

## **Insecticidal properties and persistence of *Berlinia grandifolia* (J. Vahl) and *Securidaca longepedunculata* Fres., two Aromatic Plants from the Far-North region of Cameroon alone or in Combination against *Sitophilus oryzae* L. (Coleoptera : Curculionidae).**

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**ABSTRACT:** The aim of the present work was to evaluate the insecticidal effects of two plants used by farmers as natural pesticide to manage stored sorghum pests in the Far-North Region of Cameroon. Dried powders of *Berlinia grandifolia* (trunk bark) and *Securidaca longepedunculata* (root bark) were tested against *Sitophilus oryzae*. Powders were tested at 0.05, 0.5, 1, and 2 g/100 g of sorghum grain for screening LD50 and LT50 on adults' mortality of each plant. Combinations of plant powder toxicity and persistence were evaluated with screening results data's on adults' weevils toxicity. Results showed that the effects varied with plant species, quantity and exposure time. *Securidaca longepedunculata* plant powder is the most toxic (LD50 = 0.07g/100g grain) and have rapid effects (LT50 = 3.72 days at 1 g/100 grain) than *Berlinia grandifolia* (LD50 = 2.046g/100g of grain; LT50 = 7.82 days at 1 g/100g of grain). The toxicity of *Berlinia grandifolia* on adults decreased of 33.87 % from 7 to 19 days post treatment. There was not decrease observed in *Securidaca longepedunculata* mortality rate in the same conditions (69.975 ± 3.072 % of mortality mean). The mortality rates induced by combinations of the powders (*Securidaca longepedunculata* + *Berlinia grandifolia*) were increased by the proportion of *Securidaca longepedunculata* used, and, after 3 to 7 days exposure time, a synergic effect was observed. These results suggest that a suitable strategy for pest management of stored sorghum is possible by using natural product.

**Keywords:** stored sorghum, *Sitophilus oryzae*, plant powder, toxicity persistence, synergic effects, Far-North Cameroon

### **INTRODUCTION**

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is the most important post-harvest insect pest of sorghum in Cameroon (Ngamo *et al.*, 2004). In a period of 100 days a single prolific female can produces 24 adults and more than 57% of grains are attacked (Ladang *et al.*, 2008). Futher more, these grains are the most common and constitute the bulk of food production in other parts of sahelian zone in Africa (Djanan, 2003 ; Hounhouigan, 2003 ; Nukenine, 2010 ; Guèye *et al.*, 2011). To prevent losses, producers usually rely on the use of chemical insecticides (Guèye *et al.*, 2011). These chemical insecticide used frequently and abusively can lead to the environmental pollution and the intoxication of consumers. (Ngamo, 2001 ; Kouninki *et al.*, 2007 ; Kaloma *et al.*, 2008, Kouninki *et al.*, 2010). There is an urgent need to develop user-friendly storage conditions with minimal adverse effects on the environment and on consumers. Since last decade, many researches on stored

products protection are based on the alternative methods to synthetic chemical pest control (Ngamo et Hance, 2007 ; Kouninki *et al.*, 2010 ; Nukenine, 2010, Afful *et al.*, 2012, Kouninki *et al.*, 2014; El Idrissi *et al.* ,2014 ). In Cameroon, many botanical insecticides have been found to control post-harvest pests: *Anonna senegalensis*, *Lippia rugosa*, *Hyptis spicigera* (essentials oils); *Azadirachta indica*, *Plectrantus glandulosus*, *Xylopi aethiopica*, *Ocimum canum*, *Vepris heterophylla* (powders) (Ngamo *et al.*, 2007 Ngassoum *et al.*, 2007; Kouninki *et al.*, 2007 ; Kouninki *et al.*, 2010 ; Nukenine *et al.*, 2010). In the Far-North Region of Cameroon, other investigations have revealed that *Berlinia grandifolia* (J. Vahl) and *Securidaca longepedunculata* Fres., two local's plants, are usually used by farmers as botanical insecticides to control insect pest during storage. These two local plants have not been tested in laboratories against the rice weevil. The objective of this study is to evaluate the insecticidal effect of these plants powders, alone or in combination on sorghum grain and its persistence effects against *S. oryzae*.

