

## INSECTICIDE ACTIVITY OF PLANT EXTRACTS OF THE NORTHEASTERN BRAZILIAN FLORA FOR THE CONTROL OF *Sitophilus zeamais* [Mots, 1885] AND *Sitotroga cerealella* [Olivier, 1819]

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### Abstract

One of the typical crops of the semiarid tropic is the maize *Zea mays* L., a rustic plant cultivated in the Brazilian northeastern semiarid region mainly by small farmers. It is one of the sources of protein and carbohydrates and an economic alternative for job creation, especially for rural populations. Among the factors limiting its cultivation are pests, among which weevil and Angoumois grain moth. This work aims to evaluate the insecticide activity of plant extracts obtained from medicinal plants of the Brazilian northeastern flora for the control of Angoumois grain moth and weevil under laboratory conditions. The methodology consisted in the production of plant powders, which were mixed with 99.8% alcohol, and then macerated and filtered. Using a rotary evaporator and applying a water bath, it was possible to separate the alcohol from the filtered solution, resulting in a creamy paste, which is the extract itself, later used in tests with insects. Preliminary tests were applied at the concentrations of 0% [control] to 100% ml of extracts. Extracts with a mortality rate  $\geq 50\%$  were analyzed by bioassays and four replicates, each consisting of a lot of five insects. The experimental design was completely randomized.

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# INSECTICIDE ACTIVITY OF PLANT EXTRACTS OF THE NORTHEASTERN BRAZILIAN FLORA FOR THE CONTROL OF *Sitophilus zeamais* [Mots, 1885] AND *Sitotroga cerealela* [Olivier, 1819]

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## Abstract

*One of the typical crops of the semiarid tropic is the maize *Zea mays* L., a rustic plant cultivated in the Brazilian northeastern semiarid region mainly by small farmers. It is one of the sources of protein and carbohydrates and an economic alternative for job creation, especially for rural populations. Among the factors limiting its cultivation are pests, among which weevil and Angoumois grain moth. This work aims to evaluate the insecticide activity of plant extracts obtained from medicinal plants of the Brazilian northeastern flora for the control of Angoumois grain moth and weevil under laboratory conditions. The methodology consisted in the production of plant powders, which were mixed with 99.8% alcohol, and then macerated and filtered. Using a rotary evaporator and applying a water bath, it was possible to separate the alcohol from the filtered solution, resulting in a creamy paste, which is the extract itself, later used in tests with insects. Preliminary tests were applied at the concentrations of 0% [control] to 100% ml of extracts. Extracts with a mortality rate  $\geq 50\%$  were analyzed by bioassays and four replicates, each consisting of a lot of five insects. The experimental design was completely randomized.*

**Keywords:** Corn weevil, Angoumois grain moth, Stored grains, Semiochemicals, Bioinsecticides.

## 1 Introduction

The Brazilian Northeast region, with an area of 1.56 million km<sup>2</sup> [18.2% of the national territory], contains most of the Brazilian semiarid region, which is the most populated semiarid climate area in the world [1]. In this region, the Caatinga is a complex of vegetal formations with several plant physiognomies [2].

According to [3], the Caatinga vegetation is extremely diverse. The number of phanerogamous species surpasses 5,000 species; 1,512 are present in the Caatinga *stricto sensu*, with at least 318 endemic species. Such biodiversity, associated with knowledge, makes this biome one of the most privileged ones as a source of resources with a bio-prospective potential.

Plants are complex living beings and, as such, have an excellent metabolism, which leads to the production of a wide variety of chemicals. Some of these substances, such as proteins, lipids, carbohydrates and nucleic acids, are common to all living beings and are used in plant growth, reproduction and maintenance [4]. However, a large number of chemical compounds produced by plants serve other purposes, for example as food deterrents and protecting plants against predators and pathogens [5].

Botanical insecticides produced using plant extracts have arisen due to the need for alternatives to the chemical control of insects that harm agricultural crops, reducing or eliminating problems of environmental contamination, food residues, harmful effects on beneficial organisms, and resistant insects [4].

Extracts are concentrated preparations of various possible consistencies obtained from dried vegetable raw materials, pre-treated or not, and prepared by a process involving a solvent. This involves two steps in the manufacturing process: the separation of specific compounds from a complex medium and the drug or part of the plant used [6].

