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Effect of hexane, acetone and methanol extracts of *Plectranthus glandulosus* on the mortality of the adults of *Callosobruchus maculatus* and *Sitophilus zeamais*

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Abstract
Plants extracted substances can constitute the best alternative to synthetic chemical insecticides and a good preservation of the environment because they are biodegradables. The leaves of *Plectranthus glandulosus* were extracted with hexane, acetone and methanol, then fractionated and tested on adults of *Sitophilus zeamais* and *Callosobruchus maculatus*. Corn and cowpea seeds were each administered three (3) different doses (1, 5 and 10 g/kg) of these extracts to assess adult mortality of *S. zeamais* and *C. maculatus*. The other percentage of mortality obtained was collected and analyzed. In all cases, the hexane fraction was more effective than that of acetone and methanol because it induced more mortality and it appears that *S. zeamais* is more vulnerable than *C. maculatus* to the different extracts. The hexane extracts induced mortality of 98.08% against adults of *S. zeamais* and 70.00% against adults of *C. maculatus* in one day of exposure with the dose of 10g/Kg. *P. glandulosus* extracts can be used as a natural solution against insect plant damage.

Keywords: *Plectranthus glandulosus, Sitophilus zeamais, Callosobruchus maculatus* plant extracts, bioactivity, maize, cowpea

Introduction
In Africa, the main purpose of agricultural production is to provide food availability during periods that are not flexible.
These main commodities in production are cereals and legumes, which constitute the main staple foods for most of the world's developing countries [1]. The most widely used are cowpeas and maize and are consumed globally [2]. These foods are the most cultivated in Africa to respond to the African food crisis [3]. Out of nearly 4 million metric tons of cowpea produced globally and 70 million metric tons of maize produced annually in Africa, West and Central Africa contribute up to 70% of this production, and Nigeria occupies alone, 66% of production [4, 5].
In Chad, the annual maize production is 176,243.9 metric tons and that of cowpea is poorly known [6].
Pests generally destroy these agricultural products during storage periods, mainly rodents, fungi, and insects [7]. Those causing the most damage to farmers are insects [8]. Among the latter, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) are pests of cowpea and maize respectively, causing extensive damage during storage. They generally integrate food from the fields and are transported to storage structures [9]. The losses caused by these weevils are both quantitative and qualitative. Estimates of these losses are highly variable and can reach 90% [10], due to the tropical climate being very favorable to their development [11]. The extent of the damage caused to stored food obliges farmers to take protective measures [12]. The method commonly used to limit post-harvest losses is the application of synthetic chemical insecticides [13]. However, the abusive use of chemical insecticides (sometimes prohibited), the failure to take precautions in their handling and their persistence on the treated foodstuff are all factors which lead to the resistance of harmful insects, the elimination of their natural enemies, health problems (to consumers and farmers) and environmental pollution [14]. Alternative control methods are needed to minimize the harmful effects of these insecticides.

Plant-based insecticides are more biodegradable and can be a more environmentally friendly source of insecticides. Hence the need to find means of protecting foodstuffs, alternatives to synthetic insecticides, effective, more respectful of human health and the environment and available. Studies carried out in several agrosystems in Africa and particularly in North Cameroon reveal that farmers, in their traditional practices, use plant extracts with an insecticidal effect for the conservation of agricultural products [15]. Various plant extracts or products such as leaf powders [16], vegetable oils [17], essential oils [18] and aqueous extracts [19] have been the subject of scientific investigations against pests of stored food. For this purpose, the effectiveness of aqueous extracts from the leaves of *P. glandulosus* has been demonstrated. *Plectranthus glandulosus* Hook F. (Lamiaceae) is one of the 18 species of the genus Plectranthus, which are indexed worldwide [20]. In Cameroon and Chad, *P. glandulosus* is known by farmers as a medicinal [21] and culinary plant [22]. It is a plant whose leaves are commonly used to protect stored cereals [16, 23, 24].

Recent studies have shown that the leaf fractions of *P. glandulosus* exhibit strong larvicidal activity against mosquitoes [25]. The essential oil from the leaves of the same plant has also been shown to be very effective against mosquito larvae and pupae [26].

Other studies have also shown that the fractions of hexane, and methanol have been shown to be toxic to *C. maculatus* [27]; similarly, the hexane, acetone fractions of chloroform and butanol have also been shown to have protective efficacy against adults of *C. maculatus*. In Chad, no study has been conducted on the effect of *P. Glandulosus* extracted on the mortality of these two beetles.