## MATERIALS AND METHODS

### Culturing of insects

The strain of *S. oryzae* was collected from infested stock of sorghum grains at the Mokolo market (Far-North region of Cameroon). Weevils were reared on sorghum in 1000 ml glass jar, in which 30 adults of mixed sex were introduced and kept under laboratory conditions to allow oviposition. The adults were removed after two weeks to enable the emergence by sieving the grain, after which the grains were reintroduced into the glass jars.

### Collection and Preparation of plant powders

Trunk bark of *B. grandifolia* (J. Vahl) and roots bark of *Securidaca longepedunculata* Fres. were collected and air-dried in Mokolo at room conditions during three months (July to September 2011). Each part of the plant were pounded in a wood mortar until the powder passed through 0,4 mm mesh sieve. The powders were stored in plastic jar in laboratory conditions for bioassays.

### LD50 and LT50 determination

Adults of Rice Weevil, *S. oryzae* were exposed with various increasing quantity of each plant separately. For each plant powder, four different rates were used: 0.5, 5, 10 and 20g /kg of sorghum seed, and five replications were made. *Sorghum bicolor* (L) Moench (Poaceae) seeds were purchased in local market of Mokolo during the harvest period (October-November 2011). Seeds were disinfested by keeping in a freezer at 5°C for 24 hours and exposed to sun light for 4 hours. The sorghum was kept in laboratories conditions. The moisture content of the seed (8.3 +/- 0.31%) was determined by using the method of AFNOR (1982). The mixture was subsequently handshaken for 2 minutes to have uniform coating. 20 unsexed weevils were introduced in each jar. Control consisted of sorghum without insecticidal product and sorghum treated by Malagrain at the standar rate. Malagrain DP 5 which contained malathion 5 % was used as positive control. This chemical insecticide was authorized for 10 years by the Ministry of Agriculture and Rural Developpement on december 30<sup>th</sup> in 2004 in Cameroon (CAPANET, 2005). The number of *adults* weevils' death was recorded after 1, 3, 7 and 14<sup>th</sup> days after weevil infestation. LD50 (rate of powder plant which induce 50% of weevil mortality) and LT50 (exposure period time which can cause 50% of weevil mortality) values were determined by Probit analysis (Finney, 1971).

### The persistence insecticide effects of *Berlinia grandifolia* and *Securidaca longepedunculata* against *Sitophilus oryzae*

500 ml glass jar containing 50 g sorghum seeds were treated using the LD50 of *B. grandifolia* and *S. longepedunculata* obtained for the persistence insecticide effects. The control consisted of 50 g of untreated sorghum seeds. For each treatment, 5 pairs of *S. oryzae* were introduced in each jar after 7, 11, 15 and 19 days after application of treatment (Table 1). For Each treatment four replications were made. The mortality of *S. oryzae* was recorded 4 days after infestation.

**Table1. Experimental protocol and days on which parameters were recorded**

Experiment	Days			
Preparation of treatments	0			
Insect introduction (infestation)	7	11	15	19
Mortality observation	11	15	19	24

**Insecticidal effect of *Berlinia grandifolia* and *Securidaca longepedunculata* combinations against *Sitophilus oryzae*.**

500 ml of jar containing 50 g sorghum seed were treated by one of the three combinations of *B. grandifolia* and *S. longepedunculata*. Three combinations with different proportions of 7-days LD50 of plant powders have been prepared (Table 2). Five pairs of weevils were introduced. Each treatment was replicated five times. Mortality of *Sitophilus oryzae* was recorded after 4<sup>th</sup>, 8<sup>th</sup> and 14<sup>th</sup> days after treatments in presence of the various plants powder combinations.

