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# Bio-efficiency of Methanolic Extracts of Leaves of *Plectranthus glandulosus* on Mortality and Offspring of *Sitophilus zeamais* $F_1$ in Maize Protection

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***Abstract:*** *To find an alternative to environmentally harmful chemical pesticides, the methanolic extracts of Plectranthus glandulosus leaves were evaluated for mortality and $F_1$ offspring production of Sitophilus zeamais. Delvap Super® was applied as a reference insecticide at the recommended dose (0.1 g / kg), while the extracts were used at four doses (5, 10, 20 and 40 g / kg). Adult mortality was recorded daily for 14 days post-treatment. One day after exposure, Delvap Super® killed 100 % of the weevils, and the extracts caused 96.66 % mortality with the highest dose (40 g / kg). The $LD_{50}$ value in four days was 3.72 g / kg. From the smallest (5 g / kg) to the largest dose (40 g / kg), the extracts significantly reduced the emergence of offspring respectively 43.87 to 100 %. Considering these results, these extracts could be recommended as alternatives to synthetic insecticides against S. zeamais in storage.*

**Key words**: offspring, *Plectranthus glandulosus*, toxicity, *Sitophilus zeamais*.

## I. Introduction

Cereals are the food base for most of the world's poor [1]. Maize cultivation is the most important after wheat and rice, especially for developing countries [2]. Because of climate impacts, maize is grown once a year, at periods that are not scalable, while its consumption is year-round [3]. This situation forces farmers to store a large portion of their crops for consumption throughout the year [4]. Unfortunately, stored products are usually attacked by insects [5]. Among these insects, one of the most damaging is the corn weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). The latter is cosmopolitan food insect in general and maize in particular whose infestation begins from the field during drying and continues during storage [6]. It is found in all the warm and tropical regions of the world, which are particularly prevalent in America, Australia, Asia and Africa [7] and are transported to temperate regions as import raw materials [8]. The methods used by peasants to limit their

damage are generally synthetic chemical insecticides that often cause insect resistance, pollute the environment and harm the health of consumers [9]. This is why studies have turned to plants that have proven effective in protecting stored commodities in several African countries [10]. These are leaf powders, essential oils [9], vegetable oils [11] and aqueous extracts [12] that were the subject of scientific investigations against pests of stored products. *Plectranthus glandulosus* is a plant belonging to the family Lamiaceae (Labiatae). It has insecticidal, culinary and medicinal properties [9]. In the Adamawa region, the essential oil extracted from this plant has been found to be very toxic to adults of *S. oryzae*, *S. zeamais* and *Prostephanus truncatus* [13]. The objective of this work is to evaluate the effect of methanolic extracts of *P. glandulosus* leaves on mortality and production of *S. zeamais* $F_1$ offspring.

## II. Materials and methods

### II.1. Culture of Sitophilus zeamais

The *S. zeamais* strain used comes from seeds infested with maize harvested at Dang in the district of Ngaoundere, Cameroon. These insects were grown on the "SHABA" variety under ambient laboratory conditions (t ≈ 23.0-30.5 ° C, RH ≈ 43.5-81.5%). After 14 days of culture, the seeds were sieved and the insects removed [14]. From the emergence of the $F_1$ generation observed 4 weeks later, a sieving was performed weekly [9]. Corn weevils up to 7 days old and of indeterminate sex were used for bioassays.

### II.2. Collection of leaves of Plectranthus glandulosus

The leaves of *Plectranthus glandulosus* were harvested in October 2015 in the district of Ngaoundere, department of Vina, Adamaoua region, Cameroon at the point of latitude 7° 25'42.6 " N, longitude 13° 35'07.8 " E and altitude 1151 m. This region belongs to the so-called Sudano-Guinean agro-ecological zone. The climate is characterized by two seasons: a rainy season (April to October) and a dry season (November to March). The annual rainfall is 1600 mm. The average annual temperature is 22° C with a maximum of 34° C in March and a minimum of 12 ° C in December. The average annual relative humidity is 70%. The identification of the plant was confirmed at the Yaoundé National Herbarium in Cameroon, where the samples were deposited. The leaves of *P. glandulosus* were dried for 7 days [13] in the shade in a room under ambient laboratory conditions (t ≈ 18.0-29.0 °C; RH ≈ 53, 3-97.7%). The resulting powder was then stored in the freezer at -18 °C before use.

