

Bioefficacy of *Plectranthus kirbii* Powder and Extracts on Stored Cowpea Pest *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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Abstract

Cowpea seed constitutes an important source of proteins for populations in Sub-Saharan Africa. But this food resource is heavily damaged by cowpea beetle *Callosobruchus maculatus*. The control of that pest is mainly carried out by using synthetic insecticides. Despite the efficacy of this method, it caused environmental and health problems. Therefore, the search for alternative methods is vivaciously needed. In this issue, the bio-efficacy of *Plectranthus kirbii* extracts was assessed on *C. maculatus* regarding adult mortality, suppression of population and grain damage as well as seed viability preservation and repellency. The leaf powder and aqueous extracts of the plant were tested at 2, 4, 8 and 16 g/kg on bruchid adult for toxicity and damage bioassays. Repellency test was carried out using the plant aqueous, methanolic and ethyl acetate extracts at 0.5, 1, 2 and 4 mg/cm². The seed viability was evaluated using seeds preserved for three months at the single concentration of 16 g/kg of each plant extract. Significant mortality of cowpea beetle was induced by the plant aqueous extract and leaf powder. LC₅₀ values decreased with the increasing exposure period, and aqueous extract and leaf powder recorded 33.42 and 9.48 g/kg respectively within 3 days whereas within 5 days, the same extracts in the same order recorded LC₅₀ of 1.31 and 8.73 g/kg respectively. These extracts significantly reduced damage by suppressing almost completely the bruchid population growth. The non-infested grain preserved recorded high grain viability compared to the infested ones. The non-treated infested recorded the lowest germination rate (11.33%). The repellency rate ranged from 38.75% to 83.75%. Ethyl acetate and methanolic

extracts were classified as the class III repellent product, while aqueous extract ranged as class IV in repellency. Considering these findings, the extracts of *P. kirbii* could favourably replace the synthetic insecticides used in the cowpea protection during storage.

Keywords

Cowpea Beetle, Plant Extracts, Mortality, Damage, Viability

1. Introduction

Pulses and cereals constitute the main staple food grain around the world. Pulses, in particular, cowpea is an important grain consumed and used in the tropic and subtropics [1]. In sub-Saharan Africa, cowpea is cultivated for different issues. The plant is cultivated in different types of soil and has the ability to improve soil fertility and prevent erosion [2]. Cowpea is the most important source of food for man; animals and it is an important source of revenue in sub-Saharan countries. Cowpea is used to complete the lack of proteins in diet of populations in developing countries which represent three-quarters of the population in the world, but they produce only a quarter of the global production of meat [3]. Then the gap in proteins due to low meat production can be compensated by cowpea cultivated in this part of the world. The high protein content (25%) of cowpea with vitamins and minerals makes it an important economic crop in sub-Saharan region [1] [4]. Cowpea constitutes an affordable source of plant protein especially for people with low income in many tropical countries in Africa and Asia where it is predominantly consumed [5]. All these uses and benefices make the conservation and storage of this grain necessary in order to guarantee its availability, since this crop is cultivated once per year but its consumption is done alone the year. During storage, the cowpea grain is heavily attacked by different insect pests, specially the bruchid cowpea *Callosobruchus maculatus*, which is the main pest of that grain during storage [6] [7]. This insect belongs to genus *Callosobruchus*, which attacks grain legumes during both pre- and post-harvest stages all over the world [8].

C. maculatus is a serious challenge at small farmers' level, village traders and middle-income households where storage conditions are characterized by their poverty and inadequacy. The high level of damage induced by this insect pest is a major cause of increasing the pulse production [9]. The increase in terms of cowpea production exacerbates the pressure on environment and cost of productivity. The bruchid beetle starts its attack right from the field prior to harvest to storage where the insect population is built up to damaging levels [7] [10]. The insect spends its entire immature stage in individual legume seeds, where it causes weight loss, decrease in germination potential and diminishes the market as well as nutritional values of the commodity. Then *C. maculatus* destroys the stored cowpea by its attack resulting in quantitative and qualitative losses [7]

[10] [11]. The damage induced by this insect in storage obliges the peasants, farmers and smallholders to search for solution in order to minimize the losses.

The protection grain in the storages mostly relies on the use of the synthetic pesticides. This technique is expensive to rural farmers, and impractical in the primitive nature of storage in many localities [12]. The persistent use of these insecticides in granaries of small-scale farmers has led to a number of problems such as killing of non-target species, user hazards, toxic residues in food, development of genetic resistance in the treated pest, increased cost of application [13] [14]. The loss of cowpea during storage and in fields is prevented by producers thanks chemical insecticides. But their widespread and intensive use has led to serious problems, including pollution of the environment, insecticide resistance, pest resurgence, pesticide residues, poisoning of workers and lethal effects on non-target organisms [15] [16] [17]. The harmful effect of this method imposes the seek for alternative methods and the use of natural substances specially plant derived products is considered one of the most promised alternatives to synthetic chemical.

