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Bioefficacy of *Aguaria salicifolia* and *Plectranthus kirbii* leaf powders to protect bean grains against infestation by *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae)

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Abstract
The bioefficacy of *Plectranthus kirbii* and *Aguaria salicifolia* leaf powders to protect stored bean against infestation of *Acanthoscelides obtectus* was assessed. The ability of these plants to induce adult mortality, inhibit progeny production, reduce grain damage and insect population growth, as well as to preserve seed viability was tested at different contents. *Plectranthus kirbii* and *A. salicifolia* caused significant mortality to adult *A. obtectus*. The LC_{50} of *P. kirbii* and *A. salicifolia* were 11.67 and 12.67 g/kg respectively after 10 days of contact. The F_1 progeny production was significantly inhibited by both powders. The two powders considerably suppressed population of *A. obtectus* and reduced weight loss of bean after six months of storage. The seeds treated with *P. kirbii* and *A. salicifolia* significantly germinated compared to the untreated ones. Considering these results, *A. salicifolia* and *P. kirbii* could be used for the protection of stored beans.

Keywords: *Acanthoscelides obtectus*, *Plectranthus kirbii*, *Aguaria salicifolia*, leaf powder, bean

1. Introduction
The food availability around the world is characterized by a serious instability. This situation is exacerbated in tropical and subtropical regions especially in developing countries. The food supply in Africa remains insecure and unsure. This situation is due to several factors. The insecurity induced by war and conflicts reduces agricultural productivity. The agricultural lands are sometimes inaccessible then reducing the culture zones in addition the number of workers is seriously reduced because some part is engaged in the conflicts another part leave their villages to escape from violence. Food supply faces lot of difficulties, even produced food distribution out of production zones constitutes a great challenge; there is a lack of infrastructures and poor routes, which considerably disturb the circulation of food from production pool to the consumers. The food production in Africa is very low compared to the population need, that is due to the low productivity caused by poor agricultural technology and lack of financial means; the agriculture nowadays is very challenging and necessitates much technology and financial to cope with demand. In addition to these factors threatening food security, the losses during food storage remain a serious problem, because after overcoming all the challenges linked to production and distribution, the harvest can be lost during storage if appropriate methods or measures are not taken.
In Africa, an estimated 10% to 30% of all food produced yearly is destroyed by insect pests [1]. *Phaseolus vulgaris* L., one of the most important legumes crops worldwide can be affected by various pests, both in fields and in storage. *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae: Bruchinae), is a major pest of common bean that begin its infestation in the field continuing the damage during storage, where it causes greatest losses, to the point of producing the loss of whole crops within a few months [2, 3]. *Acanthoscelides obtectus* exhibits high tolerance to various degrees of temperature, thus, it is found in cool highland areas as well as the warmer parts of the tropics [4].
The common bean availability can be increased by reducing storage losses. Then the control of insect pest in storage such as *A. obtectus* appears very necessary. Damage caused by *A. obtectus* on bean could be reduced through chemical, biological, physical control and host

plant resistance, which are important components of integrated pest management strategies. Globally, farmers largely rely on chemical substances to protect their crops as well as stored agricultural products against insect pests, and diseases [5]. Concerns about rapid evolution of insecticide resistance and the impact of these chemicals in environmental pollution and human health have intensified the search for alternative eco-friendly strategies of pest management [6, 7]. Several studies indicate that a wide range of health problems are closely related to the widespread use of toxic chemicals on crops. These problems include birth defects, hormonal problems and nervous system damage [8]. Therefore, an environmentally safe and economically feasible pest control practice needs to be available. Botanicals are relatively environmentally safe. They are generally considered to be more biodegradable leading to less environmental problems [9, 10]. Biopesticides especially plant extracts are biodegradable, cheaper and more accessible in developed countries, and also less demand in term of technology.

The insecticide-based plant extract is considered as alternative to chemical synthetic pesticide in term of cost, protection of environment, low technology demand and availability. These insecticidal extracts can be used in different formulations, and they can be employed as powder, solvents extracts, essentials oils, vegetable oils depending on purpose, plant species or the part of plant used and type of substrate. *Aguaria salicifolia* (Ericaceae) and *Plectranthus kirbii* (Lamiaceae) are two plants found in Cameroon flora precisely in Mount Cameroon. These plants are largely available and easily accessible by smallholders for their own use, then it is necessary to carry out research work to assess their ability to protect one of the most stored and consumed pulses in this country. *Agauria salicifolia* is a plant found in Central Africa, South Africa and Madagascar [11], it is used in traditional medicine to treat snake bite, skin problems, rhumatism etc [12]. As insecticide this plant revealed toxic against mustard weevil, *Phaedon cochleariae*, but in our knowledge less data showed the insecticidal effect of this plant on stored insect pests. The same observation is done for *P. kirbii* which belongs to the Lamiaceae family whose members are characterized by its richness in essential oils which contain volatile compounds conferring to these plants their insecticidal properties. Therefore, it is becoming important, useful even imperative to evaluate the ability of these plants especially their leaves to protect stored bean *P. vulgaris* against infestation by *A. obtectus*.

