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EFFECTS OF INSECTICIDAL EFFICACY OF *SECURIDACA LONGEPEDUNCULATA* (POLYGALACEAE) POWDER ROOT BARK EXTRACTS AGAINST *SITOPHILUS ZEAMAIS* (CURCULIONIDAE) FOR PEST CONTROL IN THE FAR NORTH REGION OF CAMEROON

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ABSTRACT

Currently, farmers are misusing synthetic pesticides to control stored food pests. These pesticides have harmful effects both for the consumers and for the environment. When harvesting their cereals, some farmers use several methods to reduce pest pressure during storage. This study investigated the use of powdered formulations of the bark of *Securidaca longepedunculata* combined with the fruit mantis of *Acacia nilotica* against *Sitophilus zeamais*. For this purpose, a granulometric study of powder particles was performed to determine the sieve mesh intervals in order to obtain different powdery extracts. These extracts, at varying proportions, were each used to formulate bioinsecticides that were assessed with respect to insecticidal activity on the different developmental stages of *S. zeamais*. Furthermore, an acute toxicity study of the most promising aqueous extract was carried out on rats. The granulometric particles size analysis permitted selection of the particle size between 400 and 100 µm. The findings of the qualitative characterization of the secondary metabolites from the different fractions showed the presence of total polyphenols, flavonoids, tannins, alkaloids and saponins. Each formulated fraction at different doses (3, 5 and 8 g/kg) resulted in mortality rates (60, 80 and 90%) that were higher than that of the negative control (without *S. longepedunculata* powder, 5.2%) but lower than positive control (treated with the reference pesticide, 100%) at a significant difference of ($P < 0.05$). The insecticidal activity was dose-dependent and time-dependent. The large particles induced lower mortality compared to the small particles. However, the 8 g/kg dose of the 100 and 200 µm fraction resulted in higher mortality rates (86.2-90%) during the experimental periods (5, 35 and 90 days). No clinical signs of toxicity or mortality of rats, as well as no changes in body weight, were observed with *S. longepedunculata* root bark aqueous extract at a dose of 2000 mg/kg. This study suggests the potential use of the formulated powder from the roots of *S. longepedunculata* as an alternative to synthetic insecticides in the fight against *S. zeamais*.

Key words: *Securidaca longepedunculata*, *Acacia nilotica*, *Sitophilus zeamais*, synthetic pesticides, toxicity

Citation: **Bayang WS, Ngamo TLS and A Goudoum** Effects of Insecticidal Efficacy of *Securidaca longepedunculata* (Polygalaceae) Powder Root Bark Extracts Against *Sitophilus zeamais* (Curculionidae) for Pest Control in the far North Region of Cameroon. *Afr. J. Food Agric. Nutr. Dev.* 2026; **26(2):** 28740-28759. https://doi.org/10.18697/ajfand.149.26115

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INTRODUCTION

The agricultural sector remains one of Cameroon's engines of growth aimed at diversifying its economy and strengthening its resilience to external shocks [1]. Generally, this sector faces numerous challenges, including post-harvest losses due to pests, which can reach up to 50% of food production within six months of storage [2, 3]. Among the animal organisms that attack stored cereals causing economic damage, insects alone can lead to losses exceeding 30% during corn storage [4, 5]. The family of Curculionidae particularly *Sitophilus zeamais*, plays a major role in the destruction of stored maize grains in Northern Cameroon [6]. Losses due to insect pests during grain storage cannot be tolerated by the population, given the importance of maize cereal as staple food [7]. Thus, to reduce the pressure from bio-aggressors during the post-harvest storage of foodstuffs, the farmers primarily use synthetic insecticides in their storage facilities [5, 8]. The excessive or repetitive use of these pesticides in agriculture has induced serious problems, the most significant of which include toxicity to humans, soil and water pollution, incidence of resistance in weeds and insects, as well as the destruction of many beneficial organisms including pollinators [9-13]. Given the current trend towards eliminating conventional chemical products, the practice of zero pesticide residue agriculture appears necessary to reduce the use of pesticides, and to promote the use of plant products with an insecticidal effect [14-16]. In addition to being safe and biogradable, these products are easier to use and can, in some cases, be as effective as synthetic pesticides against a particular pest [17]. In addition, several studies have demonstrated the use of various parts of aromatic plants in powdered form to protect stored *Zea mays* L. [18, 19]. *Securidaca longepedunculata* a plant in Cameroon's flora, is an interesting plant for farmers to investigate and has been the subject of several studies including repellent, insecticidal, larvicidal and ovicidal properties [20-23]. The properties of the different parts of the plant could be attributed to their bioactive secondary metabolites with neurological effects [24]. Numerous studies have been underway for some time to isolate or identify secondary metabolites from plant extracts which have insecticidal effects [25]. The difficulty to extract bioactive compounds from plants and to separate them from their original plant matrix constitutes a major constraint for extracting/separating various active compounds, with many processes relying on organic solvents. However, these solvents can impact bioactive molecules, the environment, and consumer health [26]. In this regard, the dry extraction technique CDS (Controlled Differential Sieving) appears necessary for extracting bioactive molecules. This technique does not use solvents and provides a broader range of active compounds [27, 28]. In some cases, adoption will require further studies on the toxicity risks to non-target organisms and the environment. Acute toxicity assessment is therefore essential to identify the harmful