**Table 2. Different proportions of the plants combinations tests.**

Plant species	7-days LD50 proportions	
	<i>Securidaca longepedunculata</i> +	<i>Berlinia grandifolia</i>
Combination1=	1/4 +	3/4
Combination2=	1/2 +	1/2
Combination3=	3/4 +	1/4

During bioassays period, the daily temperature and humidity of laboratory ranged from 26.26 to 28°C and 20 to 20.61% RH.

**Data analysis**

Data were subjected to the ANOVA procedure using STATGRAPHIC PLUS software. Chi square test was applied to evaluate insecticide combination effects. Probit analysis (Finney, 1971) was applied to determine lethal concentration causing 50% (LD50) mortality of *S. oryzae* at different exposure time. Abbot's formula (Abbott, 1925) was used to correct control mortality before probit analysis and ANOVA.

**RESULTS**

**LD50 and LT50 determination**

Results obtained showed that insecticidal effects varied with plant species, dose rate and exposure time. Within 1 day exposure time, and for all applied rates, no insect mortality was observed. *Securidaca longepedunculata* is the most toxic (7-days LD<sub>50</sub> = 0.07g/100g grain) and have the most rapid effects (LT<sub>50</sub> = 3.72 days at 1 g/100 grain) than *B. grandifolia* (7-days LD<sub>50</sub> = 2.046g/100 g grain; LT<sub>50</sub> = 7.82 days at 1 g/100 grain) Table 4 and 5. The *S. oryzae* mortality caused by malagrain (100% at standard rate) was similar for all exposures times.

Depending on the post exposure time, mortality tended to increase with the increasing of the quantity of powder rates for *S. longepedunculata* (and LT<sub>50</sub> 10,490 to 3,489 days respectively for 0.05 to 2 % of powder rate), *B. grandifolia* (46,918 to 6,728 days respectively for 0.05 to 2 % of powder rate) (Table 4). High mortality rates of *B. grandifolia* (74 %) were gradually achieved within 14 days exposure time. Starting from 7 to 14 days exposure, higher mortality rates (94.6 +/-6.51 to 99 +/- 2.33%) were achieved for *S. longepedunculata*. These higher mean of toxicity do not differ significantly (Table 3).

**Table 3. Corrected cumulative mortality (mean ± SE)<sup>(a)</sup> of *Sitophilus oryzae* exposed to four concentrations of *Berlinia grandifolia* and *Securidaca longepedunculata* powder**

Product and concentration	Exposure period (days)			
	1	3	7	14
<b><i>B. grandifolia</i> (g/kg)</b>				
0	0.00 ± 0.00	0.00 ± 0.00 <sup>d</sup>	6 ± 4.1 <sup>d</sup>	7 ± 5.7 <sup>f</sup>
0.5	0.00 ± 0.00	0.00 ± 0.00 <sup>d</sup>	37 ± 14.83 <sup>c</sup>	12 ± 5.7 <sup>e</sup>
5	0.00 ± 0.00	17 ± 9.74 <sup>cd</sup>	52 ± 16.8 <sup>bc</sup>	61 ± 13.41 <sup>c</sup>
10	0.00 ± 0.00	28 ± 14.4 <sup>bc</sup>	52 ± 8.36 <sup>bc</sup>	56 ± 17.81 <sup>b</sup>
20	0.00 ± 0.00	34 ± 12.94 <sup>b</sup>	64 ± 21.9 <sup>b</sup>	75 ± 9.35 <sup>b</sup>
F value	-	66.07 <sup>**</sup>	26.85 <sup>**</sup>	60.51 <sup>*</sup>
<b><i>S. longepedunculata</i> (g / kg)</b>				
0	0.00 ± 0.00	0.00 ± 0.00 <sup>d</sup>	6 ± 4.1 <sup>d</sup>	7 ± 5.7 <sup>d</sup>
0.5	0.00 ± 0.00	24 ± 7.41 <sup>c</sup>	45 ± 16.95 <sup>c</sup>	54 ± 8.21 <sup>c</sup>
5	0.00 ± 0.00	58 ± 6.7 <sup>b</sup>	95 ± 5 <sup>b</sup>	96 ± 2.23 <sup>b</sup>
10	0.00 ± 0.00	69 ± 7.41 <sup>ab</sup>	94 ± 6.51 <sup>b</sup>	99 ± 2.23 <sup>ba</sup>
20	0.00 ± 0.00	74 ± 19.17 <sup>a</sup>	97 ± 6.7 <sup>a</sup>	99 ± 2.23 <sup>a</sup>
F value	-	53.11 <sup>**</sup>	107.87 <sup>**</sup>	379.77 <sup>**</sup>
Malagrain (0,5g / kg)	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>

