



Natural Products: Are They Efficient Alternatives against the Stored Grain Pest *Sitophilus zeamais*?

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cereal crops strongly contribute with the world economy and because of this the reduction of their productivity may lead to irreparable economic consequences worldwide. The maize weevil *Sitophilus zeamais* is the main pest of maize in the field or during processing, storage and commercialization of grains, also attacking processed food. The population control of *S. zeamais* uses a restrict panel of synthetic insecticides which are associated with environmental contamination, selection of resistant individuals and toxicity to non-target organisms, including humans and other animals. This scenario has stimulated the search for new insecticides and plant metabolites stand out because of their high degree of biodegradability and more selective toxicity. This work provides a review of the effect of plant compounds on *S. zeamais* to encourage the use of these ingredients in more ecofriendly strategies for pest control. Plant insecticides can exert their

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toxicity by ingestion, contact and/or fumigation resulting in damage to the development, survival and reproductive potential of *S. zeamais*, or may present food deterrent activity, which protects the substrates from the attack by the insects. The data reviewed contribute to consolidate the use of natural products in control of *S. zeamais*.

Keywords: Maize weevil; natural insecticides; plant metabolites.

1. INTRODUCTION

Cereal grains such as corn, wheat and rice represent important components of basic diets for most people around the world. So, their production accounts for a significant portion of the world economy [1]. The reduced productivity or poor quality during storage due to the presence of insect pests may lead to irreparable economic consequences [2,3].

Sitophilus zeamais (Coleoptera: Curculionidae), the maize weevil, represents a primary pest adapted to warm and preferentially tropical environments where it stands out as one of the main corn pests in the field or in post-harvest processing, storage or commercialization. It also attacks processed food including macaroni and cookies [4-7].

The *S. zeamais* females damage cereal grains by using their rostrum perforating a cavity to deposit a single egg and cover it with a gelatinous material synthesized by them. The

larvae hatch from the eggs and go through four instars to become prepupae to pupae, from which the adults emerge (Fig. 1) [8-10].

In addition to the direct damage to grains, infestation by *S. zeamais* can facilitate the entry of mites, other insects or fungi because of the increase of temperature and humidity, resulting in the drastic reduction in viability, nutritional and market values, food security and germination capacity of the grains [11-15].

The population control of *S. zeamais* has used synthetic insecticides that include bifenthrin and fenitrothion [16-17]. Their indiscriminate use has been associated with serious soil and water contamination, selection of resistant individuals, persistence in the environment and in the cereal grains, as well as toxicity to non-target organisms, including humans and other animals [18,-21]. There are several reports on resistance of *S. zeamais* against recommended active chemical ingredients available in the market [22].

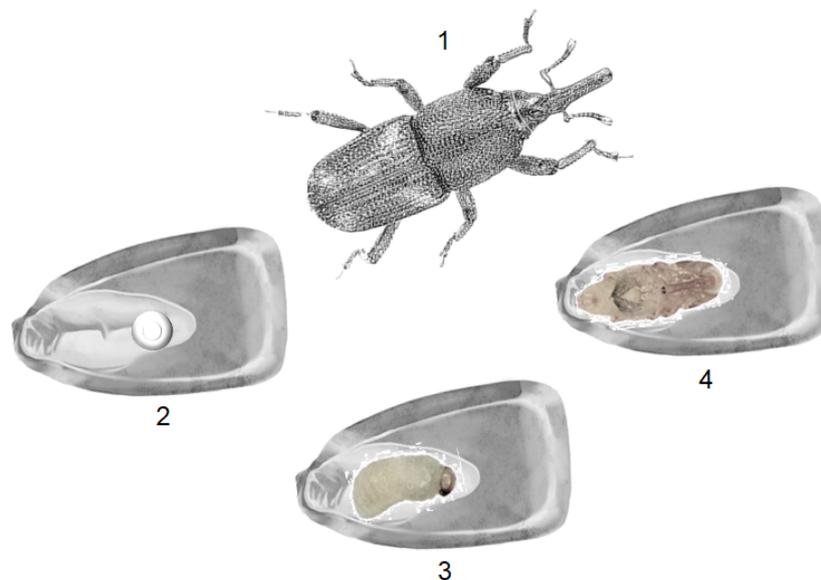


Fig. 1. Life stages of *S. zeamais*: 1. Female 2. Egg 3. Larva 4. Pupa

The consequences of using a restrict panel of synthetic insecticides have driven the search for new agents to control *S. zeamais*, with emphasis on plant compounds such as aqueous extracts, essential oils and isolated ingredients that generally have a higher degree of biodegradability, and show a more selective toxicity [23-29]. This work provides a review of the effect of these products on *S. zeamais* to encourage their uses in more ecofriendly strategies for pest control. Plant compounds with insecticidal activity can exert their toxicity by ingestion, contact and/or fumigation, or food deterrent activity. The Fig. 2 presents a scheme of procedures for investigating these effects, which will be discussed below [1,8,17,24,28,30].