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effects of a single oral dose of a substance or of several doses administrated over 24 hours [29]. Indeed, few particles size and phytochemical studies of active molecules have been performed to improve endogenous knowledge. However, the particle size research of powdered particles and potential active compounds of the plant represents a major issue that could enhance insecticidal activity. A formulation based on *S. longepedunculata* root bark powders combined with *Acacia nilotica* fruit powders has not been studied. Its significance lies in its potential as an alternative to chemical products and as a complementary method for preserving stored foodstuffs for local communities. This work evaluates the insecticidal effect of root bark from *S. longepedunculata* against *Sitophilus zeamais*.

MATERIALS AND METHODS

Plant harvesting and powder preparation

Samples of the root barks from *Securidaca longepedunculata* and the fruits of *Acacia nilotica* were collected in Kalfou and Maroua, respectively, in the Far North region of Cameroon in February 2023. The plant materials were cleaned with a brush, washed with tap water, shade dried at room temperature (24.2 ± 0.7°C; RH ≈ 67.4 ± 3.2%) for two weeks, and then ground to powder by a wooden mortar. The obtained powders were sieved using a 1 mm mesh sieve, stored in hermetically sealed glass bottle, labelled and kept in the dark in the laboratory to prevent oxidation.

Experimental Animals, *Rattus rattus* L. 1758

White Wistar rats (*Rattus rattus*) weighing approximately 100 g, used for the acute toxicity study, were purchased from the National Veterinary Laboratory (LANAVET) in Garoua Cameroon. Animals were acclimatized in the Applied Zoology laboratory and fed with a standard diet (a mix of corn bran, palm oil, cottonseed meal and fish meal).

Powder fractionation by controlled differential sieving protocol

The particle size distribution of the obtained powder was carried out by using a laser granulometer (Mastersizer 2000) [30] before fractionation. The fractionation into particle size classes was carried out according to the CDS (Controlled Differential Sieving protocol) (JNSY200) [27, 28], which involved successive sieving of a mass of powder with decreasing mesh sieves stacked on top of each other (from the top sieve with the largest mesh to the bottom sieve with the smallest mesh). Thus, 100 g (initial mass) of powder was placed on the first sieve. A vertical vibrational movement with amplitude of 0.5 mm was applied to the various sieves for 10 minutes. The different fractions of powders were used for various analyses and preparation of the extract for the formulation of the bio insecticide. The yield of each fraction powder of *S. longepedunculata* was determined according to the following

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formula [31]: $\boldsymbol{Rdt}\ (\%) = \frac{\mathbf{m2}}{\boldsymbol{m1}}\ \boldsymbol{X}\ \mathbf{100}$, (Rdt = Yield), where m_1 is the mass of the powder initially introduced for fractionation and m_2 is the mass of the powder retained on the sieve or in the collecting basin.

Phytochemical screening of *Securidaca longepedunculata* root bark fractions

Phytochemical screening with different solvents (Hexane, Acetone, and Methanol) was performed to highlight the presence of active compounds in the powders of each fraction.