In the present study hexane, acetone and ethanol extracts from the leaves of *P. glandulosus* were tested, at different concentrations, on the adult mortality of *S. zeamais* and *C. maculatus*.

Material and methods

Plant material

Collecting and drying *Plectranthus glandulosus* leaves

The young leaves of *P. glandulosus* going from the terminal bud towards the base of the plants greater than or equal to one meter in height were harvested between 9 a.m. and 11 a.m. in the month of October to December 2018 in the Sudano-Guinean zone. from Chad mainly in the Department of Lake-lere, at the point of latitude 9° 35 and 9° 41 North and of longitudes 14° 06 and 14° 19 East and altitude 233 meters. The harvested *P. glandulosus* leaves were dried for 7 days [28] in the shade under ambient laboratory conditions [29] (t ≈ 23.0-32.0 °C; HR ≈ 40.2-80.3%). Then they were crushed and sieved through a sieve with a mesh size of 0.4 mm [30], then stored in the freezer at -18 °C before use.

Extraction methods

Three thousand grams (3000 g) of *P. glandulosus* powders were mixed separately with 8 L of hexane, combined for 30 minutes and left for 24 hours after which they were stirred. 48 hours later, under laboratory ambient conditions (t ≈ 32.0-38.5 °C; RH ≈ 80.5-90.5%) the mixture was then filtered through Whatman qualitative filter paper N°. 6. The residue obtained after filtration was put through the above method again and the filtrate was mixed with that obtained initially [31]. After performing the hexane extraction, the remaining paste was dried for 10 hours at room temperature in the laboratory (t ≈ 32.0-38.5 °C; RH ≈ 80.5-90.5%) and then extracted successively with acetone and methanol, following the same method used for hexane [31]. The filtrates obtained with hexane, acetone and methanol were then concentrated separately in a Rotavapor of type R – 110 at 70 °C, 60 °C and 65 °C [31]. After complete evaporation of the solvents, the final crude extracts were weighed and placed in dark colored vials, then kept in the refrigerator at 4 °C protected from light before use for bioassays on insects [19].

Corn and cowpea

Maize, variety "SHABA" and cowpea (*Vigna unguiculata*) variety Mokolo were obtained from the stock of the Chadian Institute of Agricultural Research for Development (ITRAD) of N'Djamena. The seeds were sorted, freed from impurities (broken pieces) and were kept in a plastic bag and then stored in the freezer at -18 °C for 15 days in order to eliminate any form of living organisms that may be in the seeds [28]. They were then brought back and stored in the laboratory under ambient conditions (t ≈ 24.0-35.0 °C; RH ≈ 75.2-90.0%) for two weeks before bioassays for acclimatization [28]. The initial moisture content of the seeds was determined according to the AFNOR method [32].

Beetles: *Sitophilus zeamais* and *Callosobruchus maculatus*

The research was carried out in Chad under laboratory ambient conditions (t ≈ 26.0-35.0 °C; RH ≈ 75.2-90.0%).

The strains of *S. zeamais* and *C. maculatus* used to come from infested corn and cowpea seeds purchased at the market of Ndjamena city.

These insects were reared on "SHABA" and Mokolo varieties under laboratory conditions (t ≈ 26.0-35.0 °C; HR ≈ 75.2-90.0) Samples of 15 adult insects of unknown sex were were reared in 20 jars of 500 ml each containing 200 g of corn seeds for *S. zeamais* and 200 g. cowpea for *C. maculatus*. The jars were covered with cotton cloths to prevent the escape of insects and closed with the perforated lids for aeration of the medium then placed in ambient laboratory conditions [16]. After 14 days of infestation for maize and six days for cowpea, the seeds were sieved using a sieve with a mesh size of 3mm to separate the insects from the seeds and the insects were removed [33]. From the emergence of the *F*1 generation observed from the 28th day for the two varieties, a sieving was carried out every week [16]. Maize weevils up to 7 days old and cowpea weevils up to 2 days old were used for the bioassays.

Phytochemical screening of extracts from *Plectranthus glandulosus* leaves

The powder from the leaves of *P. glandulosus* which was obtained after grinding was used to carry out the preliminary phytochemical screening (phenolic compounds, alkaloids, steroids, flavonoids, tannins, and saponins). Standard qualitative methods as described by Tiwari and *al.* [34] and taken up by Kosini and *al.* [31] were used.

Toxicity test

Each extract was diluted in the solvent used for its extraction to obtain solutions at 0.25 g / ml. Three different volumes (0.2 ml; 1 ml and 2 ml) of each extract were mixed separately corresponding to the content of 1; 5 and 10 g / kg respectively. Each extract was mixed separately with 50g of maize and cowpea into 500 ml jars. Each jar was shaken for 2 minutes for uniform adhesion of the extracts to the seeds [28].