2. INSECTICIDAL ACTIVITY OF NATURAL PRODUCTS AGAINST *S. zeamais*

2.1 Toxicity by Ingestion

There are many reports of plant preparations that exert toxicity post ingestion to *S. zeamais* (Table 1). Methods for assessment of toxicity by ingestion (Fig. 2A) can use natural food, such as

maize grains soaked with the sample, or synthetic seeds, made with wheat flour [30]. These assays monitor the insect survival and determinate mortality rates as a parameter to reflect an acute effect. The occurrence of chronic effect can also be monitored in these assays through nutritional indices that include the biomass gain rate (BGR); The Relative Consumption Rate (RCR) and the Efficiency in Conversion of Ingested Food (ECIF); BGR reflects the daily increase of insect biomass, RCR indicates the amount of food daily ingested per milligram of biomass, and ECIF represents how much the food mass ingested by insects is converted into biomass [31].

It was reported that the ingestion of a saline extract of *Schinus terebinthifolia* leaves at 100, 200 and 250 mg/g during 07 days caused mortality rates of 32%, 40% and 51% of *S. zeamais* adults, respectively [30]. Twenty days after treatment, all the insects that ingested the *S. terebinthifolia* leaf extract were dead and the authors attributed this effect to the flavonoid and gallic acid content of the extract.

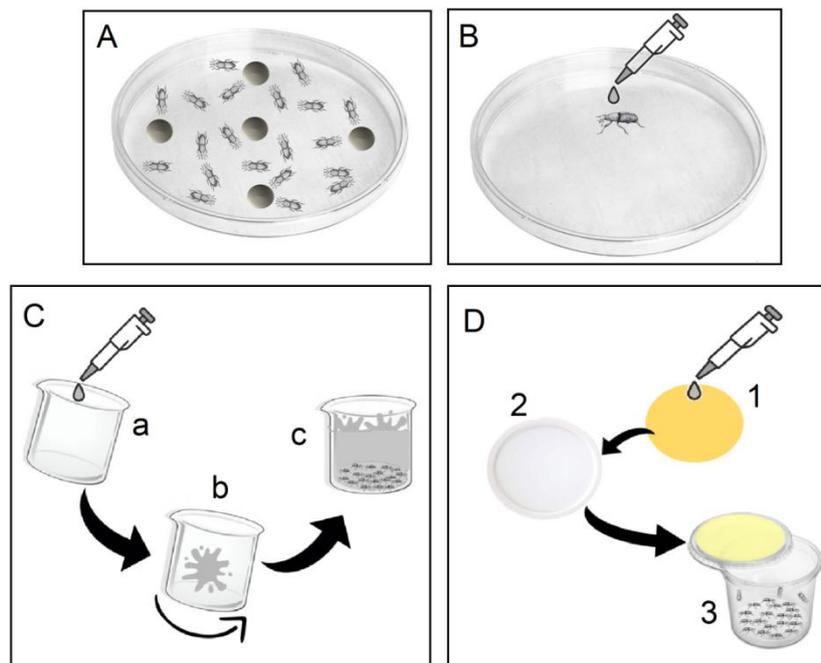


Fig. 2. Outline of the procedures used to investigate the toxicity of plant compounds by ingestion (A), where natural or artificial grains are impregnated with a sample for further analysis of insect survival and nutritional parameters; contact (B) by applying the sample directly to the surface of insects body; and fumigation (C) with the application of the sample directly on the inner surface of a container and subsequent conditioning of the insects, or (D) with impregnation of the sample in filter paper placed on the lid of the container that will receive the insects, remaining closed

An artificial diet containing the water extract of *Moringa oleifera* seeds at 60, 115, 245 and 640 mg/g caused mortality of 21.7%, 40.0%, 55.0% and 82.5% of adult *S. zeamais*, respectively [1]. After 7 days of the treatment, a LC₅₀ value (concentration that kills 50% of the insects) of 214.6 mg/g was recorded for the extract, which stimulated α -amylase and endoglucanase activity from the insect midgut. After a phytochemical characterization of the extract, the authors attributed its insecticidal effect to the presence of rutin and ellagic acid. The lectin (carbohydrate binding protein) isolated by the *M. oleifera* seed extract (WSMoL, *M. oleifera* water soluble lectin) didn't show acute effect, but reduced TGB and ECAI and stimulated trypsin activity from the insect midgut.