Identification of Alkaloids

To highlight the alkaloids, 2 mg of extract from each powder fraction was dissolved in 6 ml of ethanol at 70°C. To this mixture, 2 drops of Dragendorff's reagent and Bouchardat's reagent were added to produce a white or orange precipitate indicating the presence of alkaloids [32].

Identification of Polyphenolic Compounds

To detect polyphenols, a reaction with ferric chloride ($FeCl_3$) was used. For this assay, 1 mg of extract from each plant powder fraction was introduced into a test tube, followed by a few drops of 10% ferric chloride. The observation of a precipitate or a green-black coloration indicated the presence of phenols, while a blue colour indicated the presence of polyphenols [32].

Identification of Flavonoids

In a test tube, 1 mg of extract from each plant powder fraction was mixed with 3 ml of methanol and a few magnesium shavings, followed by 1ml of concentrated hydrochloric acid. The appearance of an orange, red, or purple colour indicated the presence of flavonoids [32].

Identification of Saponins

The formation of persistent foam beyond 15 minutes indicated the presence of saponins when 0.5 g of powder from each fraction was vigorously mixed in 4ml of distilled water [33].

Identification of Steroids and Triterpenes

Sterols and triterpenes were characterized using the Liebermann-Burchard reaction. Thus, 5 mg of powder from each fraction was dissolved in 10 ml of chloroform. This mixture was filtered before adding 2 ml of acetic anhydride and concentrated sulphuric acid. The appearance of a blue-green or purple ring revealed the presence of steroids, while a violet colour indicated the presence of triterpenes [33].

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Identification of Tannin

Plant powder from each fraction in an amount of 1 mg was introduced into 2 ml of distilled water, and 2 to 3 drops of 1% $FeCl_3$ were added. A greenish or blue-black coloration developed, indicating the presence of tannins [33].

Insecticide formulation

Each powder fraction of *Securidaca longepedunculata* was used to formulate a powdered insecticide with *Acacia nilotica* fruit powder as the excipient. A mixing plan for the particles was created to obtain a homogeneous powder and tested in three replicates for each simplex lattice design. The most promising trial was selected based on its efficacy, showing a higher mortality rate.

Obtaining adult *Sitophilus zeamais*

The adult individuals used in the study came from a permanent mass rearing facility in the laboratory. They were selected and introduced into a stock of disinfected corn obtained from IRAD: Institute of Agricultural Research for the Development of Cameroun (of the hybrid variety (CMS 8504). This now-infested corn stock was cleared of adult individuals after 32 days post-infestation. The emerging adults (F1) after 5 days were used for subsequent experiments.

Determining the insecticidal effect of each formulated powder

In 500 mL glass jars containing 97, 95 and 92 g of disinfected corn, 3, 5 and 8 g of formulated powder (70% *S. longepedunculata* root powder and 30% *A. nilotica* fruit powder) were added, respectively. A negative control not treated and only treated with *A. nilotica* fruit powder, as well as a positive control treated with "Antouka Super" (Pirimiphos-methyl 16% and Permithrin 3%), were also used. After homogenization, each jar was infested with 10 pairs of *S. zeamais* that had been starved for 48 hours. All tests were conducted in four replicates, and the count of dead individuals was performed 5, 35- and 90-days post-treatment. Corrected mortality (Mc) was determined using the following formula: $Mc = (Mo-Mt) / (20-Mt) \times 100$, where (Mo) is the mortality in the treated jars and (Mt) is the natural mortality observed in the control jars. The calculation of the DL_{50} was derived by plotting the mortality/dose regression line where the corrected mortality percentages were transformed into probit [21].

Insecticidal activity of *Securidaca longepedunculata* on different stages of development

To obtain the different stages of the pest's development cycle, five batches of corn seeds (500 g each) were placed in five 10 L pots. Each pot was infested with populations of *S. zeamais* (600) for 24 hours to ensure a large number of eggs on the seeds, and then these adults were removed by sieving. Treatments were applied to the infested seeds at the following intervals: 7, 14, 20, 23 and 25 days,

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corresponding to the different ages of the developmental cycle: L 1 (eggs aged 7 days), L 2 (eggs aged 14 days), L 3 (20 days post-oviposition), L 4 (23 days post-oviposition) and nymphs (more than 23 days post-oviposition) [34]. Treatments with each formulation (3, 5 and 8 g) were applied to the infested grains at a rate of 100 g per breeding jar. The control jar consisted solely of infested corn grains for 24 hours. For each treatment, the emergence of a new generation was observed once after 35 days from the oviposition date, and all four replicates were checked to verify the ovicidal, larvicidal and nymphalidal effects of the different formulations.