The treated seeds were left for two hours in the laboratory until total evaporation of the solvent [35].

The negative control consisted of 50 g of untreated seeds for each extract and each repeat. Delvap Super® (Reference Insecticide) was used as a positive control at the recommended dose of 0.1 g / kg. Then, 20 adults of *S. zeamais* 7 days old or less and *C. maculatus* 1 to 2 days old at most of the undetermined sex were introduced separately into the jars and kept under laboratory ambient conditions. Hexane, acetone and methanol extracts were applied separately. Each treatment was repeated 3 times.

The jars were then covered with cotton fabrics to prevent the escape of insects [14], then closed using perforated lids to allow good ventilation [16]. Adult mortality was recorded every day for seven (7) days for *C. maculatus* and 14 days for *S. zeamais* with the different extracts. Dead insects were removed from the jars after each observation and live insects were reintroduced. Any insect that does not move either the leg or the antennae after several delicate touches with the end of the entomological forceps was considered to be dead [36].

Statistical analysis of data

The percent mortality data was transformed into arcsin√ (x / 100). These transformed data were subjected to the Analysis of Variance (ANOVA) procedure using the Statistical Package for Social Science (SPSS v.17.0) for Windows software [37, 38]. The Tukey test was used to separate the means. Lethal doses resulting in 50% (LD50) and 95% (LD95) mortality of *S. zeamais* and *C. maculatus* in seeds were determined using Probit analysis [39, 37]. Abbott's formula [40] was used to correct for control mortality prior to the application of ANOVA and Probit analysis:

Pc = ((Po - Pt) / (100 - Pt)) × 100; With Pc: mortality corrected in percentage, Pt: mortality observed in the control, Po: mortality observed in the tests.

Results

Chemical constituents of hexane, acetone and methanol extract from the leaves of *Plectranthus glandulosus*

Table 1 show the results of the phytochemical screening of the extracts of the leaves of *P. glandulosus.* This table reports the secondary metabolites detected by screening methods. They are mainly phenolic compounds, alkaloids, steroids, flavonoids, tannins and saponins.

This result revealed that the number of compounds in the plant increases with the polarity of the solvents used for the extraction. The leaf's hexane extracts did not have the same constituents as the acetone and methanol extracts. Thus, these results showed a complete absence of phenolic compounds and alkaloids in the hexane extract but were found mostly in acetone and methanol extracts. The alkaloids in acetone extract are more present than in methanol. Regarding steroids and saponins, there is a very abundant presence in hexane extracts. Steroids are moderately abundant in acetone and methanol extracts, unlike saponins which are more abundant in acetone and methanol extracts.

Flavonoids are very abundant in methanolic extracts, moderately abundant in acetone extracts, and weak in hexane extracts. As for tannins, they are absent in the extracts of hexane and acetone but present weakly in the extracts with methanol.

Steroids and saponins were the main constituents of hexane extracts, unlike acetone, which is made up of phenolics, alkaloids and saponins. Those extracted with methanol are mainly composed of phenolic compounds, flavonoids and saponins.

Table 1: Phytochemical composition of extracts from *Plectranthus glandulosus* leaves

Chemical compounds	*Plectranthus glandulosus*		
	Hexane extract	Acetone extract	Methanol extract
Phenolic compounds	(-)	(+++)	(+++)
Alkaloids	(-)	(+++)	(++)
Steroids	(+++)	(++)	(++)
Flavonoids	(+)	(++)	(+++)
Tannins	(-)	(-)	(+)
Saponins	(+++)	(+++)	(+++)

-: Absent; +: weak presence; ++: moderately abundant; +++: very abundant

Mortality of *Sitophilus zeamais* adults

The extracts from the different solvents induced a mortality significantly different from that of the negative control (P<0.001) (Table 2) with regard to *S. zeamais* on corn.

For all the extracts tested, the mortality obtained increases proportionally and gradually with the dose (F = 50.87-402.86; 38.87-302.84; 18.87-63.74) and the period of exposure (F = 644.90 - 412.59; 584.90-381.79; 584.90-381.79) respectively for extracts of hexane, acetone and methanol).