### II.3. Preparation of methanolic extracts

For the preparation of the methanolic extracts, 1 kg of powder of the leaves of *Plectranthus glandulosus* was macerated in 2.5 L of methanol for 48 hours and the solution was stirred three times a day (morning, noon and evening). The maceration process was repeated twice to maximize extraction [12]. After 48 hours, the mixture was filtered with Whatman N° 2 filter paper. The filtrate was roto-massed (BUCHI R-124) at 65 ° C for evaporation of the methanol and then evaporated. In an oven for 48 hours at 40 ° C in order to complete the evaporation of methanol [12]. A pasty substance of 56.5 g of *P. glandulosus* (a yield of 5.6%) was obtained, which is put in a dark bottle, then kept cool (4 ° C) and sheltered light before bioassays. The phytochemicals (tannins, steroids, terpenoids, saponosides and alkaloids) of extracts of *P. glandulosus* leaves were identified by screening according to the method of [15].

### II.4. Adult mortality test and $F_1$ offspring production

The maize variety "SHABA" was cleared of impurities and then kept in the freezer at -18 ° C for 15 days to eliminate any form of living organisms that may be in the seeds. It is then brought back and stored in the laboratory for two weeks prior to the acclimation bioassays

[13]. For bioassays, four concentrations (0.25, 0.5, 1 and 2 g / ml) of methanolic extracts of *P. glandulosus* leaves corresponding to doses of 5; 10; 20 and 40 g / kg were used. They were each diluted in 5 ml of methanol and separately introduced into 500 ml jars containing 50 g of corn. Then, each jar was shaken for 2 minutes for uniform adhesion of the seed extracts and left open for four hours for complete evaporation of the solvent [13]. The negative control consisted of 50 g of seeds treated with methanol. Delvap Super® was used as a positive control at its recommended dose (0.1 g / kg). Then, 20 adults of *S. zeamais* not older than 7 days of indeterminate sex were introduced into these jars and kept under ambient laboratory conditions (t ≈ 23.0-28.5 ° C, HR ≈ 66.0 -82.0%). Each treatment was repeated 3 times. The count of dead insects and live insects was done daily for 14 days of exposure. Dead insects were removed from the jars after each sighting and live insects were reintroduced. Any insect that does not move either leg or antenna after several delicate touches with the entomological forceps has been considered dead [13]. After the toxicity tests, the corn of each jar was cleared of weevils. These jars were maintained under ambient laboratory conditions (t ≈ 21.5-29.0 ° C, RH ≈ 67.0-84.5%) and observed weekly. The emergence of individuals from $F_1$ offspring was observed on the 28th day. From this day, the cumulative number of offspring was recorded every three days until the end of emergence.

### II.5. Statistical analysis of the data

Data on percent mortality and offspring reduction were converted to arcsin$\sqrt{}$ (x / 100). These transformed data were subjected to the Variance Analysis (ANOVA) procedure using SPSS v.17.0 (Statsoft 1995, USA, Zar 1999). The Tukey test was used to separate the averages. Lethal doses resulting in 50% ($LD_{50}$) and 95% ($DL_{95}$) mortality of *S. zeamais* in seeds were determined using Probit analysis (Finney, 1971, SPSS Inc., Ltd., Statsoft 1995, USA). Abbott's (Abbott, 1925) formula was used to correct control mortality prior to the application of ANOVA and Probit analysis:

$$Pc = ((Po - Pt) / (100 - Pt)) \times 100;$$

With Pc: percentage corrected mortality, Pt: mortality observed in the control, Po: mortality observed in the tests.

## III. Results

### III.1. Toxicity

The methanolic extracts of *P. glandulosus* leaves caused a highly significant mortality of *S. zeamais* compared to the negative control ($P < 0.01$). This mortality increases proportionally with the dose ($F = 674.90 - 398.68$) and the period of exposure ($F = 40.87 - 1.00$). In 14 days of exposure, all doses greater than or equal to 10 g / kg, the extracts caused 100 % mortality of *S. zeamais* which is comparable to Delvap Super®. The smallest dose (5 g / kg) induced 74.14 % mortality in 14 days of exposure. However, from the 11th day post-infestation, no significant difference in mortality was observed for all doses tested. The extracts were toxic to *S. zeamais* adults because these results indicate that lethal doses 50 ($LD_{50}$) and 95 ($LD_{95}$) decrease with increasing exposure periods. At 1day, the $LD_{50}$ and $DL_{95}$ values obtained were 29.03 and 38.83 g / kg, respectively. At 14 days, these values were 3.72 and 7.13 g / kg respectively (Table 1).