(a)=Means in the same column followed by the same lower case letter do not differ significantly at ANOVA test. \*\* P<0.00001 ; \* P <0.0001  
 Each data represents the mean of four replicates.

**Table 4. Speed action of *Securidaca longepedunculata* and *Berlinia grandifolia* powders on *Sitophilus oryzae*.**

Concentration	n	R <sup>2</sup>	Equation of regression	LT50 Days (Hours)
<b><i>B. grandifolia</i></b>				
0.05	4	0.976	y = 1.923x + 1.786	46.918 (1126.03)
0.5	4	0.955	y = 2.868x + 2.163	9.754 (234.09)
1	4	0.924	y = 2.974x + 2.258	8.355 (200.52)
2	4	0.913	y = 3.243x + 2.315	6.728 (161.47)
<b><i>S. longepedunculata</i></b>				
0.05	4	0.912	y = 2.694x + 2.250	10.490 (251.76)
0.5	4	0.919	y = 4.206x + 2.397	4.157 (99.76)
1	4	0.943	y = 4.621x + 2.359	3.725 (89.4)
2	4	0.892	y = 4.523x + 2.545	3.489 (83.52)

**Table 5. *Securidaca longepedunculata* and *Berlinia grandifolia* toxicity on *Sitophilus oryzae* at 3, 7 and 14 exposure days.**

Plant species	N	R <sup>2</sup>	Equations regression	of LD <sub>50</sub> (in % = g/100g of seeds)
<b>3 days</b>				
<i>B. grandifolia</i>	3	0.936	Y = 2.909x + 4.060	2.104
<i>S. longepedunculata</i>	3	0.944	y = 0.363x + 7,27	0.30
<b>7 days</b>				
<i>B. grandifolia</i>	3	0.785	y = 0.1268x + 5.219	2.046
<i>S. longepedunculata</i>	3	0.752	y = 0,59x+ 4.795	0.07
<b>14 days</b>				
<i>B. grandifolia</i>	3	0.357	y = 0.405x + 4.874	0.671
<i>S. longepedunculata</i>	3	0.752	y = 0.659x + 4.495	0.04

**The insecticidal effects of *Berlinia grandifolia* and *Securidaca longepedunculata* against *Sitophilus oryzae* in combination**