With the hexane extracts, on the 14th day of exposure, all the doses (1, 5 and 10 g / kg) induced a highly significant mortality of *S. zeamais* like the reference insecticide which induced 100% of mortality. As for acetone extracts, this mortality is obtained with the dose of 5 and 10 g / kg. While with the methanol extracts, it is only obtained on the 13th day with the dose of 10g / kg. Decreasing mortality is observed from hexane extract to methanol. The smallest dose (1 g / kg) of the hexane extracts induced 84.14% mortality after 14 days of exposure unlike the acetone extract, which induced 70.14%. That of methanol induced a mortality of 46.14% after 14 days. In one day of exposure, the larger dose of hexane extract induced a significant mortality of 98.08%, however, the acetone extracts induced 82.80% and those of methanol 76.60% mortality. From the 7th post-infestation day, no significant difference in mortality was observed for doses of 5 and 10 g / kg of hexane extract. This result is obtained on the 12th day with the acetone extracts. Delvap Super® induced 100% mortality after one day of exposure at its recommended dose of 0.1 g / kg (Table 2). This same mortality rate (100%) was obtained 2 days after treatment with the extract with hexane at a dose of 10 g / kg, 8 days after at a dose of 5 g / kg and 14 days later at the dose of 5 g / kg. dose of 1g / kg with no significant difference. As for the acetone extracts, this result is obtained 8 days later with the dose of 10 g / kg and 12 days later with the dose of 5 g / kg. With the methanol extracts, this mortality is obtained 12 days later with the highest dose (10 g / kg).

Mortality of *Callosobruchus maculatus* adults

Extracts of *P. glandulosus* leaves from hexane, acetone, and methanol solvents induced highly significant mortality against *C. maculatus* in cowpea. This induced mortality is different from that of the negative control (P<0.001) (Table 3).

The mortality obtained from the three-solvent extracts of the leaves of *P. glandulosus* increases in a correlated and gradual manner with the dose ((F = 21.88-32.80; 5.21-38.04; 12.07-30,25) and the exposure period ((F = 4.12 - 234.76; 29.98-192.03; 7.48-159.50) respectively for the extracts of hexane, acetone and methanol)).

In one day of exposure, the hexane extract induced a significant mortality of 70% with the largest dose (10g / kg), however, the acetone extracts induced 61.90% and those of methanol 53, 90% mortality. In 7 days of exposure, the highest dose (10 g / kg) of the hexane extracts induced a highly significant mortality of *C. maculatus* comparable to that of the reference insecticide with 100% mortality. Unlike acetone and methanol extracts which induced a mortality not comparable (respectively 63%; 69.32%) to that of Delvap Super. The smallest dose (1 g / kg) of hexane extracts induced 35.42% mortality after 7 days of exposure, unlike acetone extracts which induced 23.44%. That of methanol induced a mortality of 29.49% after 7 days of exposure.

Table 2: Corrected mortality (%) of adult S. zeamais exposed to different solvent leaf extract of P. glandulosus in the laboratory