2. Materials and methods

2.1. Insect rearing

The strain of *A. obtectus* used in this study was obtained from the culture maintained in the laboratory of Biological Sciences, Faculty of Science of the University of Bamenda. The bruchids were reared on disinfected common bean in 900 mL glass jars and kept under fluctuating laboratory conditions. Since the life span of adult *A. obtectus* is short, the insects used were aged ≤ 3 days to allow a better assessment of the plant powder efficacy.

2.2. Plants harvest and processing

Green leaves of *P. kirbii* and *A. salicifolia* were collected in September and october 2020 at Lebialem in the South-west region of Cameroon, precisely at Magha-Atuallah Road, latitude 5°40'46.1''North and longitude 10°03'39.2''East, at an altitude of 2522 m above sea level. The identity of the plant was confirmed by an Ethno-botanist of the University of Bamenda who accompanied the researchers during plant collection. The identification of these plants was confirmed at the Cameroon National Herbarium in Yaounde, *A. salicifolia* with voucher number N°33530SRF Cam and *P. kirbii* was identified in comparison with the Lamiaceae of Gabon flora. The leaves were dried at room temperature for 10 days and then crushed. The crushed leaves were ground using locally made pestle and mortar until the powder passed through a 0.20 mm sieve. Then, the powder was stored in a freezer at −4 °C until needed for bioassays.

2.3. Grain conditioning

The bean variety used was a locally cultivated variety in North-West Cameroon. Before experimentation, broken grains, the pieces of stone, sand and other foreign materials were removed from the stock. Then, the seeds were kept in the freezer at −20 °C for 14 days to allow its disinfestation. After disinfestation from all types of living organisms, the bean was kept in ambient conditions of laboratory for 14 days for its acclimatisation. After all these steps, the bean was ready for use as substrate for insect rearing and bioassays. The moisture content of bean was determined before experiment, it was 11.65±0.42%.

2.4. Phytochemical screening of *Plectranthus kirbii* and *Aguaria salicifolia* leaf

The leaf plant powder was firstly dissolved in mixture methylene chloride/methanol in the proportion of 1/1. The extract obtained for each plant was used for phytochemical screening to detect alkaloids, phenolic compounds, terpenoids and sterols, tannins, glucosides, anthraquinones, coumarins, anthocyans and saponins using the standard methods described by Harbone [13].

2.5. Toxicity and progeny tests

Different quantities of *P. kirbii* or *A. salicifolia* leaf powder (0.2; 0.4; 0.8 and 1.6 g) were added to 50 g of common beans in 450 mL glass jars covered with perforated lids to allow aeration. These different masses of leaf powder constituted the different contents used; 4, 8, 16 and 36 g/kg. The mix of bean and plant powder was shaken manually for five minutes to allow uniform coating of powder on seeds. Twenty adults of *A. obtectus* of non-determined sex were added in each glass jar. The control was constituted of beans without plant powder and infested with the same number (20 insects) of bruchids. After adding insects, the jars were covered and displayed on shelves in ambient laboratory conditions. In order to determine mortality, the observations were carried out 1, 3, 5, 7 and 10 days. During these observations, the dead and live *A. obtectus* were counted.

After recording of 10 days mortality, all insects and products were discarded. The grains were left inside the jars and the counting of F_1 adults was carried out once a week for five weeks commencing from first insect emergence. The observations were done every week up to the last emergence of F_1 progeny. After each counting session, the insects were removed from the jars and their number recorded. The inhibition F_1 progeny (%IR) was calculated as

$$\%IR = Cn-Tn/Cn \times 100$$

Where Cn is the number of newly emerged insects in the untreated jar and Tn is the number of insects in the treated jar.

2.6. Population growth and damage bioassay

The masses of 0.4, 0.8, 1.6 and 3.2 g of leaf powder of *P. kirbii* and *A. salicifolia* were added to 100 g of common bean, these quantities constituted the contents of 4, 8, 16 and 32 g/kg respectively. The jars containing bean and plant powder were hand shaken to permit uniform distribution of powder on bean surface.