The use of plant extracts gains momentum in agronomic science as a promising and differentiated option for integrated plant protection management. Combined with other practices, it can contribute to the reduction of doses and applications of synthetic chemical insecticides, which, among other problems, affect pollinating insect groups and the environment [7] and [8].

In Brazil, maize is one of the main agricultural crops, with extensive cultivated areas and wide use in human and animal food [9]. The importance of maize to human food varies from region to region because, in certain regions, the highest consumption of this grain and its derivatives is by low-income families. It is also traditional in the culinary of some cultures, such as in the Northeast. Worldwide, for Mexicans, for example, the use of this cereal and its derivatives in their cooking is a rich source of energy for the population [10].

Brazil ranks third in the world ranking of harvested area of corn grains, producing an average of 12 million hectares in each harvest, surpassed only by the United States and China [11].

Because it is a crop with a good adaptation to different ecosystems, Brazil is a country that has a high potential in the production of this grain. The 2011/2012 harvest was estimated at 165.92 million tons, 3.12 million tons above the previous year's crop [12]. These data confirm the importance of this culture in Brazil.

In 2012, 50.81 million hectares were planted, increasing the planted area by 1.9% over the year before, generating revenues and jobs for the country [12].

Along with the effort to increase productivity, the harvesting process and grain storage conditions must be improved. A positive feature of grains is that they can be stored for a long time without significant quality losses. However, a prolonged storage can only last when correct practices of harvesting, cleaning, drying, insect control and fungal prevention are adopted [13].

The conservation of the quality of this cereal is greatly influenced by the presence of insects, especially in tropical climates, which have ideal characteristics for development of grain mass [14]. Insects feed on maize grains, but weevil, *Sitophilus zeamais*, and Angoumois grain moth, *Sitotroga cerealella*, account for most losses [13].

Dozens of insect species are associated with maize, but relatively few have characteristics of a key pest, such as regularity of occurrence, geographic coverage and potential to cause economically significant damage [15].

Pests cause losses from the early stage of development by reducing sowing density, causing direct and indirect damage during the vegetative and reproductive phase and attacking grains and their derivatives during storage, which is an essential step in the production of high quality seeds. Otherwise, the development effort in production will be affected [16].

Dozens of insect species are associated with maize crops [6], among them corn weevil, *Sitophilus zeamais* [Coleoptera, Curculionidae], and Angoumois grain moth, *Sitotroga cerealella* [Lepidoptera, Gelechiidae], stand out. These insects may be responsible for the deterioration of stored corn quality, thus resulting in a high rate of grain loss [17].

*Sitophilus zeamais* [corn weevil] is a primary pest. The adults measure 2.0 to 3.5 mm, and are dark brown with light spots on the ellipses [anterior wings]. This species has a cross-infestation, which is the ability to infest grains both in the field and stored, with a high multiplication potential. It has many hosts such as wheat, rice, corn, barley, and triticale [LORINI, 2008].

The species *Sitotroga cerealella*, known as Angoumois grain moth, belongs to the order Lepidoptera, comprising the family Gelechiidae [18]. According to [19], it is a primary pest. The adults are butterflies measuring 10 to 15 mm. However, there is a certain polymorphism in this species: not all individuals have the same color, size and pattern. This pest attacks whole grains, which affects the surface of the grain mass. They may infest developing or maturing grains in the field, but infestations may be related to the proximity to the storage location of seeds.

The objective of this work is to analyze, by performing ethnobotanical and chemosystematics studies, medicinal plant species used by human populations in the São Francisco Valley region as possible sources for obtaining plant extracts that can be used to control insect populations attacking corn crops.

## 2 Material and Methods

The work was developed at the Animal Sciences Laboratory of the Department of Technology and Social Sciences [DTCS], campus III, of the State University of Bahia [UNEB], Juazeiro, Bahia, and at the

Chemical Laboratory of the Federal Institute of Sciences and Technology of the Sertão Pernambucano, campus Zona Rural, Petrolina, Pernambuco, Brazil.

## 2.1 Selection and drying of plants

The plants were selected by ethnobotanical studies in two relevant databases in the scientific-academic field: *Scielo* and *Lilacs*. For the search in the databases, the descriptors "botanical extracts," "natural insecticides" and "agroecology in the northeastern semiarid" were used. Eight species of plants, listed in Table 01, were used.