Preparation of the administered solution

The mother solution of aqueous extracts from the formulated powder was obtained by dissolving 100 g of dry powder in 1 L of distilled water for 48 hours. The mixture was then separated by centrifugation at 1000 rpm for 10 minutes, and the supernatant was collected and filtered using filter paper (Whatman N°.1). The mother solution, with a concentration of 100 mg/mL, was placed in a bottle and stored at +4°C in a refrigerator. The dose was prepared shortly before administration. Knowing the limit dose to be administered, the weight of the animal, and the concentration of the dilution, the volume to administer the extract to the animals was determined using the following formula: Volume to be administered (ml/kg) = Dose (mg/kg) × Weight (g) / (1000 × Concentration (mg/kg).

Evaluation of acute toxicity

The toxicity test was conducted following the "dose adjustment" method from OECD (Organization for Economic Cooperation and Development), guideline 425 [29] and involved testing the aqueous extract of the most effective formulation at a dose of 2000 mg/kg. The test was performed on 9 female Wistar rats over a period of 14 days. After 15hours of fasting, they were divided as follows: a control group consisting of 3 females receiving distilled water at a rate of 10 mL/kg and experimental group consisting of 6 females receiving the aqueous extract. Behavioral observations were made 4hours after administering the substances. Subsequently, hydration and feeding were performed daily for 14 days. During this period, signs of toxicity such as changes in fur, mobility, tremors, grooming and respiration sensitivity to noise after a metallic shock, stool appearance, mobility and death were noted.

Data analysis

The various data obtained in terms of percentage mortality were subjected to analysis of variance using one-way ANOVA at a significance level of 5% ($P < 0.05$) using Satgraphic Plus 5.0 software. In the case of differences, the least significant difference T-test was performed for the comparison of two means. Calculations of means, standard deviations and graphical representations of the parameters were

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performed using Microsoft Excel 2013. The SPSS (Statistical Package for Social Sciences) 16 full software was used to determine the DL_{50} values of the different fractions.

RESULTS AND DISCUSSION

Powder fractionation and collection for testing

The study of the particle size distribution of the root powder from *S. longepedunculata* (Fig. 1) revealed that 50% and 90% of the powder particles had diameters less than 161.5 µm and 376.3 µm, respectively, thus justifying the extreme range of sieve sizes chosen (100 to 400 µm). Superfine and conventional grinding methods were used to produce four types of *S. longepedunculata* powder size fractions with different particle size 100 µm, 200-300 µm, 300-400 µm and ≥ 400 µm) and varying yields (41, 38, 12 and 09 g). This variability in yield among the four extracts could be due to modifications in the surface properties of the particles compared to the initial powder. Analyses of the different powder particle size classes clearly indicate that the CDS process improves the quantity of powders in particle size between 161.5 µm and 376.3 µm. This process is generally used to enhance the properties of powdered particles in research and development [32].