### III.2. Production of $F_1$ offspring

Significant inhibition of emergence of *S. zeamais* $F_1$ offspring from negative control and dose was observed ($F = 845.66$, $P < 0.001$). The reference insecticide, Delvap Super®, at its recommended dose (0.1 g / kg) completely inhibited the emergence of *S. zeamais* $F_1$ offspring. All doses greater than or equal to 10 g / kg yielded results statistically similar to

that of Delvap Super®. The smallest dose (5 g / kg) inhibited almost 50 % of S. zeamais F1 offspring (Table 2).

## IV. Discussion

The mortality induced by the methanolic extracts of the leaves of *Plectranthus glandulosus* is due to the chemical composition of the extract which has insecticidal compounds, which are tannins, terpenoid and steroids, saponosides, and alkaloids. These results are similar to those obtained by [12]. The increase in mortality according to dose and period of exposure is due to the increase in the quantity of active ingredients and their persistence with the duration of exposure. Extracts of *P. glandulosus* may partly cause mortality of *S. zeamais* by ingestion through the digestive tract when they perforate the seeds to lay their eggs or feed. They could also act on the insects by direct contact during their movements between the seeds, which would cause their adhesions to the cuticle and consequently the desiccation of the insect then ensues the death. Obtaining 100 % mortality with the highest dose (40 g / kg) from the 2nd day after infestation is due to the insecticidal activity of the various chemical compounds present in the extract. These results are different from those obtained by [16] after a day of exposure with the powders of the same plant on the same insects. This difference in mortality is explained by the high sensitivity of *S. zeamais* to methanolic extracts as well as powders. Methanol specifically extracts chemicals that may have high toxicity compared to powders. According to [17], the biological activity of a plant varies according to the types of formulations.

The results obtained in the present work with the highest dose (40g / kg) of the extracts in one day of exposure (96.66 %) are similar to those obtained by [12] who reported that methanolic extracts of *P. glandulosus* resulted in significant mortality of *Callosobruchus maculatus*. The high mortality (74.14 %) recorded with the smallest dose on the 14th day could be explained by the high toxicity of the extracts. According to [18], toxic products to adult insects are those that cause high mortality at low doses. *Plectranthus glandulosus* showed inhibitory effect on $F_1$ offspring production at all dose levels and increased with dose and exposure period. It could be explained by the presence of phytochemicals present in the extract that would have an inhibitory action on the offspring of *S. zeamais*. [12] reported that the crude methanol extract of *P. glandulosus* leaves had a significant effect with the 40 g / kg dose reducing *C. maculatus* $F_1$ offspring to 98.25 %. [19] reported that methanolic extracts of *Securidaca longepedunculata* roots contain sterols, alkaloids, saponosides and terpenes which are insecticidal inhibitors and reduce the occurrence of *C. maculatus* and *S. zeamais* $F_1$ offspring. This would justify in the present work, the high presence of compounds with ovicidal and larvicidal properties contained in the leaves of the plant that would be responsible for the inhibition of offspring $F_1$. It would have disrupted the self-regulating functions of the endocrine insect system, which would block the release of growth hormones and cause the arrest or slowdown of the insect's morphogenetic development [20]. According to [21], the adhesion of seed extracts could prevent insects from depositing their eggs; therefore, there is inhibition of emergence.

***Table 1**. Cumulated corrected cumulative mortality (%) induced by extracts on Sitophilus zeamais adults under ambient laboratory conditions (t ≈ 23.0-28.5 ° C, RH ≈ 66.0-82.0%).*