COMMON NAME	SCIENTIFIC NAME	FAMILY
Brazilian orchid tree	<i>Bauhinia variegata</i> L.	<i>Fabaceae</i>
Klip dagga	<i>Leonotis nepetifolia</i> R. Br.	<i>Lamiaceae</i>
Bitter melon	<i>Momordica charanthia</i> L.	<i>Cucurbitaceae</i>
Brazilian peppertree	<i>Schinus terebinthifolius</i> Raddi.	<i>Anacardiaceae</i>
Black jurema	<i>Mimosa tenuiflora</i> Willd. Poir.	<i>Fabaceae</i>
Wormseed	<i>Chenopodium ambrosioides</i> L.	<i>Amaranthaceae</i>
Lemongrass	<i>Melissa officinalis</i> L.	<i>Lamiaceae</i>
Passion fruit	<i>Passiflora cincinnata</i> Mast.	<i>Passifloraceae</i>

**Table 01:** Medicinal plant species selected from ethnobotanical studies

The plant material was collected at the UNEB/DTCS experimental area [09°24'50" S, 40°30'10" W; altitude: 368 m]. Leaves of the selected species were used because they are the most abundant plant material available. At the time of collection, phytosanitary aspects of the leaves were observed, giving preference to leaves with a good conservation status.

The collections were made using pruning pliers and scissors, trays, newspapers, leather gloves, and plastic bags.

After collection of plant species, they were taken to the laboratory, where the leaves were separated from branches, identified and put to dry at room temperature [Figure 01 A and B]. At each leaf collection, a fertile branch was collected from each plant used in the experiment. These branches were pressed, dried and identified for directing them to the UNEB/DTCS *Herbarium*.

**Figure 01:** A and B: Leaves put to dry at room temperature. UNEB/DTCS Animal Science Laboratory.



**Source:** Authors' collection [2018]

After about 20 days of collection of each completely dried plant material, they passed through a forage chopper with 5-mm blades [Figure 02]. The material was reduced to dust [Figure 03] and packed in previously sterilized containers in autoclave [Figure 04], then hermetically sealed and identified.

**Figure 02:** Electric forage mincer.



**Source:** Authors' collection [2018]

**Figure 03:** Vegetable powder from minced leaves



**Source:** Authors' collection [2018]

**Figure 04:** Wrapped vegetable powders awaiting the production of extracts



**Source:** Authors' collection [2018]

Thereafter, the extracts were prepared and the biological activity tests were conducted using the insect species of this study.



## 2.2 Production of plant extracts

At the first stage, the powder was macerated. Two-liter pots and ten-liter gallons were used. The powders were placed therein; the empty space of the container was filled with 99.8% alcohol solution, and then mixed.

After 72 hours in maceration, the powders were ready for the second stage: filtration. The decanted powder was separated from the liquid above the surface. The contents remaining in the container underwent further maceration to use most of the metabolite residues present in the plant material. The filtrate was sent to the production of ethanolic extracts. There were five macerations and five filtrations of each plant species. This stage was performed at the IF Sertão de Pernambuco Chemistry Laboratory, campus Zona Rural.

Using a rotary evaporator and a water bath, it was possible to separate the alcohol from the filtered solution, resulting in a creamy paste, which is the extract itself, later used in tests with insects.

Solubility tests were performed with different reagents for each species. For this, rods and test tubes were used together with each reagent individually. The entire procedure was performed in a flow chamber, as it may be toxic to humans.

The reagents tested were methyl alcohol and chloroform. Thus, we identified the most suitable reagent [Table 2], i.e., the one that best solubilized the extract of each species, leaving only the extract in its purest form ready to be diluted at different concentrations.

**Table 02:** Reagents used in the solubilization of plant extracts, indicating the best solubilization.

	<b>Methyl alcohol</b>	<b>Chloroform</b>
Brazilian peppertree [ <i>Schinus terebinthifolius</i> Raddi]	X	
Klip dagga [ <i>Leonotis nepaetefolia</i> R. Br.]		X
Lemongrass [ <i>Melissa officinalis</i> L.]	X	
Black jurema [ <i>Mimosa tenuiflora</i> Willd. Poir.]		X
Passion fruit [ <i>Passiflora cincinnata</i> Mast]		X
Wormseed [ <i>Chenopodium ambrosioides</i> L.]		X
Bitter melon [ <i>Momordica charantia</i> L.]		X
Brazilian orchid tree [ <i>Bauhinia variegata</i> L.]		X

## 2.3 Insect Selection and Breeding

The adult insects of *Sitophilus zeamais* were obtained from the corn storage shed of the Animal Science Laboratory of the Federal University of São Francisco Valley [UNIVASF] from stored corn bags. The insects were placed in 250-ml glass jars and in nylon and plastic bags kept at the DTCS III/UNEB Animal Science Laboratory Insectary inside glass jars with corn available, so that insects were multiplying at a mass breeding level.