The use of plant constitutes an old aged practice in grain protection by peasants in sub-Saharan Africa. Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, which are selective with little or no harmful effect on non-target organisms and the environment [18] [19]. Due to several advantages of plant-derived insecticides, like biodegradable, environmentally friendly, less toxic to other animals among others, are becoming the most popular method in the management of insect pests around the world [20].

Plectranthus kirbii (Lamiaceae) is a plant largely grown in the Mount of the South West region of Cameroon. The plant is largely available and easily accessible by smallholders for their own use. The availability and easy accessibility of this plant motivated to carry out research work to assess the insecticidal ability and promote it as biopesticide candidate in the protection of stored grains. Then, it is necessary to evaluate the insecticidal activity of this plant against *C. maculatus* in the protection of cowpea, which is one of the most consumed pulses nationwide and seriously damaged during storage. *P. kirbii* 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 with wide spectrum against insect pests. In addition, in our knowledge no literature reported the insecticidal activity of *P. kirbii*.

Therefore, the bio-efficacy of *P. kirbii* extracts was assessed against *C. maculatus* regarding the mortality of adults, suppression of population growth, reduction of grain damage, repellency and the ability to preserve the viability of cowpea seeds after three months of storage.

2. Materials and Methods

Cowpea grain

The cowpea used in this present study was “Fekem” variety obtained from the

peasants of Gobo subdivision in Mayo Danay Division, Far-North region of Cameroon. This genotype is one of the most cultivated and consumed variety in that locality due to its good yield and grain size. Unfortunately, this cowpea variety is characterized by a poor resistance against bruchid attack. Before the use for experimentation, decayed grains and impurity materials were discarded from the stock and the cleaned cowpea was kept in the freezer at -20°C for disinfestation. After 14 days, the grain was removed from the freezer and kept in the ambient laboratory conditions for 14 days for acclimatisation. The moisture content of the grain was determined by using the electronic moisture tester (Pfeuffer HE 50 Mess-und prüfgeräte, Hoh-express, Germany), and it was 11.7%.

Insect rearing

The insect used for this experiment was the main cowpea insect pest *Callosobruchus maculatus*, collected from the infested cowpea in the storage facilities around Dang, Vina Division, Adamawa region of Cameroon. The insects were reared in five 900 mL glass jars containing cleaned and non-infested cowpea. Insects used in the experiment were those obtained from the fourth generation, accommodated to their new environment. Since the life duration of *C. maculatus* adult is short, the insects used were aged ≤ 3 days for a better assessment of the plant extracts efficacy.

Plant leaf harvest and processing

Green leaves of *Plectranthus kirbii* were collected at Magha-Atuallah Road (Lebialem) in September 2020 in the South-west region of Cameroon, precisely at latitude $5^{\circ}40'46.1''\text{North}$; longitude $10^{\circ}03'39.2''\text{East}$, and altitude of 2522 m. The identity of the plant was confirmed in comparison with the Lamiaceae of Gabon flora under collection number of 1243 TWN. The leaves were dried at room temperature for 14 days, crushed and then ground using wood mortar until the powder passed through a 0.20 mm mesh size sieve. Then, the powder was stored in a freezer at -4°C until needed for preparations of extracts and bioassays.

Preparation of plant solvent extracts

The plant extracts were prepared according to the method described by Mansoor-ul-Hassan *et al.* [21]. Water, ethyl acetate and methanol solvents were used for plant extraction. The plant Leaf powder (300 g) was macerated in 3 L of different solvents separately; the maceration was stirred manually for 30 minutes and then left for 24 hours. Then, each maceration was filtrated through Whatman N°1 filter paper. The filtrate obtained with solvents except water was kept in the ambient laboratory conditions for 14 days to allow complete evaporation of solvent, then the extracts were kept in dark closed glass tube and stored in the freezer at -4°C until needed for bioassays. The aqueous maceration was filtrated, and the filtrate was kept in the freezer at -18°C for 24 hours before a lyophilisation process to obtain dried extract which was stored as the previous ones.

Determination of chemical composition of *P. kirbii* leaf

The different extracts of *P. kirbii* were directly submitted to chemical screen-

ing by using the standard methods [22]. Only the leaf powder, since it was a solid, was firstly dissolved in equivalent solution made up of the mixture of methylene chloride/methanol (1/1) before applying the screening methods. The presence of main chemical families' compounds involved in insecticidal activity was determined for the different solvent extracts (aqueous, ethyl acetate and methanol) and leaf powder.