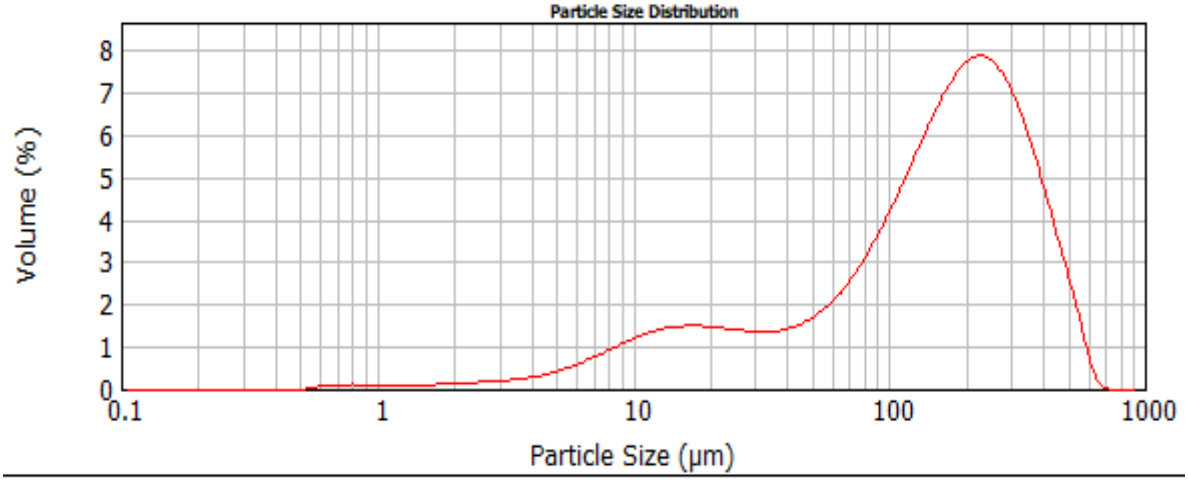

Figure 1: Particles size distribution of *Securidaca longepedunculata* root powderPhytochemical constituents of *Securidaca longepedunculata* powder

Table 1 presents the phytochemical screening of the extracts from the fractions of *S. longepedunculata* powder. The results of the chemical characterization of the extracts with different organic solvents reveal the presence of polyphenols, flavonoids, alkaloids, tannins, terpenes, steroids and saponins. Polyphenols, flavonoids, tannins and alkaloids were absent in all hexane extracts, while steroids were absent in the methanolic extracts. Terpenes and saponins were present in all

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extracts, regardless of the solvent type used. These studies corroborate the test results obtained from the root of *S. longepedunculata*, highlighting the presence of flavonoids, tannins, coumarins, alkaloids, terpenes, saponins and phenols [23, 35].

Determination of insecticidal efficacy of each fraction against *Sitophilus zeamais*

The mortality of adult *S. zeamais* in response to each insecticide formulation used was observed. All powdered doses of the plant tested (3, 5 and 8 g/kg) resulted in significant mortality ($P < 0.05$) of adult *S. zeamais* compared to the negative control (without powder). The insecticidal activity of the powders on the mortality of adults increases with dose, exposure time as well as with decreasing particles size of the plant powders. Figure 2 shows that the fractions ≤ 100 µm, 300-200 µm and 400-300 µm induced a dose-dependent effect, while the fraction ≥ 400 µm exhibited a non-dose-dependent effect, as the 5 g/kg dose resulted in higher mortality than the 8 g/kg dose ($P > 0.05$). After 90 days of treatment, the insecticidal activity of all fractions remained variable depending on the doses used (Fig. 4). However, the observed mortality was lower than that obtained in the positive control jar (Antouka super), which showed a total mortality of 100 ± 0.0% by the fifth day of treatment at a dose of 0.5 g/kg. Thus, the 8 g/kg dose of the ≥ 400 µm fraction resulted in mortality rates of 50 ± 10.6% (Fig. 2), 65 ± 0.7% (Fig. 3), and 38.7 ± 4.9% (Fig. 4) respectively after 5, 35 and 90 days. At the highest dose, the 300-200 µm and 100 µm fractions also induced significant mortalities of 85 ± 9.8%, 86.2 ± 7.0% and 67 ± 0.7% respectively after 5, 35 and 90 days (Figs. 2, 3 and 4). The observed mortalities in the different particles size fractions may be attributed to the biochemical composition of the powdered extracts. The mortalities observed after 5, 35 and 90 days of treatment may be attributed to the toxic effects and the chemical nature of the active molecules contained in the plant powders [15, 21]. Several previous studies have shown that powders from the leaves of *S. longepedunculata* have insecticidal effects on *Callosobruchus maculatus* [21]. In fact, their findings indicated that the leaf powder at a dose of 8 g/kg caused 86.1% mortality of *C. maculatus* on day 5. This mortality rate was lower than that of the fraction ≤ 100 µm (90%) used at the same dose and experimental periods. These differences could be explained by the varying sensitivities of different insect species to the various metabolite compounds found in the powders of different plant parts and the method of obtaining the powders or extracts. The phenolic compounds from various parts of the plant have insecticidal properties. Among these studies, research showed that polyphenols disrupt the natural motility of insects [36]. Additionally, tannins have a toxic effect on certain pest insect species by influencing their growth, development and fertility [15]. Furthermore, the mortality observed may be due to the content of alkaloids, which possess repellent or anti-feedant properties that inhibit pest insects