The adult insects of *Sitotroga cerealella* were obtained from pest-infested sheds at the Animal Science Laboratory of the Federal University of Vale do São Francisco [UNIVASF]. The insects were placed into nylon plastic bags. There was no multiplication and mass breeding of insects.

## 2.4 Laboratory Bioassays

The extracts were diluted to provide 200 ml of solution. Immediately after dilution, solutions were prepared at 0% [control], 25%, 50%, 75% and 100% for insect application. The extracts that presented a mortality rate  $\geq 50\%$  were considered as having a great lethal capacity [LC]

### 2.4.1 *Sitophilus zeamais*

In the laboratory, Petri dishes were prepared by autoclaving. Corn and ten insects were deposited per plate. Each experimental treatment received five different concentrations of extracts from each plant. The plates were sprayed with the solutions, and sealed with PVC film paper. They were then placed in a completely randomized BOD [biochemical oxygen demand] chambers at  $25 \pm 2^\circ\text{C}$  and relative humidity of  $40 \pm 5\%$ .

### 2.4.2 *Sitotroga cerealella*

The preliminary bioassays were separated into plots consisting of five Petri dishes per plant species containing five adult insects. The plates were sprayed with the test solutions, capped, labeled and transferred to an incubator oven under controlled temperature and humidity, and observed for 48 hours. The experimental design was completely randomized.

## 3 Results

### 3.1 Control of *Sitophilus Zeamais*

The results show that all medicinal plants tested showed insecticidal activity, with a mortality higher than 80% [Table 03], with the exception of Brazilian orchid tree, whose lethality did not reach 50%.

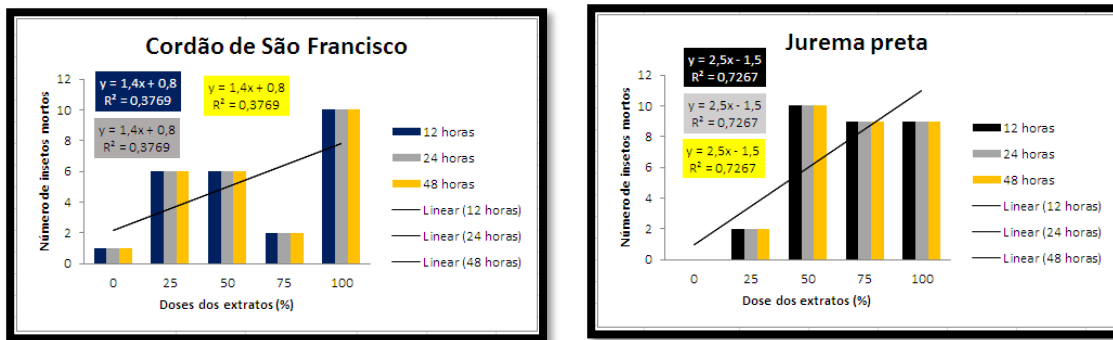
**Table 03:** Mortality/Insecticide activity of medicinal plant extracts against *Sitophilus Zeamais*

MEDICINAL PLANTS	MORTALITY RATE [%]				
	0%	25%	50%	75%	100%
Brazilian peppertree [ <i>Schinus terebinthifolius</i> Raddi]	0%	30%	60%	90%	90%
Klip dagga [ <i>Leonotis nepaetefolia</i> R. Br.]	0%	60%	60%	20%	100%
Lemongrass [ <i>Melissa officinalis</i> L.]	0%	40%	40%	80%	70%
Black jurema [ <i>Mimosa tenuiflora</i> Willd. Poir.]	0%	20%	100%	90%	90%
Passion fruit [ <i>Passiflora cincinnata</i> Mast]	0%	10%	40%	50%	100%
Wormseed [ <i>Chenopodium ambrosioides</i> L.]	0%	40%	70%	50%	70%
Bitter melon [ <i>Momordica charantia</i> L.]	0%	10%	70%	90%	100%
Brazilian orchid tree [ <i>Bauhinia variegata</i> L.]	0%	20%	30%	30%	40%