Then 20 adult bruchids of non-determined sex were introduce in the glass jars. The control was constituted by the same quantity of bean and same number of insect but without plant powder. The treatments were displayed on the shelves. The storage time was six months; from May to November 2021. After this period the number of insects in each jar was recorded in order to determine the population growth. Damage assessment was performed by measuring the weight loss of bean using the method of Adams and Schulten [14].

2.7. Viability test

In order to assess the viability of seeds, seed germination was tested using 30 randomly picked beans from each jar especially the non-perforated seeds. These seeds were selected from among those stored for 6 months with leaf powder of *P. kirbii* and *A. salicifolia* after separation of the perforated grains from the non-perforated ones in each jar. The seeds were placed on moistened paper in 9 cm petri dishes. The preparations were watered every two days. The number of germinated and non-germinated seeds was recorded after 10 days [15].

2.8. Data analysis

Abbott's formular [16] was used to correct for control mortality before analysis of variance (ANOVA) and probit analysis. Data on cumulative corrected mortality, reduction in F_1 progeny, damage, weight loss and germination percentage were arcsine-transformed [(square root(x /100)], and the number of F_1 progeny was log transformed (x+1). The transformed data were subjected to the ANOVA procedure using the statistical analysis system [17, 18]. Probit analysis [18, 19] was conducted to determine lethal dosages and mortality of *A. obtectus* at 1, 3, 5, 7 and 10 days after treatment application. The probit analysis was also used to determine the effective content causing 50% (EC_{50}) and 95% (EC_{95}) reduction in *A. obtectus* F_1 progeny.

3. Results

3.1. Chemical composition of *Plectranthus kirbii* and *Aguaria salicifolia* leaf

The phytochemical analysis was carried out on ten chemical families that were alkaloids, phenolic compounds, flavonoids, terpenoids and sterols, tannins, glucosids, anthraquinones, coumarines, anthocyanes and saponins (Table 1). This analysis revealed that glucosids and saponins compounds were not found in the leaves of *P. kirbii* and *A. salicifolia*. The phenolic compounds were very abundant in the leaves of the two plants species whereas alkaloids, terpenoids and sterols were abundant in both plants' species. Anthraquinones and coumarines were very abundant in *A. salicifolia* compared to *P. kirbii* where analysis showed their presence but very low. The anthocyanes compounds were more abundant in *A. salicifolia* than *P. kirbii* leaves.

Table 1: Phytochemical screening of leaf powder of *Aquaria salicifolia* and *Plectranthus kirbii*

Compounds	*Aguaria salicifolia*	*Plectranthus kirbii*
Alkaloids	++	++
Phenolic compound	+++	+++
Flavonoids	++	+
Terpenoids and sterols	++	++
Tanins	++	++
Glucosids	-	-
Anthraquinones	++	+
Coumarins	++	+
Anthocyanins	+++	++
Saponins	-	-

+: present (but low); ++: Abundant; +++: very abundant; -: Absent.

3.2. Toxicity induced by *Plectranthus kirbii* and *Aguaria salicifolia* leaf powders

Plectranthus kirbii and *Aguaria salicifolia* induced significant mortality of *A. obtectus* adult (Table 2). This mortality was content and period dependent, as the dosage increased the mortality increased too. *Aguaria salicifolia* at its lowest content (4 g/kg) registered 6.84% adult mortality within 3 days, but within 10 days at same content of this plant caused 26.14% mortality. *Plectranthus kirbii* recorded 5.09% mortality within same exposure period (3 days), this mortality considerably increased to reach 30.72% in 10 days. The highest mortality was achieved by the two plant leaf powders within 10 days of exposure; it was 80.72 and 81.15% respectively for *P. kirbii* and *A. salicifolia*.

Table 2: Mortality of *Acanthoscelides obtectus* induced by the two plants powders within 1, 3, 5, 7 and 10 days of exposure in the fluctuating laboratory conditions (Temp.=15-26°C; RH=74-92%)

