André Luís Lopes da Silva.

Methodology: Airton Rodrigues Pinto Junior, André Luís Lopes da Silva.

Resources: Airton Rodrigues Pinto Junior, André Luís Lopes da Silva, Kelen Haygert Lencina, Micheli Angelica Horbach, Paula Kielse.

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Writing – review & editing: Airton Rodrigues Pinto Junior, André Luís Lopes da Silva, Kelen Haygert Lencina, Micheli Angelica Horbach, Paula Kielse.

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