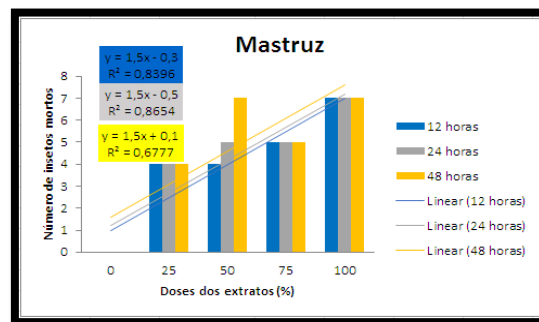


At the concentration 25%, the klip dagga extract [Figure 05 A] was the only one that had a lethality higher than 50%. For the concentration 50%, the best result was presented by black jurema extract [100% lethality, Figure 05 B], followed by wormseed [Figure 06] and bitter melon, which reached a 70% lethality, and klip dagga and Brazilian peppertree, both with a 60% lethality.

**Figure 05 A, and 05 B:** Results for efficiency of klip dagga and black jurema in laboratory bioassays for the control of *Sitophilus Zeamais*.

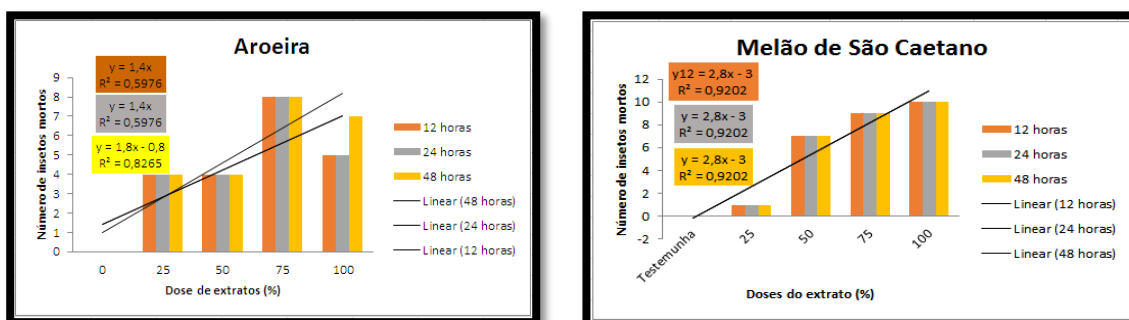


**Figure 06:** Results for efficiency of wormseed in laboratory bioassays for the control of *Sitophilus Zeamais*.



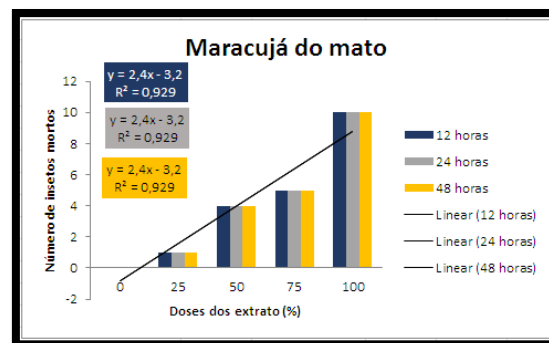
For the concentration 75%, Brazilian peppertree [Figure 07 A], black jurema [Figure 05 B] and bitter melon [Figure 07 B] obtained a 90% efficiency in the control of *S. zeamais*.

**Figure 07 A, and 07 B:** Results for efficiency of Brazilian peppertree and bitter melon in laboratory bioassays for the control of *Sitophilus Zeamais*.



At the concentration 100%, with the exception of Brazilian orchid tree [40%], all other extracts had their lethal capacity above 50%, especially the klip dagga [Figure 05 A], passion fruit [Figure 08] and bitter melon [Figure 07 B], which had 100% of efficiency. However, we do not recommend the use of extracts at high concentrations if it, at a lower concentration, obtains satisfactory results, such as klip dagga [Figure 05 A], which has a lethality at 25% and 50%. We emphasize that, in the experimental field of DTCS III/UNEB, the cultivation of this species, which presented a low local population, is recommended.