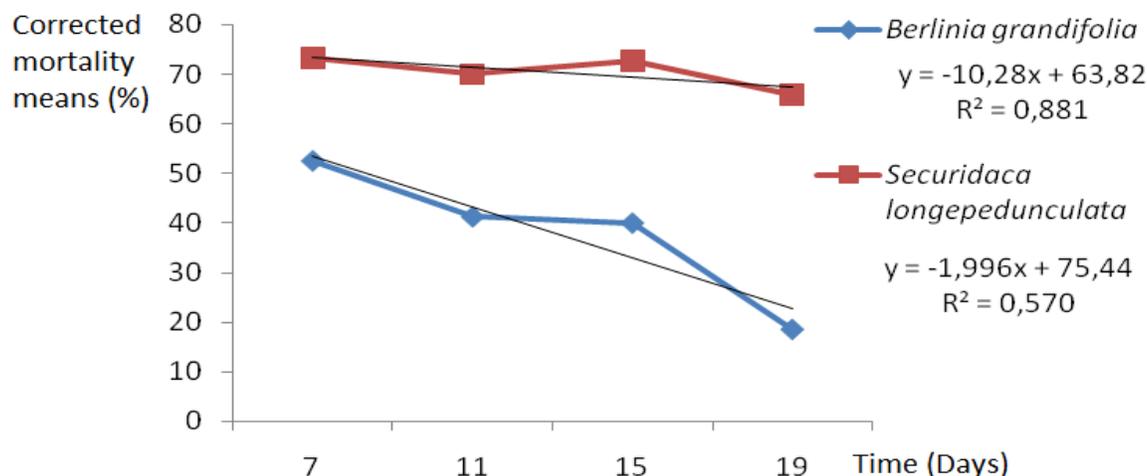
The insecticidal effect of *B. grandifolia* and *S. longepedunculata* against *S. oryzae* in combination depends on the proportions of each plant powder and the exposure time of *S. oryzae*. Mortality obtained were higher than expected within 7 exposure days (5% more in combination<sub>2</sub> and 13% combination<sub>3</sub>) but less than expected within 14 days exposure ( $\chi^2 \alpha=0.001$ ) (Table 6). Toxic effects increase with proportion rate of *S. longepedunculata* in all combinations.

**Table 6. Insecticidal activity of the three combinations of *Securidaca longepedunculata* and *Berlinia grandifolia* towards *Sitophilus oryzae*.**

Combinations	Cumulative mortality (%)		CHI <sup>2</sup> (α = 0,001)
	expected	observed	
<b>3 days</b>			
combination 1	25	25	4
combination 2	25	27.5	52
combination 3	25	40	284
<b>7 days</b>			
combination 1	50	41	44.625
combination 2	50	63	22.512
combination 3	50	55	108
<b>14 days</b>			
combination 1	100	65	56.619
combination 2	100	76	29.191
combination 3	100	87.5	11

**Persistence effects of *Berlinia grandifolia* and *Securidaca longepedunculata* toxicity against *Sitophilus oryzae*.**

The percentages of mortality according to the delay of the introduction of *S. oryzae* after application of the treatment are given in figure1. Mortality induced by *S. longepedunculata* led to 69.75 ± 3.072 % between 7 to 19 exposure days. Slight decrease observed was not significant ( $\chi^2 = 7.75$  p>0.05). *Berlinia grandifolia* powder mortality decreased from 52 to 18.63 % during the same observation period ( $\chi^2 = 25.27$ , p<0.05).



**Figure 1. Mortality of *Sitophilus oryzae* in relation with the day of its introduction after treatment of grain with *Berlinia grandifolia* and *Securidaca longepedunculata*.**

### DISCUSSION

Higher toxic effects observed with *S. longepedunculata* against *S. oryzae* justify the use of this plant as natural pesticide to protect stored grain in Cameroon, Ghana and Senegal (Seck, 1994; Belmain and Stevenson, 2001; Boeke *et al.*, 2004, Stevenson *et al.*, 2009, Afful *et al.*, 2012). Previous authors are demonstrated efficiency of root bark extracts in controlling the progeny of *S. zeamais* and *C. maculatus*. Securidacasides A and B (Stevenson *et al.*, 2009) and salicylate of methyl (Orwa *et al.*, 2009) are volatile compounds found in the roots of *S. longepedunculata*. These compounds are toxic against *C. maculatus*, *S. zeamais*, *R. dominica*, and *P. truncatus* (Seck, 1994; Jayasekara *et al.*, 2005; Afful *et al.*, 2012). After one day of exposure, neither *S. longepedunculata* nor *B. grandifolia* (0.5 to 2 %) has induced *S. oryzae* mortality. This result could be associated with the fact that applied rates were very low, comparatively to Ileke *et al* (2014), which has obtained 50 to 100% of weevils' mortality, respectively for 2.5 to 12.5% both with powders of *Sygygium aromaticum* and *Anacardium occidentale* on *S. oryzae*. The shortest time of contact with the plant can also be explained the obtained result, because within 3 days, mortality is increase significantly. Similar results were obtained with powders of *Azadirachta indica* and *Zanthoxylum zanthoxyloides* on *S. oryzae*, *Oryzaephilus mercator* and *Ryzopertha dominica* (Kayode et Olaniyi, 2014), essential oils of *X. aethiopicum* on *S. zeamais* (Kouninki, 2005), oil of *Arachis hypogea*, *Pinari macrophylla*, *Balanites aegyptiaca* on *Caryedon serratus* (Camara, 1997) and neem, eucalyptus, water hyacinth and guava powder on *P. truncatus* (Mukanga *et al.*, 2010).