Content (g/kg)	1 day	3 days	5 days	7 days	10 days	$F_{(4; 10)}$
	Aguaria salicifolia					
0	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{E}	0.00 ± 0.00^{C}	
4	0.00 ± 0.00^{Bc}	6.84 ± 1.58^{BCbc}	7.12 ± 1.71^{CDbc}	18.13 ± 3.52^{Dab}	26.14 ± 7.19^{Ba}	7.76***
8	0.00 ± 0.00^{Bb}	8.51 ± 3.25^{Bb}	14.23 ± 3.42^{BCb}	32.75 ± 3.26^{Ca}	41.29 ± 6.15^{Ba}	20.86***
16	5.00 ± 0.00^{Ac}	12.02 ± 1.49^{Bcd}	21.44 ± 0.39^{Bc}	49.03 ± 2.41^{Bb}	67.76 ± 4.47^{Aa}	124.51***
32	6.67 ± 1.67^{Ae}	20.70 ± 0.35^{Ad}	32.16 ± 0.58^{Ac}	67.25 ± 3.26^{Ab}	81.15 ± 1.71^{Aa}	295.22***
$F_{(4; 10)}$	19.00***	18.70***	51.73***	86.67***	46.76***	
	Plectranthus kirbii					
0	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{D}	0.00 ± 0.00^{C}	
4	0.00 ± 0.00^{Bd}	$5.09\pm2.89B^{Ccd}$	12.28 ± 1.75^{Bbc}	21.73 ± 2.80^{Cab}	30.72 ± 3.64^{Ba}	23.77***
8	0.00 ± 0.00^{Bd}	$6.84\pm1.58B^{Ccd}$	14.04 ± 3.51^{Bc}	30.90 ± 4.82^{BCb}	44.23 ± 1.70^{Ba}	40.01***
16	5.00 ± 0.00^{Ac}	12.02 ± 1.49^{Abc}	19.30 ± 1.75^{Bc}	43.57 ± 5.17^{ABb}	65.14 ± 6.32^{Aa}	43.01***
32	6.67 ± 1.67^{Ac}	17.19 ± 1.40^{Acd}	31.58 ± 3.04^{Ac}	60.04 ± 3.38^{Ab}	80.72 ± 5.26^{Aa}	88.52***
$F_{(4; 10)}$	19.00***	14.49***	23.72***	36.95***	58.34***	

Mean ± S.E. followed by the same capital letter in a column and the same lower letter in a line do not differ significantly at $P < 0.05$ (Tukey's test). Each datum represents the mean of three replicates of 20 insects each. ^{ns} $P > 0.05$; *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$; Temp.: temperature; RH: relative humidity.

The lethal contents 50 (LC_{50}) and 95 (LC_{95}) reduced when the exposure periods increased (Table 3). The LC_{50} of *P. kirbii* and *A. salicifolia* were 51.48 and 45.56 g/kg respectively in 5 days. But in 10 days the LC_{50} of the same plants in the same order were 11.67 and 12.67 g/kg respectively. The same tendency was observed with LC_{95}, the lowest values were recorded within 10 days exposure; *P. kirbii* and *A. salicifolia* leaf powder recorded 45.98 and 43.30 g/kg respectively.

Table 3: Toxicity parameters of *Aguaria salicifolia* and *Plectranthus kirbii* on *Acanthoscelides obtectus* adult in the ambient laboratory conditions (Temp.=15-26°C; RH=74-92%)

Products	Slope ± SE	R^2	LC_{50} (95% FL) g/kg	LC_{95}(95% FL) g/kg
5 days				
A. salicifolia	0.031±0.004	0.888	45.56(37.63; 60.99)	99.31(78.13; 142.30)
P. kirbii	0.025±0.002	0.800	51.48(42.88; 67.12)	118.41(94.57; 162.86)
7 days				
A. salicifolia	0.044±0.003	0.892	20.02(17.01; 23.84)	57.18(47.62; 73.92)
P. kirbii	0.035±0.004	0.834	23.38(19.51; 29.28)	70.44(56.21; 98.85)
10 days				
A. salicifolia	0.053±0.003	0.789	12.49(8.95; 16.25)	43.30(34.80; 60.94)
P. kirbii	0.048±0.005	0.828	11.67(7.68; 15.09)	45.98(37.35; 62.87)

FL: Fudicial limit; LC: lethal content; FL: Fuducial limit; Temp.: temperature; RH: relative humidity.

3.3. F_1 progeny production inhibition

The F_1 progeny production of *A. obtectus* was significantly inhibited by the leaf powder of *P. kirbii* and *A. salicifolia* (Table 4). This inhibition was dose dependent, the production of F_1 progeny decreased as the content increased. The lowest content reduced the progeny production by 36.92% (65.00 bruchids) and 48.46% (48.46 bruchids) in bean treated with *P. kirbii* and *A. salicifolia* respectively compared to the control (105 bruchids). The highest inhibition of F_1 progeny was performed by each plant powder with the highest content (32 g/kg); 70.12% for *P. kirbii* and 86.92% for *A. salicifolia*. The reduction was more important with *A. salicifolia* than *P. kirbii* as confirmed by their effective contents 50 and 95 (EC_{50}; EC_{95}); the EC_{50} value of *P. kirbii* leaf powder was two times more (9.98 g/kg) than that obtained with *A. salicifolia* (4.46 g/kg).