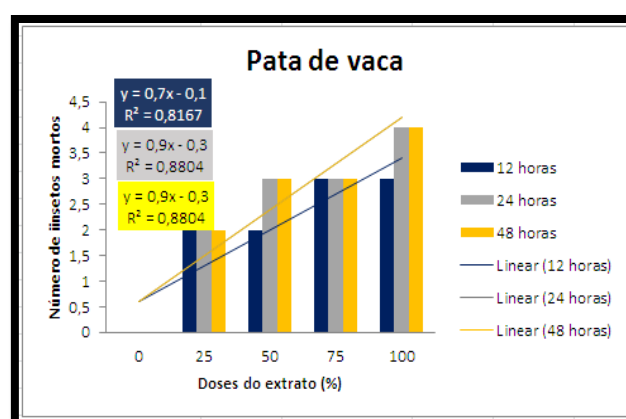
**Figure 08:** Results for efficiency of passion fruit in laboratory bioassays for the control of *Sitophilus Zeamais*.



The bitter melon [Figure 07 B] showed a great efficacy at the concentration 50% [with a 70% mortality] and at the concentration 75% [90% lethality], making its use more economical at these concentrations.

The lowest mortality rate was presented by Brazilian orchid tree [Figure 09] at the tested concentrations.

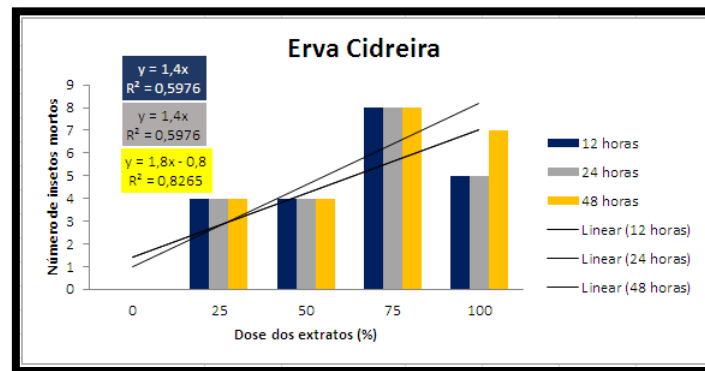
**Figure 09:** Results for efficiency of Brazilian orchid tree in laboratory bioassays for the control of *Sitophilus Zeamais*.



In general, the efficiency of insecticide activity increases as the concentration increases, as observed for lemongrass [Figure 10], passion fruit [Figure 08], Brazilian peppertree and bitter melon [Figures 07 A and B, respectively]. However, some species decrease their efficiency at the concentration 100%, such as black jurema [Figure 05 B], which obtained a better efficiency at the concentration 50% [100% lethality].

It is considered in this experiment as the most suitable species for the control of *S. zeamays* since it had a higher mortality rate at lower concentrations.

**Figure 10:** Results for efficiency of lemongrass in laboratory bioassays for the control of *Sitophilus Zeamais*.



Importantly, the usefulness of plants for insect control is not limited to the use of the substances obtained from them or their extracts. These active substances can usually be used for the synthesis of new active ingredients. This knowledge has aided in the development of insect control methods less aggressive to the environment.

### 3.2 Control of *Sitotroga cerealella*

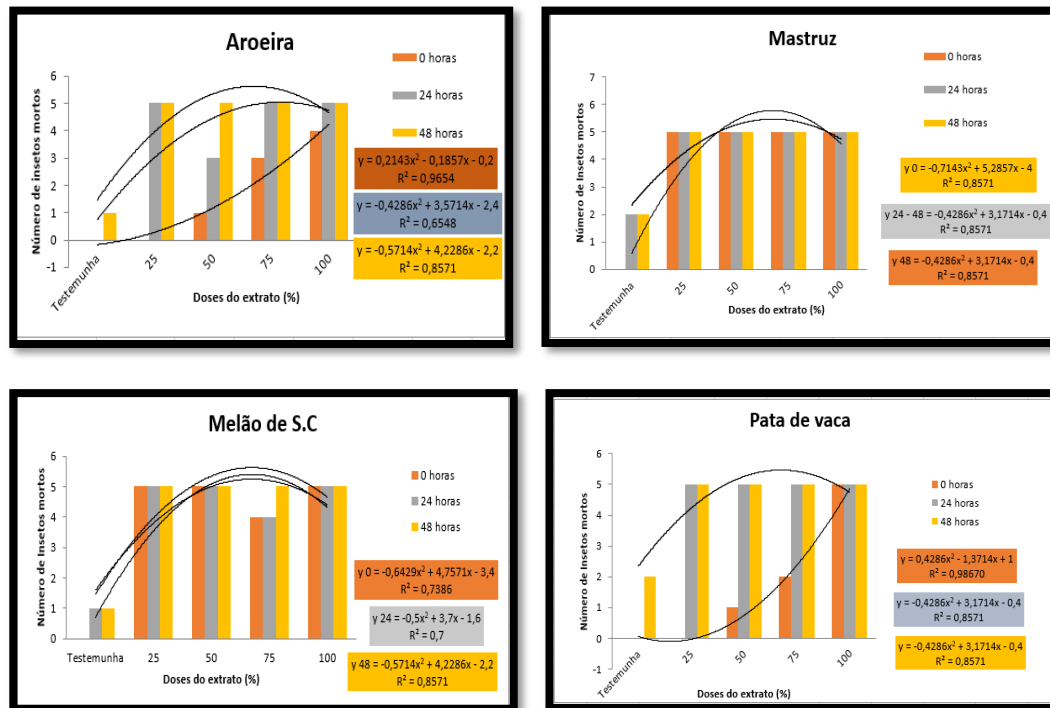
The results show that all medicinal plants tested showed insecticidal activity, with a mortality higher than 60% [Table 04].