The essential oil of *Croton rudolphianus* leaves killed *S. zeamais* adults with a LC₅₀ of 102.66 μ L/g, recorded after 7 days of the insect exposition [17]. The ether extract of the *Ramalina complanata* lichen (12 mg extract/g of diet) containing divarictic acid caused mortality of 33.33 \pm 11.55 % of *S. zeamais* adults, while the isolated divarictic acid (12 mg/g) killed 52.5 \pm 9.57% of the insects [32]. The authors also described that the extract and the divarictic acid caused a reduction of insect biomass by impairing the conversion of ingested food.

2.2 Contact Toxicity

The contact toxicity can be investigated by applying the product directly to the insect body surface (Fig. 2B), or to the inner surface of a container (Fig. 2C) where the insects are maintained [33, 24]. The insect survival is monitored and, if there is contact toxicity, the death may occur after a short incubation period, usually 24 to 72 h. The results are generally expressed as an average lethal dose (LD₅₀). Values lower than the LD₅₀, referred to as sublethal concentrations, despite not causing immediate death, can be used in the investigations aiming to determine the consequences of a chronic effect [34].

Some plant preparations toxic by contact to *S. zeamais* are shown in Table 1. The essential oil of *Lippia sidoides* leaves containing 68.5% of thymol killed *S. zeamais* adults after 72 h of being applied in prothoracic region of the insects from Maracaju/MS (LD₅₀ of 7.10 μ g/mg) or Jacarezinho/PR (LD₅₀ of 19.93 μ g/mg) Brazilian municipalities [35], while the isolated Thymol reduced the survival of the insects from Maracaju

and Jacarezinho with LD₅₀ values of 17.08 μ g/mg and 25.71 μ g/mg, respectively. The authors also evaluated nanoformulations containing 18% of the oil or isolated thymol, which killed the insects of Maracaju (LD₅₀ 26.44 μ g/mg and 20.75 μ g/mg, respectively) and Jacarezinho (LD₅₀ of 35.96 μ g/mg and 27.71 μ g/mg, respectively). In addition, the nanoformulations remained active after being stored for seven months, provided controlled release of the active principles and increased their solubility.

Essential oils of *Annona senegalensis* leaves, *Hyptis spicigera* flowers and *Lippia rugosa* flowers at concentrations ranging from 0.1 x 10⁻² to 5 x 10⁻² mL/mL were toxic by contact to *S. zeamais*, and the mortality rates ranged from 68 to 100 % (*H. spicigera*), 50% to 99% (*L. rugosa*) and 51% to 100% (*A. senegalensis*) after 24 h of treatment [36]. The insects that survived after contact with the essential oils showed reduced fertility, since the average number of eggs laid by females during 10 days was 5.4, 2.2 and 1.6 for insects treated with essential oils of *A. senegalensis*, *H. spicigera* and *L. rugosa*, respectively, while in the control an average of 18.8 eggs was recorded.

The *Croton rudolphianus* leaf essential oil (0.75 to 75 μ L/mL) containing methyl chavicol (22.96%), eugenol (4.03%), (E)-caryophyllene (4.22%), bicycloelemene (3.96%), bicyclogermacrene (3.81%) and spathulenol (2.79%) interfered with the survival of *S. zeamais* adults in a dose-dependent way when applied in the insects chest, and the LC₅₀ was 70.64 μ L/mL [17]. Methyl chavicol and eugenol were toxic to *S. zeamais* with LC₅₀ of 76.1 and 186.2 μ g/cm², respectively [37]. The essential oil of *Lippia javanica* leaves, its constituents perylaldehyde and linalool, as well as a mixture of perylaldehyde and linalool at 10 mg/mL caused 85%, 99.5%, 100% and 100% mortality of *S. zeamais*, respectively [38].