Table 4: Inhibition of Progeny production of *Acanthoscelides obtectus* by *Plectranthus kirbii* and *Aguaria salicifolia* leaf powder under ambient laboratory conditions (Temp. =15-26°C; RH=74-92%)

Content (g/kg)	Plant		t value
	Plectranthus kirbii	*Aguaria salicifolia*	
Mean number of F_1 progeny (Mean±SE)			
0	105.00±4.73^{a}	105.00±4.73^{a}	
4	65.00±11.24^{b}	54.33±4.91^{b}	0.66^{ns}
8	51.67±8.67^{b}	39.67±5.90^{b}	1.56^{ns}
16	36.33±6.06^{b}	34.00±5.90^{bc}	0.36^{ns}
32	31.00±4.48^{b}	14.00±3.77^{c}	2.12^{ns}
$F_{(4; 14)}$	15.52***	49.84***	
Inhibition of adult emergence relative to control (%)			
0	0.00±0.00^{c}	0.00±0.00^{d}	
4	36.92±13.13^{ab}	48.46±2.30^{c}	-0.75^{ns}
8	49.89±10.37^{a}	62.09±6.01^{bc}	-1.33^{ns}
16	65.07±6.77^{a}	67.80±3.33^{b}	-0.42^{ns}
32	70.12±5.47^{a}	86.92±3.09^{a}	-1.98^{ns}
$F_{(4; 14)}$	11.06**	86.28***	
EC_{50} (95% FL) g/kg	9.98(1.81; 15.18)	4.61(2.62; 6.35)	
EC_{95} (95% FL) g/kg	67.24(41.60; 464.96)	45.46(36.85; 62.28)	

Mean ± S.E. followed by the same lower-case letter in a column do not differ significantly at $P < 0.05$ (Tukey's test). ^{ns}: $P > 0.05$; *: $P < 0.05$; **: $P < 0.001$; ***: $P < 0.0001$; Temp.: temperature; RH: relative humidity; EC: effective content.

3.4. Suppression of population growth and reduction of grain damage

Plectranthus kirbii and *A. salicifolia* leaf powder considerably suppressed population growth of *A. obtectus* and reduced weight loss of stored bean (Table 5). The weight loss reduction decreased as the plant powder content increased, the same tendency was also observed for population suppression. *Aguaria salicifolia* leaf powder at its lowest content (4 g/kg) suppressed more than the half (460.75 insects) of population in untreated bean (935.25 insects at 0 g/kg). *Plectranthus kirbii* at its lowest content (4 g/kg) considerably reduced population growth of *A. obtectus* compared to the control (it was 662.50 insects against 935.25 insects recorded in non-treated bean). Lowest population growth was observed for both plants at their highest content (32 g/kg); 232.50 and 295.25 bruchids respectively for *A. salicifolia* and *P. kirbii* leaf powder against 935.25 for control (non-treated bean).

Table 5: Weight loss of conserved bean induced by the population increase of *Acanthoscelides obtectus* under ambient laboratory conditions (Temp. =15-26°C; RH=74-92%)

Contents (g/kg)	Plant		t value
	Plectranthus kirbii	*Aguaria salicifolia*	
Insect number Mean±SE			
0	935.25±30.36^{a}	935.25±30.36^{a}	
4	662.50±24.92^{b}	460.75±47.01^{b}	2.81^{ns}
8	612.50±31.19^{b}	417.50±31.68^{cd}	3.19*
16	485.00±9.94c	322.25±7.10^{de}	31.88***
32	295.25±3.66^{d}	232.50±10.40^{e}	4.47*
$F_{(4; 15)}$	105.70***	86.60***	
Weight loss (%)			
0	54.47±1.80^{a}	54.47±1.80^{a}	
4	41.61±0.54^{b}	34.84±2.00^{b}	3.47*
8	36.32±1.92^{b}	27.15±0.40^{bc}	4.97*
16	33.37±2.46^{b}	24.76±1.41^{c}	2.80^{ns}
32	24.04±2.77^{c}	14.51±3.82^{d}	1.97^{ns}
$F_{(4; 15)}$	30.00***	46.41***	

Mean ± S.E. followed by the same lower-case letter in a column do not differ significantly at P < 0.05 (Tukey's test). ^{ns}: P > 0.05; *: P < 0.05; **: P < 0.001; ***: P < 0.0001; Temp.: temperature; RH: relative humidity.