| Mortality (mean ± standard error) / Dose (g / kg) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Doses (g/kg) | 0 | 5 | 10 | 20 | 40 | Delvap Super®(0,1) | F | $LD_{50}$ | $LD_{95}$ |
| Exposure period (day) | *Plectranthus glandulosus* | | | | | | | | |
| 1 | 0,00 ± 0,00$^{Ba}$ | 0,00 ± 0,00$^{Be}$ | 0,00 ± 0,00$^{Be}$ | 6,66 ± 3,33$^{Be}$ | 96,66 ± 3,33$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 674,90$^{***}$ | 29,03 | 38,83 |
| 2 | 0,00 ± 0,00$^{Ca}$ | 0,00 ± 0,00$^{Ce}$ | 0,00 ± 0,00$^{Ce}$ | 35,00 ± 2,88$^{Bd}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 1731$^{***}$ | 21,24 | 26,78 |
| 3 | 0,00 ± 0,00$^{Ca}$ | 0,00 ± 0,00$^{Ce}$ | 1,66 ± 1,66$^{Ce}$ | 61,66 ± 1,66$^{Bc}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 2646$^{***}$ | 18,78 | 25,51 |
| 4 | 0,00 ± 0,00$^{Ca}$ | 1,66 ± 1,66$^{Ce}$ | 3,33 ± 1,66$^{Ce}$ | 85,00 ± 2,88$^{Bb}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 1142$^{***}$ | 16,07 | 22,62 |
| 5 | 0,00 ± 0,00$^{Ca}$ | 1,66 ± 1,66$^{Ce}$ | 20,00 ± 0,00$^{Be}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 5684$^{***}$ | 12,26 | 17,14 |
| 6 | 0,00 ± 0,00$^{Ca}$ | 1,66 ± 1,66$^{Ce}$ | 46,66 ± 6,00$^{Bd}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 368,98$^{***}$ | 10,2 | 14,22 |
| 7 | 0,00 ± 0,00$^{Ca}$ | 1,75 ± 1,75$^{Ce}$ | 59,29 ± 8,68$^{CBd}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 190,75$^{***}$ | 9,63 | 12,03 |
| 8 | 0,00 ± 0,00$^{Ca}$ | 1,75 ± 1,75$^{Ce}$ | 68,68 ± 9,34$^{Bbcd}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 157,29$^{***}$ | 9,06 | 12,23 |
| 9 | 0,00 ± 0,00$^{Ca}$ | 14,03 ± 4,64$^{Cde}$ | 78,94 ± 9,11$^{Babc}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 122,58$^{***}$ | 7,86 | 12,23 |
| 10 | 0,00 ± 0,00$^{Ca}$ | 31,34 ± 2,78$^{Bcd}$ | 85,34 ± 7,74$^{Aabc}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 165,26$^{***}$ | 6,58 | 11,93 |
| 11 | 0,00 ± 0,00$^{Ca}$ | 48,45 ± 5,22$^{Bbc}$ | 90,81 ± 4,73$^{Aab}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 203,43$^{***}$ | 5,14 | 11,15 |
| 12 | 0,00 ± 0,00$^{Ca}$ | 52,17 ± 9,50$^{Bbc}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 114,89$^{***}$ | 4,93 | 7,02 |
| 13 | 0,00 ± 0,00$^{Ca}$ | 55,99 ± 7,69$^{Bab}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 171,65$^{***}$ | 4,8 | 6,98 |
| 14 | 0,00 ± 0,00$^{Ca}$ | 74,14 ± 4,91$^{Ba}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 100 ± 0,00$^{Aa}$ | 398,69$^{***}$ | 3,72 | 7,13 |
| F | / | 40,87$^{***}$ | 63,74$^{***}$ | 402,86$^{***}$ | 1,00$^{*}$ | / | / | / | / |

*The values assigned to the same lowercase letter in the same column and the same capital letter on the same line does not differ significantly at the 5% threshold. *: P >0.05 (not significant); ***: P <0.001 (highly significant); /: F values were not determined because of total mortality.*

***Table 2**. Production of $F_1$ offspring under ambient laboratory conditions (t ≈ 21.5-29.0 ° C, RH ≈ 67.0-84.5%).*

| Products and doses (g / kg) | | Offspring $F_1$ | Reduction |
|---|---|---|---|
| *Plectranthus glandulosus* | | | |
| | 0 | 53,00 ± 1,52$^{a}$ | 0,00 ± 0,00$^{c}$ |
| | 5 | 29,66 ± 2,08$^{b}$ | 43,87 ± 3,36$^{b}$ |
| | 10 | 3,00 ± 1,00$^{c}$ | 94,34 ± 1,09$^{a}$ |
| | 20 | 0,00 ± 0,00$^{c}$ | 100 ± 0,00$^{a}$ |
| | 40 | 0,00 ± 0,00$^{c}$ | 100 ± 0,00$^{a}$ |
| Delvap Super® | 0,1 | 0,00 ± 0,00$^{c}$ | 100 ± 0,00$^{a}$ |
| | F | 722,42$^{***}$ | 845,661$^{***}$ |

*Values with the same letter in the same column for each parameter are not significantly different at 5%; ***: P <0.00*

## V. Conclusion

The methanolic extracts of leaves of *Plectranthus glandulosus* and Delvap Super® were tested on *Sitophilus zeamais*. These extracts were revealed to be toxic to *S. zeamais* adults by inducing a mortality which varies according to dose and period of exposure. These extracts also significantly reduced the emergence of offspring $F_1$ compared to control. After three months of storage, these extracts significantly suppress $F_1$ offspring at high dosages. The toxicity and inhibitory power of the $F_1$ offspring revealed by extracts of *P. glandulosus* with respect to *S. zeamais*, indicate that they may be suggested to contribute to the protection of stored seeds by causing the death of insects. During storage. Considering these results, we can say that these extracts could be recommended as alternative to synthetic insecticides against S. zeamais in storage.

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