Conc (g/kg)	0	1	5	10	DelvapSuper®(0,1)	*F*
EP (Day)	**Hexane extract**					
1	0,00 ± 0,00^{Ca}	0,00 ± 0,00^{Ce}	1,00 ± 1,00^{Cd}	98,08 ± 3,33^{Aa}	100 ± 0,00^{Aa}	644,90***
2	0,00 ± 0,00^{Ca}	1,00 ± 1,00^{Ce}	1,66 ± 1,66^{Cd}	89,08 ± 2,88^{Aa}	100 ± 0,00^{Aa}	1521***
3	0,00 ± 0,00^{Ba}	1,00 ± 1,00^{Be}	2,66 ± 1,33^{Bd}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	3412***
4	0,00 ± 0,00^{Ba}	1,33 ± 1,33^{Be}	3,33 ± 1,66^{Bd}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	1024***
5	0,00 ± 0,00^{Ca}	1,45 ± 1,45^{Ce}	20,00 ± 0,00^{Bc}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	5964***
6	0,00 ± 0,00^{Ca}	2,66 ± 1,33^{Ce}	56,66 ± 6,00^{Bb}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	378,98***
7	0,00 ± 0,00^{Ca}	2,75 ± 1,33^{Ce}	65,29 ± 8,68^{Bb}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	129,75***
8	0,00 ± 0,00^{Ba}	2,75 ± 2,75^{Be}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	177,29***
9	0,00 ± 0,00^{Ca}	16,03 ± 5,64^{Bde}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	129,56***
10	0,00 ± 0,00^{Ca}	41,34 ± 3,78^{Bcd}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	195,23***
11	0,00 ± 0,00^{Ca}	58,55 ± 6,22^{Bbc}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	241,66***
12	0,00 ± 0,00^{Ca}	62,17 ± 9,50^{Bbc}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	164,29***
13	0,00 ± 0,00^{Ca}	65,99 ± 7,69^{Bab}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	184,25***
14	0,00 ± 0,00^{Ca}	84,14 ± 4,91^{Ba}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	412,59***
F	/	50,87***	63,74***	402,86***	/	/
Acetone extract						
1	0,00 ± 0,00^{Ba}	0,00 ± 0,00^{Be}	0,00 ± 0,00^{Bf}	82,80 ± 1,66^{Bb}	100 ± 0,00^{Aa}	584,90***
2	0,00 ± 0,00^{Ca}	0,00 ± 0,00^{Ce}	1,00 ± 1,00^{Cf}	15,33 ± 1,84^{Bd}	100 ± 0,00^{Aa}	1341***
3	0,00 ± 0,00^{Ca}	0,00 ± 0,00^{Ce}	1,66 ± 1,66^{Cf}	38,40 ± 4,00^{Bc}	100 ± 0,00^{Aa}	2129***
4	0,00 ± 0,00^{Ca}	1,00 ± 1,00^{Ce}	2,20 ± 1,66^{Cf}	55,22 ± 3,31^{Bbc}	100 ± 0,00^{Aa}	1004***
5	0,00 ± 0,00^{Da}	1,33 ± 1,33^{De}	15,00 ± 2,00^{Cf}	64,25 ± 4,69^{Bb}	100 ± 0,00^{Aa}	4384***
6	0,00 ± 0,00^{Da}	2,00 ± 0,56^{De}	36,33 ± 5,00^{Ce}	70,22 ± 5,15^{Bb}	100 ± 0,00^{Aa}	278,98***
7	0,00 ± 0,00^{Da}	1,75 ± 1,66^{De}	45,30 ± 5,68^{Cd}	79,54 ± 1,88^{Bb}	100 ± 0,00^{Aa}	210,80 ***
8	0,00 ± 0,00^{Ca}	2,75 ± 2,75^{Ce}	50,66 ± 2,22^{Bcd}	87,19 ± 5,12^{Aa}	100 ± 0,00^{Aa}	157,35***
9	0,00 ± 0,00^{Ca}	15,03 ± 4,64^{Cde}	68,50 ± 4,10^{Bc}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	112,60***
10	0,00 ± 0,00^{Da}	31,34 ± 2,78^{Ccd}	72,00 ± 3,66^{Bbc}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	135,26***
11	0,00 ± 0,00^{Ca}	48,55 ± 4,22^{Bbc}	85,00 ± 2,58^{Ab}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	153,45***
12	0,00 ± 0,00^{Ca}	52,17 ± 7,20^{Bbc}	95,00 ± 1,86^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	134,79***
13	0,00 ± 0,00^{Ca}	62,50 ± 4,69^{Bab}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	171,65***
14	0,00 ± 0,00^{Ca}	70,14 ± 4,91^{Ba}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	381,79***
F	/	38,87***	43,24***	302,84***	/	/
Methanol extract						
1	0,00 ± 0,00^{Ba}	0,00 ± 0,00^{Be}	0,00 ± 0,00^{Be}	76,60 ± 0,00^{Bc}	100 ± 0,00^{Aa}	584,90***
2	0,00 ± 0,00^{Ba}	0,00 ± 0,00^{Be}	0,00 ± 0,00^{Be}	50,00 ± 0,00^{Bd}	100 ± 0,00^{Aa}	1341***
3	0,00 ± 0,00^{Ba}	0,00 ± 0,00^{Be}	0,00 ± 0,00^{Be}	1,66 ± 1,66^{Be}	100 ± 0,00^{Aa}	2129***
4	0,00 ± 0,00^{Ba}	1,00 ± 1,00^{Be}	1,66 ± 1,66^{Be}	3,33 ± 1,66^{Be}	100 ± 0,00^{Aa}	1004***
5	0,00 ± 0,00^{Ca}	1,33 ± 1,33^{Ce}	1,66 ± 1,66^{Ce}	20,00 ± 0,00^{Be}	100 ± 0,00^{Aa}	4384***
6	0,00 ± 0,00^{Ca}	1,66 ± 0,56^{Ce}	1,85 ± 1,66^{Ce}	43,63 ± 4,00^{Bd}	100 ± 0,00^{Aa}	278,98***
7	0,00 ± 0,00^{Ca}	1,66 ± 1,66^{Ce}	2,75 ± 1,33^{Ce}	58,39 ± 5,68^{Bc}	100 ± 0,00^{Aa}	210,80 ***
8	0,00 ± 0,00^{Ca}	2,12 ± 2,15^{Ce}	2,75 ± 1,33^{Ce}	69,68 ± 3,34^{Bbc}	100 ± 0,00^{Aa}	157,35***
9	0,00 ± 0,00^{Ca}	3,03 ± 1,61^{Cde}	4,03 ± 1,64^{Ce}	79,94 ± 4,11^{Bab}	100 ± 0,00^{Aa}	112,60***
10	0,00 ± 0,00^{Ca}	5,34 ± 1,78^{Cd}	33,34 ± 2,75^{Bcd}	88,34 ± 3,67^{Aabc}	100 ± 0,00^{Aa}	135,26***
11	0,00 ± 0,00^{Ca}	13,12 ± 2,11^{Cbc}	45,45 ± 3,22^{Bc}	90,81 ± 3,74^{Aab}	100 ± 0,00^{Aa}	153,45***
12	0,00 ± 0,00^{Da}	15,15 ± 2,20^{Cbc}	53,27 ± 7,50^{Bbc}	94,00 ± 4,00^{Aa}	100 ± 0,00^{Aa}	134,79***
13	0,00 ± 0,00^{Da}	25,70 ± 3,62^{Cb}	56,99 ± 5,69^{Bb}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	171,65***
14	0,00 ± 0,00^{Da}	46,19 ± 4,91^{Ca}	74,15 ± 4,89^{Ba}	100 ± 0,00^{Aa}	100 ± 0,00^{Aa}	381,79***
F	/	18,87***	40,87***	63,74***	/	/