The reduction of weight was significant, from the application of leaf powder. The highest reduction of weight loss was induced by the highest content of the plant (32 g/kg). At this content the bean stored for six months recorded 24.04 and 14.51% weight loss when treated with *P. kirbii* and *A. salicifolia* respectively compared to non-treated bean (control) with 54.47% weight loss. During storage, bean weight loss and insects' growth were positively correlated (Figs 1A and AB), as bruchids increased the bean weight loss increased too. Then weight loss was greatly affected by the number of insects.

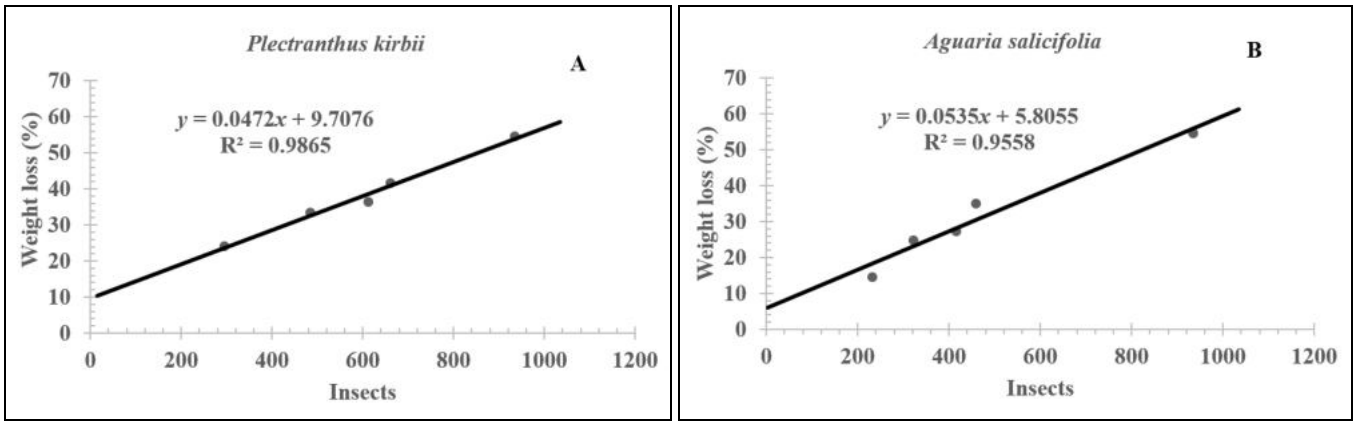

Fig 1: Corelation between bean weight loss and population increase of *Acanthoscelides obtectus* during storage

3.5. Germination of conserved seeds

The seeds of *P. vulgaris* conserved by the leaf powder of *P. kirbii* and *A. salicifolia* significantly germinated compared to the untreated seeds or control (Table 6). The bean treated with *P. kirbii* at different contents statistically recorded the same germination rate, however its highest rate (87.50%) was reached at its highest content (32 g/kg). The germination rate of bean preserved with *A. salicifolia* varied with contents, as content increased the viability rate increased too. The highest germination rate (90%) was obtained by *A. salicifolia* at its highest content (32 g/kg). The lowest germination rate of 53.33%. Was observed in non-treated (control). The seeds treated with the *A. salicifolia* and *P. kirbii* leaf powder at each content did not show statistical difference (t=-0.52−2.91; P>0.05).

Table 6: Germination of seeds bean treated with the leaf powder of *Plectranthus kirbii* and *Aguaria salicifolia* and stored for six months under fluctuating laboratory conditions (Temp.=15-26°C; HR=74-92%)

Content (g/kg)	Plants		t value
	Plectranthus kirbii	*Aguaria salicifolia*	
0	53.33±4.08^{b}	53.33±4.08^{c}	
4	78.33±2.90^{a}	74.17±2.10^{b}	1.06^{ns}
8	83.67±1.36^{a}	74.20±2.50^{b}	2.90^{ns}
16	86.67±1.36^{a}	85.83±4.38^{ab}	0.19^{ns}
32	90.00±2.36^{a}	87.50±2.50^{a}	0.52^{ns}
$F_{(4; 15)}$	28.69***	19.49***	

Mean ± S.E. followed by the same lower-case letter in a column do not differ significantly at P < 0.05 (Tukey's test). ^{ns}: P > 0.05; ***: P < 0.0001; Temp.: temperature; RH: relative humidity.