**Table 04:** Mortality/Insecticide activity of medicinal plant extracts against *Sitotroga cerealella*

MEDICINAL PLANTS	MORTALITY RATE [%]				
	0%	25%	50%	75%	100%
Brazilian peppertree [ <i>Schinus terebinthifolius</i> Raddi]	0%	100%	100%	100%	100%
Klip dagga [ <i>Leonotis nepaetefolia</i> R. Br.]	0%	100%	60%	80%	100%
Lemongrass [ <i>Melissa officinalis</i> L.]	0%	60%	100%	100%	100%
Black jurema [ <i>Mimosa tenuiflora</i> Willd. Poir.]	0%	60%	100%	100%	100%
Passion fruit [ <i>Passiflora cincinnata</i> Mast]	0%	80%	80%	100%	80%
Wormseed [ <i>Chenopodium ambrosioides</i> L.]	0%	100%	100%	100%	100%
Bitter melon [ <i>Momordica charantia</i> L.]	0%	100%	100%	100%	100%
Brazilian orchid tree [ <i>Bauhinia variegata</i> L.]	0%	100%	100%	100%	100%

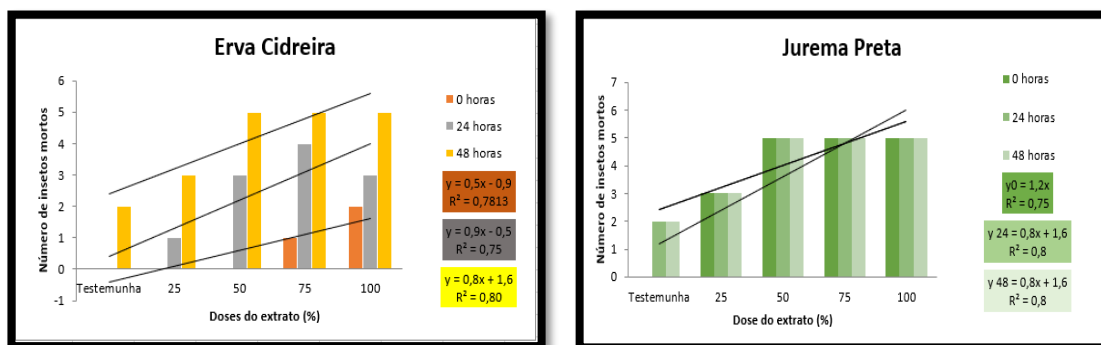
Brazilian peppertree, wormseed, bitter melon and Brazilian orchid tree [Figures 11 A, B, C and D] had a better performance at 25%, 50%, 75% and 100% concentrations for the control of *S. cerealella*. For these species, we recommend the use of extracts at the concentration 25% because they have maximum lethality and low cost.

**Figure 11 A, B, C and D:** Efficiency results of Brazilian peppertree, wormseed, bitter melon and Brazilian orchid tree in laboratory bioassays for the control of *S. cerealella*.