Mortality bioassay

The mortality of cowpea beetle test was carried out in the fluctuating laboratory conditions ($t = 23.71^{\circ}\text{C} \pm 1.03^{\circ}\text{C}$; $\text{RH} = 81.38\% \pm 2.03\%$). During experiment, temperature and relative humidity were recorded by datalogger (Data logger Model EL-USB-2, LASCAR, China). In the 450 mL glass jars, 50 g of cowpea (Fekem genotype) was introduced, then 0.1 g, 0.2 g, 0.4 g and 0.8 g of aqueous extract and leaf powder corresponding respectively to 2, 4, 8 and 16 g/kg were separately added. The mixture of cowpea and plant extract was manually shaken for 2 minutes to allow uniform coating of extract on grain. Twenty (20) *C. maculatus* adults aged ≤ 3 days old were added in jars containing treated grains. 50 g of cowpea grains infested 20 pea beetles without plant treatment constituted the negative control. All glass jars containing preparation were covered with perforated lids and displayed on shelves in the same laboratory conditions. The number of dead and alive insects were recorded after 1-, 3-, 5- and 6-days post infestation. The insect was considered dead, after several delicate contacts with entomological forceps without any reaction.

Population growth and cowpea damage

After recording mortality within 6 days post-infestation from the previous experiment (mortality bioassay), each jar experiment was maintained in the same laboratory conditions for further observations. After three months of storage, the emerging bruchids were counted. At same time, the number of damaged and undamaged cowpea grain was determined. The weight loss of cowpea grains after three months of storage was assessed according to counting and weighing method [23].

Repellency experiment

The repellent action of methanolic, ethyl acetate and aqueous extracts of *P. kirbii* on *C. maculatus* was carried out according to McDonald *et al.* [24]. The arenas test consisted of 7 cm Whatman N°1 filter paper cut in half (19.25 cm^2). Four dosages of each extract were prepared by dissolving 10, 20, 39 and 77 mg of extract in 1 ml of ethanol. The different plant solvent extracts were applied to a half filter paper disc as uniformly as possible with a pipette corresponding to the dosages 0.5; 1; 2 and 4 mg/cm^2 . The other half filter paper was treated with 1 mL of ethanol alone. Methanolic, ethyl acetate and aqueous extracts of *P. kirbii* treatments, and control half discs were air-dried for 15 min to allow complete evaporation of ethanol. Each Full disc was subsequently reassembled by attaching treated half to untreated half with clear adhesive tape. Each remade filter paper disc was placed into 7 cm Petri dish and 20 adult insects aged ≤ 3 days were released at the centre of the filter paper disc. The Petri dishes were then covered

and left under the ambient laboratory conditions. Four replications were made for each treatment. The number of insects present in the control (N_c) and treated (N_t) strip were recorded 30, 60, 90 and 120 min after exposure. Percent repellency (PR) was calculated according to the following formula:

$$PR = \left[\frac{(N_c - N_t)}{(N_c + N_t)} \right] \times 100$$

The mean repellency values of each plant extract were calculated and assigned to repellency classes [24]: class 0 ($PR < 0.1\%$), class I ($PR = 0.1\% - 20\%$), class II ($20.1\% - 40\%$), class III ($40.1\% - 60\%$), class IV ($60.1\% - 80\%$), class V ($80.1\% - 100\%$).

Viability test

In order to assess the viability of seeds, 50 g of cleaned cowpea was introduced in 450 mL glass jar. The different extracts of *P. kirbii* (leaf powder, aqueous extract, ethyl acetate and methanolic extracts) at highest content (16 g/kg) were added separately to each jar containing grain. Two batches of different treatments were made; one was infested with *C. maculatus* adult and other was non-infested. Four replications were maintained for each lot containing different treatments. After three months of storage, 30 non-perforated seeds were randomly picked up from each jar. The seeds were placed on moistened paper in 9 cm petri dishes and kept in the ambient laboratory conditions ($t \approx 23.22^\circ\text{C} \pm 1.04^\circ\text{C}$; $\text{RH} \approx 82.81\% \pm 1.48\%$). The preparations were watered every two days. The number of germinated and non-germinated seeds was recorded after 10 days [25].

Data analysis

The investigation of bioefficacy of *P. kirbii* was carried out from May to October 2021 and data on different parameters were collected. Abbott's formula [26] was used to correct for control mortality before analysis of variance (ANOVA) and probit analysis. Data on cumulative corrected mortality, damage, weight loss, repellency and germination rate were arcsine-transformed [(square root ($x/100$))], and the number of emerging bruchids was log transformed ($x + 1$). The transformed data were subjected to the ANOVA procedure using SPSS package Version 20.0 [27] [28]. Probit analysis [28] [29] was conducted to determine lethal concentration (LC) and mortality of *C. maculatus* within 1-, 3-, 5- and 6-days post treatment. The graphs were plotted by SigmaPlot 14.0 [30].