4. Discussion

The dose dependent mortality of *A. obtectus* obtained from the present study showed that the two plant species leaves contained insecticidal compounds whose quantity increased with increasing mass of powder and their efficacy is improved with the ascending exposure periods. The toxicity increased as the contact of insect with plant powder was multiplied due to the prolonged time of contact of insects with the plant powder. The toxicity of these plants may be attributed to their chemical compounds. The phytochemical screening showed that the two plants contained the compounds such as alkaloids, phenolics, flavonoids, terpenoids, tannins, coumarins and anthocyanins, where previous studies revealed their insecticidal properties. Adebowale and Adedire [20] reported that the toxic effect of *Jatropha curcas* seed could be due to the presence of several sterols and terpene alcohols which have been known to exhibit insecticidal properties to *Callosobruchus maculatus*. In the same order, some studies found that the plant species contain secondary metabolites which are important source of chemical compounds such as the steroids, phenolic compounds and tannins with wide range of biological activity reported to have great impact on insecticidal activities [21, 22]. Ileke *et al*. [23] showed that the presence of alkaloids, flavonoids, saponins and tannins in the powders and ethanolic extracts of *Acanthus montanus, Acanthospermum hispidum, Argyreia nervosa* and *Alchornea laxiflora* confers to these plant species their insecticidal activity against *S. zeamais* the main insect pest of maize grain. Several mechanisms and modes of action can explain the bioactivity of the leaf powder of *A. salicifolia* and *P. kirbii*. Researches have revealed that the complex mixtures in plant powders, extracts or oils inhibited acetyl cholinesterase enzyme (AChE) action [24]. The inhibited acetyl cholinesterase enzyme (AChE) activity interferes with the neuromodulator octopamine [25, 26], it can also block GABA-gated chloride channels of the insect pest resulting to their death [27]. In addition to the disturbance of nervous system by the active compounds contained in plant powder, the asphyxia could be evoked when the powder reduces and even suppress the respiratory gas exchange. Most insects breathe by means of trachea which usually opens at the surface of the body through spiracles. These openings or air chambers might have been hindered from receiving enough oxygen into the body of the insects which eventually led to their asphyxiation and death [28].

The inhibition of progeny production of insect pest by insecticidal substance is carried out though reduction of fecundity and fertility, and disturbance of immature stages' development. *Aguaria salicifolia* and *P. kirbii* leaf powder could act by these ways since they significantly inhibited the production of *A. obtectus* F_1. This inhibition was also dose dependent; it means that the quantity of inhibiting compound is increased by increasing of leaf powder quantity. These phytochemical compounds can affect the reproductive potential, as reported by Kaur and Rup [29], coumarins, besides the mortality that they induce, may exert other negative effect on insect such as decreasing reproductive potential. The plant powders used in the present study could disrupt the egg development, for this effect the leaf powders must have passed through the eggs chorion and by this mean disrupt normal developmental stages from eggs to adults as suggested by Ileke and Olotuah [30] when the insect was able to lay egg. Concerning egg hatching, the larva of insect pest can be killed by the active compounds of plant powder. Such observation was done by Velu *et al*. [31] concerning mosquito vectors of chikungunya and malaria. These authors observed that the alkaloids from *Arachis hypogea* extract have larvicidal activity. In addition, the uniform coating of seeds with powder may had prevented egg laying and this could be also a raison of reduction of progeny. The inhibition of progeny production may be almost the same for both plants powders, but *A. salicifolia* was a bit more effective than *P. kirbii*, this could be explained by their chemical composition, even though the same, the presence of bioactive compounds such as flavonoids, coumarins, anthraquinons and anthocyanes were more marked in *A. salicifolia* than *P. kirbii*.

Plectranthus kirbii and *A. salicifolia* leaf powder significantly suppressed population of *A. obtectus* and reduced damage on *P. vulgaris* seeds. The bean weight loss was positively correlated with the insect population growth; therefore, it becomes important even capital to suppress population growth in order to reduce grain weight loss. The protection of pulses and cereals against bruchids and weevils are documented for many plants species but no information in our knowledge has been found for the plants used in this research work. Delobel and Malonga [32] reported that *Eupatorium odoratum*, *Nicotiana tabacum* leaf powder are used by farmer to protect beans against *A. obtectus* and *C. maculatus*. Hikal *et al*. [22] showed that seed powder of *J. curcas* and *Annona muricata* are good protectant of stored rice grain from the attack of *S. zeamais*. In addition to other insecticidal mechanisms already mentioned, the leaf plant can render the treated bean improper for consumption by the insect pest thanks to its chemicals, when ingested some of these chemicals may act negatively on digestive system. Agrawal *et al*. [33] and Vattikonda *et al*. [34] reported the inhibition of digestive enzymes and delaying of development by anthocyanins and phytoalexins. These two chemical compounds act as insect growth inhibitors, which are mediated by the limited assimilation of dietary proteins. Flavonoids can also act on insect nutrition, precisely as deterrents [35]. After six months of storage there was observed an important increase of population growth, this can be explained by the reduction of active compounds due the fluctuating laboratory conditions for example the humidity which increases grain moisture content and absorption of humidity by plant powder which diluted the quantity of available active ingredients. The combination of several active compounds found in these plants' powders reduce the arise of resistance. Coumarin, one the compounds found in the used plants is able to alter the detoxication capability of insect, by reversibly or irreversibly inhibiting cytochrome P450 detoxication enzymes [36, 51]. This cytochrome confers to the insect the ability to produce enzyme responsible for the degradation of the insecticidal molecule, which induce resistance to the insecticidal product. But the plants tested in this work were constituted by many molecules that reduce the potential of insect pest to develop resistance.