The values assigned the same lowercase letter in the same column and the same uppercase letter on the same row do not show a significant difference according to the Tukey test at the 5% level. *: P >0.05 (not significant); ***: *P*<0.001 (highly significant); /: F values have not been determined due to total mortality.

Table 3: Corrected mortality (%) of adult *C. maculatus* exposed to different solvent leaf extract of *P. glandulosus* in the laboratory

	Conc. (g/kg)	Exposure period							
		1	2	3	4	5	6	7	*F*
Hexane extract	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	
	1	1.97 ± 1.28^{Dd}	2.95 ± 1.49^{Dad}	4.33 ± 2.14^{CDd}	6.98 ± 1.58^{Cd}	14.71 ± 1.95^{Bd}	31.12 ± 2.28^{Ac}	35.42 ± 3.28^{Ac}	21.88***
	5	9.72 ± 3.32^{Ec}	14.10 ± 3.27^{Eec}	25.32 ± 4.33^{CDEc}	27.40 ± 3.71^{BCDc}	34.52 ± 2.52^{BCc}	54.32 ± 4.12^{Ab}	59.16 ± 3.43^{Ab}	22.17***
	10	70,00 ± 2,16^{Bb}	38,63 ± 1,66^{Db}	53,54 ± 4,00^{BCb}	56,22 ± 5,11^{BCb}	62,55 ± 3,33^{Bb}	65,21 ± 4,78^{Bb}	95,4 ± 4,21^{Aa}	32,80***
Delvap Super	(0,1g/kg)	100 ± 0,00^{Aa}	100 ± 0,00A^{a}	100 ± 0,00A^{a}	100 ± 0,00A^{a}	100 ± 0,00A^{a}	100 ± 0,00A^{a}	100 ± 0,00A^{a}	/
	F	4.12*	5.2*	25.28***	129.89***	136.43***	140.24***	234.76***	
Acetone extract	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{cd}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	
	1	1.12 ± 1,12^{Cc}	2.43 ± 1.12^{Cd}	7.24 ± 2.00^{Bd}	7.50 ± 2.55^{Bd}	8.32 ± 3.20^{Bd}	12.38 ± 4.03^{Bd}	23.44 ± 3.66^{Ad}	5,21**
	5	12.03 ± 2.50^{Cb}	13.32 ± 2.44^{Cc}	22.43 ± 2.00^{Bc}	26.34 ± 2.40^{Bc}	29.45 ± 2.39^{Bc}	47.39 ± 1.90^{Ac}	49.98 ± 4.88^{Ac}	30.60***
	10	61,90 ± 2,55^{Bb}	38,67 ± 3,49^{Cb}	50,00 ± 2,32^{Bb}	53,22 ± 1,78^{Bb}	58,12 ± 4,00^{ABb}	61,99 ± 3,66^{Ab}	63 ± 4,00^{Ab}	38,04***
Delvap Super	(0,1g/kg)	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	/
	F	29.98***	32.21***	24.30***	50.05***	69.27***	79.00***	192.03***	
Methanol extract	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	
	1	0,00 ± 0.00^{Cd}	1.41 ± 0.23^{Cd}	3.59 ± 1.66^{Cd}	11.22 ± 3.16^{Bd}	8.66 ± 2.00^{Bd}	23.38 ± 2.21^{Ad}	29.49 ±2.98^{Ad}	12.07***
	5	7.65 ± 2.32^{Dc}	11.35 ± 1.50^{Dc}	15.54 ± 2.61^{CDc}	20.66 ± 1.55^{Cc}	26.15 ± 2.77^{Cc}	40.66 ± 2.32^{Bc}	59.29 ± 3.44^{Ac}	38.55***
	10	53,90 ± 2,20^{Bb}	36,16 ± 2,20^{Db}	45,00 ± 1,44^{CBb}	50,00 ± 2,33^{ABb}	54,11 ± 4,00^{ABb}	60,00 ± 3,75^{Ab}	69,32 ± 4,02^{Ab}	30,25***
Delvap Super	(0,1g/kg)	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	100 ± 0,00^{a}	/
	F	7.48**	159.19***	140.89***	323.45***	158.64***	167.75***	159.50***	