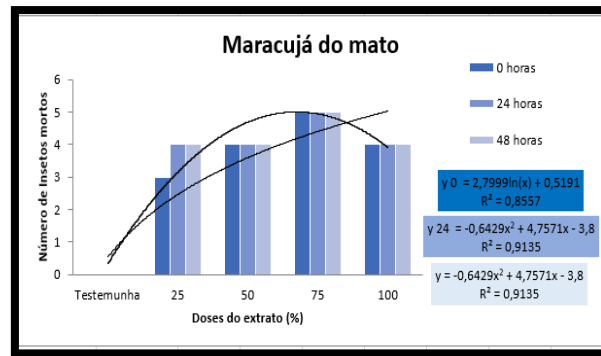
The lowest mortality rate recorded was 60%, presented by lemongrass and black jurema [Figure 12 A and B] at the concentration 25%.

**Figure 12:** Efficiency Results of **A:** Lemongrass and **B:** black jurema in laboratory bioassays for control of *S. cerealella*.



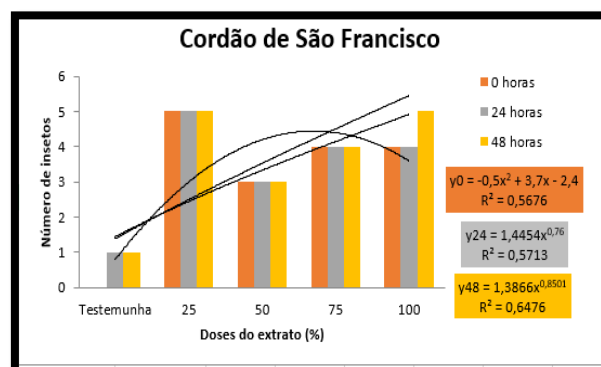
In general, the efficiency of insecticide activity increases as the concentration increases, as observed for Brazilian peppertree [Figure 11 A], lemongrass and black jurema [Figure 12 A and B]. However, some species decrease their efficiency at high concentrations, such as passion fruit [Figure 13], which obtained only 80% effectiveness [pure extract = 100%] and 100% lethality [at a concentration of 75% of extract].

**Figure 13:** Results for efficiency of passion fruit in laboratory bioassays for the control of *S. cerealella*.



The klip dagga [Figure 14] is 100% effective at the concentration 25%, and it is an economically viable and effective species for controlling *S. cerealella*.

**Figure 14:** Results for efficiency of passion fruit in laboratory bioassays for the control of *S. cerealella*.



The use of plants for insect control is not limited to the use of the substances obtained from them or their extracts. These substances can and should be used for the synthesis of new active principles, helping to control insect populations aggressive to various agricultural crops.

Our results prove the effectiveness of plant extracts that have been researched and largely with already proven effects for pest control [20]. The insecticide activity of plant extracts can be manifested through direct mortality, repellency, sterility, interference in development, and modification of arthropod behavior [21].

## 4 Conclusions

The continuous growth of the world population increases the demand for food. As a result, it is urgent to reduce losses in the different stages of food production, especially losses resulting from the action of insects at either the production and/or storage [22] and [23]. Insect control techniques still largely use agrochemicals, which are quick solutions to increase production, but without taking into account the harmful effects of these substances on living organisms and the environment. However, currently, when

designing an integrated pest management, a set of control methods and strategies are used to reduce losses due to the biological action of insects [24].

One of such strategies is the identification of plants that can be used as bioinsecticides. They can control the growth of insect populations in addition to causing less side effects and being economically viable.

Medicinal plants, as they are an abundant and easily accessible natural resource, can and should be used to obtain natural insecticides as an alternative to synthetic insecticides. The advantages are a low production cost, which makes them more affordable for small producers, and the non-cumulative effects on food and the environment, promoting the isolation of active ingredients that allow the synthesis of new phytosanitary products.

Thus, studies highlighting the use of plant species that have metabolites capable of mitigating impacts caused by pests on grains are important because they allow obtaining better bioinsecticides [25]. Thus, the production of extracts in this study aimed to find new ways to control *Sitophilus Zeamais* and *Sitotroga cerealella* in order to increasingly mitigate losses resulting from the action of this pest, reducing pest control costs and consequently increasing the profit margin for the producer by providing a bioinsecticide as a tool more environmentally friendly and more efficient in insect control.

Plants with a high potential can also be used for the production of different compounds, such as vegetable oils. According to studies on other plant species, they have a high potential for the control of various insects, producing natural products that, after field and laboratory testing, can be marketed at competitive prices [26] and [27]. This work aims a continuity of studies and the use of the same species studied for the production of extracts for the control of fungi and pathogenic bacteria. It also aims the production of vegetable oils following the same line of research.

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