One of the objectives for grain storage in the developing countries such as Cameroon, is to provide not only grain for meal but also seed for sowing. The seed of *P. vulagris* protected by the leaf powder of *P. kirbii* and *A. salicifolia* conserved their germination rate during the six months of storage. All leaf powder contents recorded higher germination rate compared to the control. No difference was

observed for the same content between the two plants. The germination rate slightly varied with contents for each plant. The loss of viability recorded by control can be explained by the attack induced by *A. obtectus*, even these seeds looked not perforated but can be infested by the immature stage of insect which consume the part of seed especially grain embryo for its development. By this action, it may damage the seed embryo and diminish viability. The increase of bean viability with ascending plant content could be attributed to the protective effect of powder which increased with content then the quantity of active plant ingredients. The plant powders did not negatively affect the germination of the conserved bean. Chukwuka *et al*. [38] found that maize (*Zea mays* L.) seed treated with extracts of *Tithonia diversifolia* and *Vernonia amygdalina* produced the same germination as the control, and the growth, development and yield were not significantly affected by all extracts. Miafo *et al*. [39] reported that the germination of cowpea seeds was unaffected after treatment with ethanolic leaf extracts of *Balanites aegyptiaca*, *Melia azedarach* and *Ocimum gratissimum.* Kasa and Tadese [40] who reported that the use of crude powders of 17 botanical plant species for the control of *S. zeamais* on sorghum had no effect on seed germination. Even though, it is not the case here, some plant extracts in other studies reduced the seed viability. Extracts of some plants have found to possess phytotoxic effect on seeds of vegetable and other crops by inhibiting germination [41-43]. The wheat seed treated with the extract of *Calotropis procera* recorded highest germination rate at 5% concentration, but with the higher concentration (7% and 10%) the seed germination percentage decreased to 40% [44]. Inhibition on seed germination affects typically result from a combination of allelochemicals which interfere with various physiological processes in the receiving plant [45]. Mangal *et al*. [46] found that seed germination and radicle length of *Vigna sinensis* L. were decreased by increasing concentration of *Calotropis gigantea* L. leaf extract. Certain compounds of plant extracts are responsible for reduction grain viability. Kumar *et al*. [47] found that flavonoids have an allelopathic effect as they are capable of increasing the levels of reactive oxygen causing inhibition of germination. In addition to allelochemical compounds, others factors can diminish even suppressed completely the viability of stored seed. The conditions of storage such as variation of temperature and relative humidity influence seed viability. These storage conditions in turn modify moisture content and this modification greatly affects grain properties. All durable stored products are hygroscopic and can therefore absorb or release moisture from and to the surroundings, this modification of moisture content out of certain ranges according to the stored grain species may easily induce loss of their viability [48]. The plant treatment sometimes improved germination rate. Salma *et al*. [49] reported that bean seeds treated with plant extracts have often showed higher emergence percentages. The poor germination of wheat (*Triticum aestivum* L.) has been improved by the application of garlic juice thanks its bioactive metabolite allicin, and also promoted seedling vigour [50].

5. Conclusion

The leaf powder of *P. kirbii* and *A. salicifolia* revealed effective to protect stored bean but the effectiveness was diminished when the storage periods becoming longer. Then the efficacy can be potentialized by avoiding the critical variation in term of humidity and even retreating when the quantity of active compound decreases. The used plants permitted to conserve the viability of bean but the effect of powder on seeds without infestation needs to be assessed in order to determine the potential allelochemical properties of these extracts. Further studies need to be carried out in order to determine the effect of these plants on mammalian toxicity before promoting their use in the protection of stored common bean for human and animal consumption.

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