The values assigned the same lowercase letter in the same column and the same uppercase letter on the same row do not show a significant difference according to the Tukey test at the 5% level. *: P >0.05 (not significant); ***: *P*<0.001 (highly significant); /: F values have not been determined due to total mortality.

Discussion

Chemical compounds extracted are different according to the solvent used.

Due to these differences in polarity in their chemical composition, extracts of many plant species have been formulated using organic solvents with different degrees of polarity as insecticidal material to achieve more extraction [41].

The extracts with different solvents from the leaves of *P. glandulosus* have good insecticidal properties thanks to the presence of their secondary phytochemical and / or metabolic constituents which have been demonstrated. These include phenolic compounds, alkaloids, steroids, flavonoids, tannins and saponins. Therefore, plants with secondary metabolites constitute a diverse group of molecules that are involved in the adaptation of plants to their ecological environment or habitat. Thus, alkaloids, saponins, steroids, terpenoids, total phenols, flavonoids and tannic acids have demonstrated investigations and have been characterized as having insecticidal properties [42, 41]. These secondary metabolites can act as repellants, toxicants, deterrents, inhibitors of reproduction and reducers of damage to foodstuffs in storage [43].

Toxic molecules are chemicals used to kill insects orally, by contact, or by fumigating.

Work has shown that around 2000 plant species have insecticidal properties, among which we can cite essential oils, aqueous extracts and powders of *P. glandulosus* which have demonstrated their insecticidal activities through their bioactive effects on several insect species, generally Coleoptera [44, 18].

The insecticidal properties of the various extracts of the leaves of *P. glandulosus* with regard to *S. zeamais* and *C. maculatus* could be linked to the phytochemical constituents demonstrated in the plant, which are phenolic compounds, alkaloids, steroids, flavonoids, tannins and saponins which have properties of toxicity regarding insects [45].

The insecticidal power of the various aqueous extracts of plants does not only vary according to the types of plant species, age, insect species, geographical location, but also the solvents used for their extraction [46].

Previous studies by Adeniyi and *al.* [47] indicate that ethanolic extracts containing several phytochemical groups are more toxic to *Acanthescelides obtectus* than other extracts containing less secondary metabolites. This confirms the effectiveness of our extracts which contain several types of secondary metabolites depending on the extraction solvents.

In fact, the hexane extract from the leaves of *P. glandulosus* which by phytochemical study indicates a significant presence of steroids is highly toxic with regard to *C. maculatus* than the acetone and methanol extracts which contain several other compounds phytochemicals and not the presence of steroids. Steroids derived from the leaves of *P. glandulosus* may be toxic to *C. maculatus* through their synergistic actions with other secondary metabolites. Steroids, known as modified triterpenoids, are biologically important natural products [48]. They have several properties, most of which is insecticide [49]. They inhibit the activity of an enzyme, acetylcholinesterase, which destroys acetylcholine after the transmission of nerve impulses and kills insects [50].

Since the hexane extract from the leaves of *P. glandulosus* contains steroids indicated as rapid-acting metabolites in *C. maculatus*, this specific property may in part be due to the presence of the steroids or to another chemical compound with a similar biological activity. The presence of steroids in acetone extracts and methanol from *P. glandulosus* leaves may be the cause of higher toxic insecticidal effects of acetone extracts than methanol compared to the presence of other secondary metabolites, mainly alkaloids by synergistic action, which are less present in methanol extracts. With the exception of the tannins which were present in the methanol extracts and absent in the acetone extracts, the two extracts were qualitatively almost identical and quantitatively different in their chemical compounds.