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[15]. In the present work, the alkaloids and terpenoids contained in the extracts are thought to be responsible for the suppression of the *S. zeamais* population. These extracts also significantly reduced the mass losses of treated maize seeds compared with the negative control. These results are similar to those obtained with methanolic extracts of *S. longepedunculata* tested on *S. zeamais* [15].

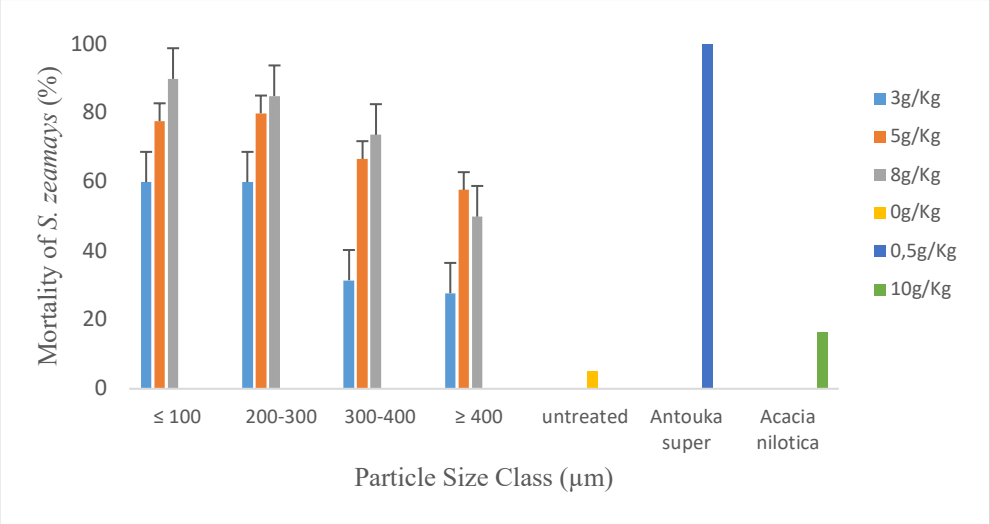

Figure 2: Evaluation of the insecticidal activity of *Securidaca longepedunculata* on adult *Sitophilus zeamais* aged no more than five days over a period of 5 days

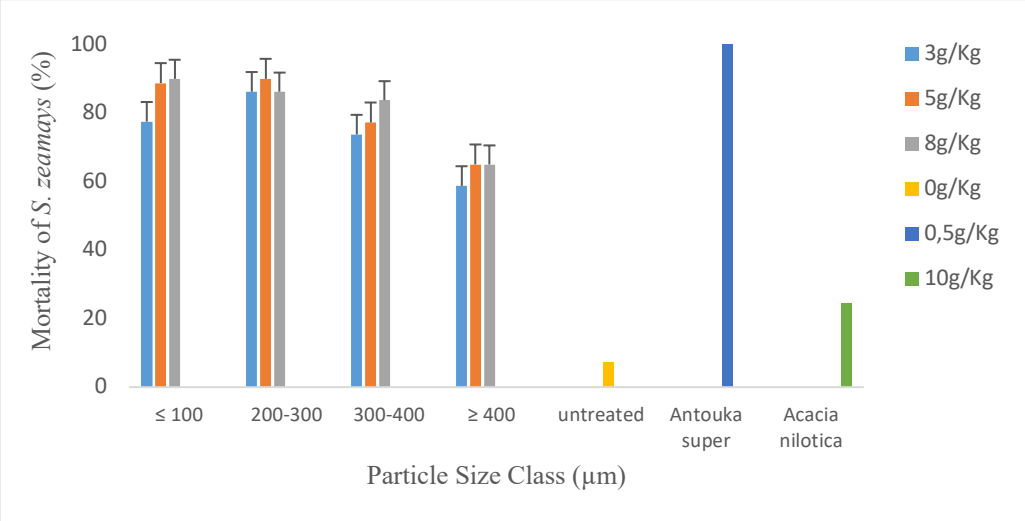

Figure 3: Evaluation of the insecticidal activity of *Securidaca longepedunculata* on adult *Sitophilus zeamais* aged no more than five days over a period of 35 days