The oil of roasted, raw or cooked seeds of *Jatropha curcas* containing palmitic acid as the major constituent (54 to 86%) killed *S. zeamais* adults after contact with mortality rates of 47.52%, 47.36% and 46, 96%, respectively after 3 h of starting the experiment [39].

Anethum graveolens seed extract also reduced the survival of *S. zeamais* with LD₅₀ of 121.5 μ L/plate [40]. The authors believed that the insecticidal ingredients in the extract were dilapiol (14.4%), limonene (16.6%) and carvone

(55.2%). The same study reported a LD₅₀ of 325.4 µL/plate to an *Azadirachta indica* seed extract toxic by contact.

The characterization of the extract of *Ola x zeylanica* leaves showed as the main constituents thyrane (24.7%) and bis(methylthio)methane (BMTM) (75.3%) [41]. The authors showed that adults of *S. zeamais* incubated with the extract (0.5%, v/v) died at rates of 60% and 83% after 3 and 12 h, respectively; when the extract (0.5%, v/v) was impregnated into corn kernels, mortality rates were similar (59.0% and 77.0% after 3 and 12h, respectively). The role of organosulfur BMTM in plant defense against a wide range of pest insects is well known [42].

2.3 Fumigation Toxicity

The toxicity of plants by fumigation to *S. zeamais* has been reported (Table 1). A method similar to that showed in Figure 2D revealed that the essential oil of *C. rudolphianus* (64µL/L) caused 43.75% mortality of *S. zeamais* adults [17]. The essential oil from *J. curcas* seeds (200 µL/L of air) roasted, cooked or raw also caused mortality of 84.68%, 15.64% and 38.8% of *S. zeamais*, respectively, after 12 hours of exposure [39]. *S. zeamais* adults also showed poisoning by fumigation (LC₅₀ of 80.01µL/L of air) after exposure to essential oil from the peel of *Citrus sinensis* fruits, containing mainly limonene (59.3%), terpineol (8.31 %), linalool (6.56%), citronellol (6.21%) and citral (1.15%) [43]. Among these active ingredients, the most toxic was citral with a LC₅₀ of 2.02µL/L of air. The ether extract of the *Ramalina complanata* lichen and the divarictic acid isolated from it were also toxic to adults of *S. zeamais* after 7 days of exposure. The effect of divarictic acid was dose dependent and the LC₅₀ was 1.6 mg/L of air [32].

2.4 Food-deterrent Effect

The data obtained in a methodology similar to that used to assess the toxicity by ingestion (Figure 2A) allow determining the occurrence of food deterrence and the calculation of the food deterrence index (IDA) through the equation: $IDA = [100 \times (C-T)]/C$, where C corresponds to the mass of ingested food by the insects from control and T corresponds to the mass of ingested food by the test group [44]. Food deterrent activity is the ability of a sample to discourage, inhibit and even block food intake, and this sample can be classified according to the IDA as no deterrent

(IDA < 20%), weakly deterrent (50% > ADI ≥ 20%), moderately deterrent (70% > ADI ≥ 50%) or strongly deterrent (IDA ≥ 70%) [45].

The saline extract of *Myracrodruon urundeuva* leaves (10 to 150 mg/g) had a weak to moderate food deterrent effect to *S. zeamais*, while the lectin isolated from it (MuLL; 3 to 150 mg/g) was a moderate to strong deterrent agent [46]. Other preparations reported at the international literature as strong food deterrents to *S. zeamais* (IDA: 91.5%) are the water extract of *M. oleifera* seeds (640 mg/g) containing ellagic acid and rutin and the saline extract of leaves of *S. terebinthifolia* (250 mg/g) containing gallic acid and flavonoids, with an IDA of 91.5% and 100%, respectively [1, 30]. The extract of *Abarema cochliocarpos* bark at 500mg/g, which contained gallic acid and ellagic acid, showed a strong deterrent effect (IDA > 90%) [47].

3. ADVANTAGES AND DISADVANTAGES OF USING NATURAL PRODUCTS FOR INSECT CONTROL

The data reviewed in this manuscript suggest that the use of natural products (extracts, essential oils, secondary metabolites and insecticidal proteins) emerges as a differentiated and promising option for integrated pest management, because it can contribute to the reduction of synthetic insecticide applications, which when overused, pose problems to non-target organisms and environment. In this sense, the application of natural products has a set of advantages that include their low toxicity to non-target organisms and the usually higher degree of biodegradability (Fig. 3).