Adult mortality of *S. zeamais* and *C. maculatus* increases with dose and period of exposure. Regardless of the types of extracts depending on the different solvents, the toxic effect depends on the constituents of the chemical molecules contained in the plant. Dessenbe and *al.* [51] obtained 100% mortality of *S. zeamais* with the dose of 10g / kg of extracts of *P. glandulosus* while [19] reported that extracts of leaves of *P glandulosus* induced 100% mortality in adults of *C.*

maculatus. The mortality induced by our extracts with regard to *S. zeamais* and *C. maculatus* on maize and cowpea (dose of 5g / kg and 10g / kg with hexane extracts on *S. zeamais* and *C. maculatus* in the 8th and 7th day of exposure respectively) in the present study corroborates the work of the aforementioned authors. However, [31, 19] obtained better efficacy with the hexane extracts of *Ocinum canum* and *P. glandulosus*; which induced 100% mortality in both cases with the dose of 5g / kg after 6 days of exposure.

As regards the differences in mortality which are observed in the present work between the different extracts with regard to *S. zeamais* and *C. maculatus*, it was observed that the extracts of hexane, acetone and methanol are more toxic to *S. zeamais* than *C. maculatus*. This fragility of *S. zeamais* to extracts could be explained by the fact that *C. maculatus* in the adult state does not feed on the seeds, unlike adults of *S. zeamais* which feed on the seeds and by this food intake ingest the extracts which are responsible for their death [52]. This result is different from that obtained by Tofel and *al*. [52], which indicates that essential oils of *Azadirachta indica* induce more mortality in adults of *C. maculatus* than adults of *S. zeamais*. It supports *A. indica* oil's appetite suppressant mechanism against *S. zeamais*; because vegetable oils are known to penetrate the cuticle of insects by blocking the stigmas, which prevents the insect from breathing and the result is death by asphyxiation [53]. Since the extracts are less volatile than the oils, the induced mortality in adults of *S. zeamais* is largely due to the dietary interference of the extracts by the plant. Owolabi and *al*. [54] have shown that the toxicological action of volatile molecules in plant extracts could be due to the poisoning of insects during food intake. Extracts of *P. glandulosus* may in part cause mortality of *S. zeamais* compared to *C. maculatus* by ingestion through the digestive tract when they puncture seeds for egg laying or foraging. The mortality of *C. maculatus* adults observed in the present study could be linked to the action of the extracts on the insect by direct contact during their movements between the seeds, which would lead to their adhesions to the cuticle and consequently desiccation of the insect and then death follows.

Prates and *al*. [55], reported that substances toxic to insects enter the joints, respiratory and digestive systems of the latter. These substances would bind to the receptors of the octopaminergic neurotransmitters causing the convulsion and death of insects [9]. Cuticle sclerotization in insects increases with age. It is characterized by the hardening of the cuticle by the formation of an additional wax layer increasing the cuticular permeability [56]. Then, the low sensitivity of *C. maculatus* compared to *S. zeamais* would be due to the non-interference of the active principle contained in the extracts since the adults do not feed, and have had limited contact with the active principle contained in the extracts.

As observed in the present study, adults of *C. maculatus* of one-day old are naturally young than adults of *S. zeamais* aged 7-14 days. The elytra of adults of *C. maculatus* partially cover the dorsal abdomen as well as the disc in adults of *S. zeamais*, the dorsal abdomen is completely covered by the elytra. In this case, adults of *S. zeamais* are more susceptible than adults of *C. maculatus* could not be attributed to the disposition of the elytra, but rather to the consumption of the food by the latter. This result is different from that obtained by Danga and *al*. [19], which obtains a high sensitivity to essential oils of adults of *C. maculatus* compared to adults of *S. zeamais* and which justifies by the increased mobility of *C. maculatus* compared to *S. zeamais*. The present work corroborates with the results of Bamaiyi and *al*. [57], who recorded a lower mortality in *C. maculatus* than in *S. zeamais* with the seed powder of *Khaya senegalensis*.

Conclusion

The phytochemical screening of the extracts of the leaves of *P. glandulosus* revealed the presence of several secondary metabolites which varied according to the solvents of extraction. From the results obtained following the toxicity tests, we can conclude that these different extracts can constitute powerful insecticides of plant origin with regard to *S. zeamais* and *C. maculatus*. In particular, that of hexane is more promising in the preservation of foodstuffs. The latter, compared to the positive control, showed similar insecticidal activity from a dose of 5 g/kg and can be suggested to contribute to the protection of stored crops, and constitute a powerful alternative to synthetic chemical insecticides.

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