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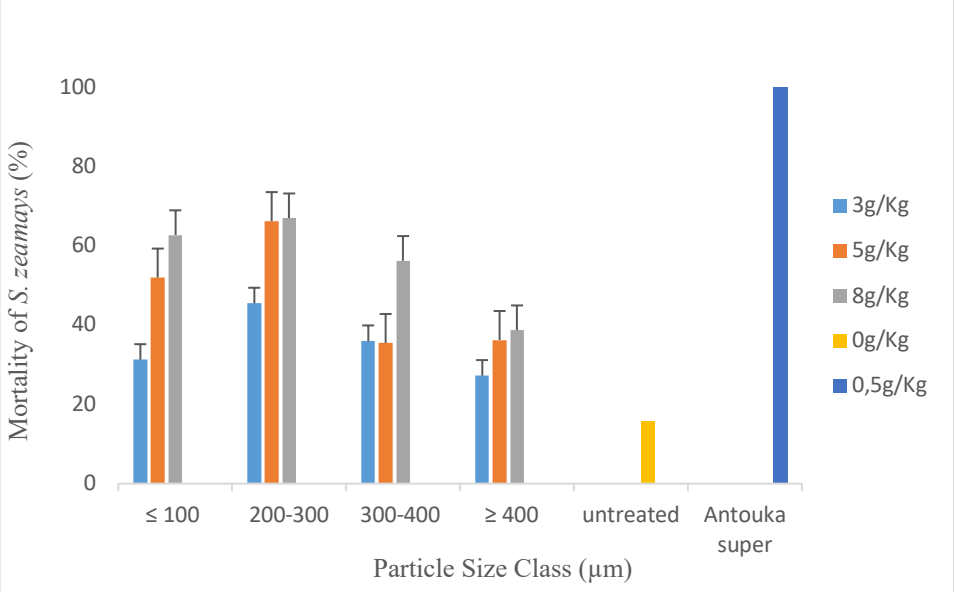

Figure 4: Evaluation of the insecticidal activity of *Securidaca longepedunculata* on adult *Sitophilus zeamais* aged no more than five days over a period of 90 days

The calculation of the LD_{50} categorizes the different particle size fractions of the powder based on their effectiveness on the mortality of the target pest. The LD_{50} values for the following fractions: ≥ 400 µm, 400-300 µm, 300-200 µm and ≤ 100 µm are 6.3 ± 0.6, 4.8 ± 0.8, 3.4 ± 0.4 and 3.3 ± 0.7 g/kg, respectively. The 300-200 µm and ≤ 100 µm fractions were the most effective across all treatments.

Insecticidal activity of *Securidaca longepedunculata* against different developmental stages

Table 2 shows the impact of the powder from *S. longepedunculata* peels individually on the development cycle of *S. zeamais*. There is a significant difference ($P < 0.05$) between the fractions and the untreated control regarding the emergence of the first-generation population. The average numbers of L 1, L 2, L 3, L 4, and nymphs emerging from the untreated seed lots are 6 ± 0.8, 11 ± 2.5, 5 ± 1.6, 8 ± 2.9 and 7 ± 2.1, respectively. All treatments with different amounts of the formulated insecticidal powder resulted in no emergence (0 adult emerging) in the treated lots at the various developmental stages (eggs, larvae and nymphs) of *S. zeamais*. Thus, aromatic plants and their bioactive molecules exert dual activity through inhalation and inhibition on different phases of the reproductive cycle [15]. The activity of the various extracts may also be attributed to triterpenoid properties that cause death and malformation in future generations of insects [15]. It is worth noting that volatile terpenic molecules have an insecticidal effect through inhalation on *Sitophilus*

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zeamais and inhibit cholinesterase [37]. Saponins have been shown to inhibit growth and ovogenesis in insects [15].