Some natural compounds still have a very effective acute action, inducing insect death a few hours after the exposure. Additionally, natural products can be incorporated into formulations to promote: 1. Controlled release of the active ingredients, 2. Reduction of lethal concentrations, 3. Greater stability to environmental conditions and 4. Greater safety in handling of preparations.

On the other hand, the use of natural products as insecticides can present disadvantages (Fig. 3), among which the difficulty of acquiring the products on a large scale stand out, which can be solved through heterologous expression (production of proteins through recombinant DNA technology), when these compounds are insecticidal proteins. Furthermore, due to lower

Table 1. Natural compounds (essential oils, extracts and isolated ingredients) with insecticidal activity against *S. zeamais*.

Preparations	Effect	Reference
Essential oils <i>Annona senegalensis</i> leaves, <i>Hyptis spicigera</i> flowers and <i>Lippia rugosa</i> flowers <i>Croton rudolphianus</i> leaves	Chronic by contact. Mortality ranging from 50 to 100% or reduced fertility	Ngamo et al. [36]
<i>Lippia javanica</i> leaves <i>Jatropha curcas</i> seeds	Chronic by ingestion, contact and fumigation. (LC50 ranging from 70.6 to 102.66 $\mu\text{L/g}$) Acute by contact. 85% mortality after 48 h at 10mg/mL Acute by contact. 47% mortality after 3h at 1.50 $\mu\text{L/cm}^2$; Acute by fumigation. 84.68% mortality after 24 h at 200 $\mu\text{L/L}$	Ribeiro et al. [17] Kamanula et al. [38] Babarinde et al. [39]
<i>Citrus sinensis</i> bark	Acute by fumigation. LC50 of 80.01 $\mu\text{L/L}$	Oyedeji et al. [43]
Extracts <i>Anethum graveolens</i> and <i>Azadirachta indica</i> seeds	Acute by ingestion. LD ₅₀ = 121.5 $\mu\text{L/placa}$ and LD ₅₀ of 325.4 $\mu\text{L/plate}$	Albiero et al. [40]
<i>Ramalina complanata</i> lichen	Chronic by ingestion. Mortality of 33.33% at 12 mg /g after 07 days	Silva et al. [32]
Isolated ingredients <i>Olex zeylanica</i> leaf bis(methylthio)methane <i>Ramalina complanata</i> divarictic acid	Acute by contact. 83.0% mortality after 12h at 0.5%, v/v Acute by fumigation. LC50 of 1.6 mg/L of air	Perera et al. [41] Silva et al. [32]

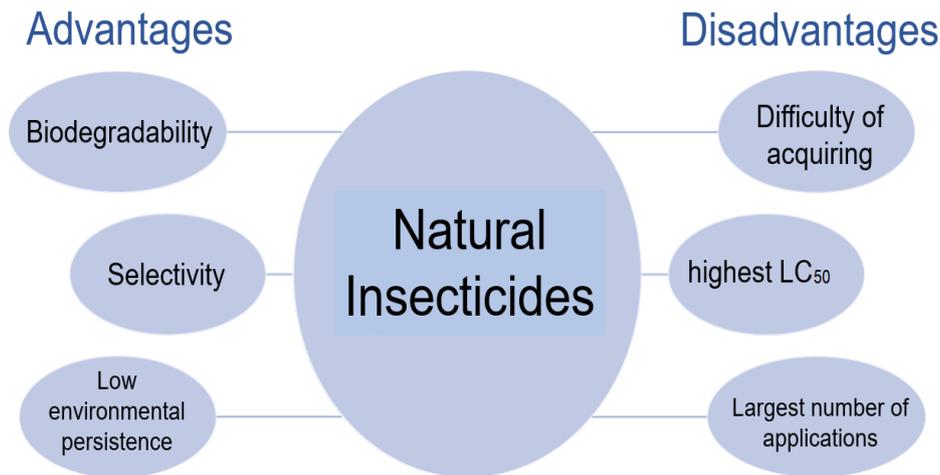


Fig. 3. Advantages and disadvantages of using natural products for insect control

environmental persistence, the use of natural products may require a greater number of applications when compared to synthetic ingredients. On the other hand, the low persistence and multiplicity of mechanisms of action can make natural products less likely to promote resistance or tolerance in pests.

4. CONCLUSION

According to the data reviewed herein, we can assume that natural products represents promising alternatives against the stored grain pest *S. zeamais* and this work can contribute to consolidate the use of these ingredients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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