Generally, the toxicity of both plants powders to stored product pest, *S. oryzae* is influenced by exposure time, applied dose and plant used (Oyeniyi *et al.*, 2015; Thambi and Cherian, 2015).

The persistence toxicity test result showed that *S. longepedunculata* has preserved toxicity (69,97 ± 3,072 %) while for *B. grandifolia* plant powder, the toxicity decreases rapidly. These results can be explained through chemical active component of the species plant used. Investigations on the essential oil of several aromatics plants in Northern Cameroon (Ngassoum *et al.* 2002a; 2002b, Jirovetz *et al.* 2002 ; Ngamo *et al.*, 2007; Goudoum *et al.*, 2013) had proven that plant species has more persistence toxic effect when they contained higher proportion of oxygenated molecules such as oxygenated monoterpenes and sesquiterpenes. Regnault-Roger *et al.* (2002) showed the lower volatility of oxygenated molecules because of their higher molecular weight. Thus, persistence effect of *S. longepedunculata* toward *S. oryzae* could be justify by their higher oxygenated sesquiterpenes contents (securidacasides A) which had been efficient against Curculionidae and Bruchidae (Seck, 1994 ; Stevenson *et al.*, 2009). Obeng-Ofori *et al.* (1997). Ngamo *et al.* (2007) and Goudoum *et al.*, (2013) showed also that persistence of insecticidal activity was in relationship with the sensitivity of the major target pest to active compound. Decreasing toxicity of *B. grandifolia* is similar to those results obtained with *X. aethiopicum* against *C. maculatus* (Kouninki *et al.*, 2010).

Combination test at different proportion of *S. longepedunculata* and *B. grandifolia* revealed synergic action both on toxicity effect and speed action of mixture (Ngassoum *et al.*, 2007; Khatun *et al.*, 2014). ( Similar results

were obtained by investigations made with essential oil combinations of *H. spicigera* + *V. heterophylla* and *O. canum* + *V. heterophylla* (Ngassoum *et al.*, 2007). Synergic effects were observed on both the toxicity on *S. oryzae* on reducing lethal time (LT50) and increased repellent properties of these combinations. The volatile nature of the compounds of the plants powder used could have induced chemical reactions and so the emergence of new toxic molecules or disappearance of antagonist compounds to toxic molecules (Ngassoum *et al.*, 2007); the combinations of low proportions of *B. grandifolia* induce higher mortality than expected and are faster than expected. *B. grandifolia* could amplify the toxic potency of *S. longepedunculata*, and therefore, the types of interaction between the combinations of different powders depends on the mixing ratios. Similar observations were made with combinations of chemical insecticides pirimiphos-methyl and other three essential oils in different proportions against *C. maculatus* by Khalequzzaman Rumu (2010). These authors have observed synergistic effects between pirimiphos-methyl and *Elettaria cardamomum* (L.) only the ratio of 1:20. Other combinations (ratios of 1:1, 1:2, 1:5, 1:10) are rather antagonistic effects.

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