Acute Toxicity Data

The use of aromatic plants for pest control has become a necessity due to the significant insecticidal effects of these plants. Moreover, the search for new biologically active substances that are less toxic and free of side effects compared to synthetic chemicals is a growing concern for consumer health. Consequently, a complementary study focused on the risks of acute oral toxicity of the aqueous extract from the bark of *S. longepedunculata* on female Wistar rats (Table 3). Results indicate that there were no signs of toxicity related to the physiology and behaviour of the animals following the administration of the aqueous extract formulation at a dose of 2000 mg/kg according to the OECD guideline 425 [29]. These experimental conditions also suggest that it may be possible to produce high-quality traditional bioinsecticides that ensure the safe use of the plant parts.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Results obtained demonstrate that the powder from the roots of *S. longepedunculata* in different particle size classes is effective for preserving stored corn grains. The formulation of the bioinsecticide using the 100 µm and 200 µm fractions shows a higher mortality percentage compared to the other fractions, highlighting the benefits of the differential and controlled sieving process that enriched certain size classes with active compounds responsible for the insecticidal, ovicidal, larvicidal and nymphalidal activity against *S. zeamais*. The complementary study on the acute oral toxicity of the bioinsecticide formulated from the bark of *S. longepedunculata* on female Wistar rats showed no signs of toxicity. It is essential to promote the cultivation of *S. longepedunculata* to meet the increasing demand and to continue studying the sub-acute or chronic toxicity of the formulated bioinsecticide.

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Table 1: Phytochemical screening of *Securidaca longepedunculata* powder based on solvents

Secondary metabolites	Hexane Extract				Acetone Extract				Methanol Extract			
	≥ 400 µm	400-300 µm	300-200 µm	≤ 100 µm	≥ 400 µm	400-300 µm	300-200 µm	≤ 100 µm	≥ 400 µm	400-300 µm	300-200 µm	≤ 100 µm
CPT	—	—	—	—	+	+	+	+	+	+	+	+
Flavonoids	—	—	—	—	+	+	+	+	+	+	+	+
Tannins	—	—	—	—	+	+	+	+	+	+	+	+
Alkaloids	—	—	—	—	+	+	+	+	+	+	+	+
Terpenes	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	—	—	—	—
Saponins	+	+	+	+	+	+	+	+	+	+	+	+

Legend: **+:** Positive; -: Negative; CPT: Total Phenolic Compounds

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Table 2: Evaluation of the insecticidal activity of *Securidaca longepedunculata* on the larvae and nymphs of *Sitophilus zeamais*

Life Cycle Stages	3 g/kg Treatment				5 g/kg Treatment				8 g/kg Treatment				0 g/kg
	≥ 400 µm	400-300 µm	300-200 µm	≤ 100 µm	≥ 400 µm	400-300 µm	300-200 µm	≤ 100 µm	≥ 400 µm	400-300 µm	300-200 µm	≤ 100 µm	
L1 (7)	0	0	0	0	0	0	0	0	0	0	0	0	6 ± 0,8
L2 (14)	0	0	0	0	0	0	0	0	0	0	0	0	11 ± 2,5
L3 (20)	0	0	0	0	0	0	0	0	0	0	0	0	5 ± 1,6
L4 (23)	0	0	0	0	0	0	0	0	0	0	0	0	8 ± 2,9
Nymph	0	0	0	0	0	0	0	0	0	0	0	0	7 ± 2,1

Legend: - Zero emerging adults

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Table 3: **Effects of aqueous extracts of the formulated powder on some physiological parameters in female Wistar rats over 14 days**

Parameter	Periods																
Physiological	**1h**	**2h**	**3h**	**4h**	**J_2**	**J_3**	**J_4**	**J_5**	**J_6**	**J_7**	**J_8**	**J_9**	**J_{10}**	**J_{11}**	**J_{12}**	**J_{13}**	**J_{14}**
Grooming	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Coat Condition	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremors	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn
Motility	AN	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Reaction to Noise	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Convulsions	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn	Nn
Stool Appearance	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Legend: N: Normal; Nn: No; AN: Abnorm, J: Jours

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