3. Results

Chemical composition of *P. kirbii* leaf

The phytochemical analysis revealed that the *P. kirbii* leaf contained different chemical compounds, and their content varied with extract (Table 1). Alkaloids and phenolic compounds were abundant in all extracts except in aqueous extract. The screening revealed that glucosides and saponins were absent except in methanolic extract for the first compound and aqueous extract for the second. Flavonoids were low in all extracts apart methanolic extract where they were very abundant. Terpenoids and sterols were abundant at the same level in ethyl

Table 1. Phytochemical screening of *Plectranthus kirbii* leaf extracts.

Compounds	Ethyl acetate	Methanol	aqueous extract	leaf powder
Alkaloids	++	++	+	++
Phenolic compounds	++	++	+	+++
Flavonoids	+	+++	+	+
Terpenoids and sterols	++	+	+	++
Tannins	++	++	++	++
Glucosides	-	+	-	-
Anthraquinones	++	++	+	+
Coumarins	+	++	+	+
Anthocyanins	++	+++	++	++
Saponins	-	-	++	-

+: low; ++: Abundant; +++: very abundant; -: Absent.

acetate extract and leaf powder of the plant. Whereas in methanolic and aqueous extracts, the same compounds were present in low concentration. Anthraquinones were abundantly present in ethyl acetate and methanolic extracts, and low in aqueous extract and leaf powder. Coumarins were low in all extracts except in methanolic extract characterised by a very marked presence. Anthocyanins were abundant in the four of *P. kirbii* extracts.

Mortality of adult *C. maculatus* induced by *P. kirbii* extracts

The mortality caused by leaf powder and aqueous extract of *P. kirbii* significantly increased as content and exposure period increased (Figure 1). Within 1 day exposure, both extracts at different contents led to lowest mortality, but the efficacy of these treatments considerably increased from 3 days to reach high performance within 6 days exposure. Even at the lowest content (2 g/kg), significant mortality rate was achieved by both extracts when exposure period prolonged. The leaf powder revealed more toxic than the aqueous extract; the powder at 16 g/kg within 6 days killed more than 80% of *C. maculatus* adult, whereas aqueous extract at same content within the same exposure period caused less mortality (about 70%) of the insect.

The Lethal concentration values for leaf powder and aqueous extract decreased as the exposure period was extended (Table 2). In general, at all exposure period except 5 days, the LC₅₀ values were lower for leaf powder than aqueous extract. The LC₅₀ of leaf powder and aqueous extract were 9.48 and 33.42 g/kg, respectively within 3 days exposure. Within 6 days exposure, the same extracts in the same order recorded the LC₅₀ of 0.32 and 0.52 g/kg respectively. Globally, no difference was observed between theoretical and experimental models since χ^2 was not significant. In term of toxicity speed, the leaf powder acted faster than aqueous extract since leaf powder slope is greater than that of aqueous extract.

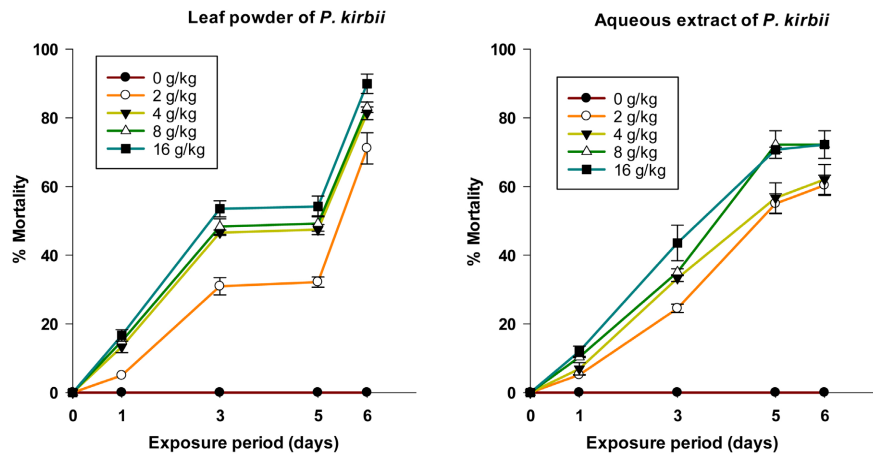


Figure 1. Cumulative corrected mortality of adult *Callosobruchus maculatus* induced by leaf powder and aqueous extract of *Plectranthus kirbii* in cowpea grain at different exposure periods under ambient laboratory conditions

Table 2. Toxicity parameters of *Plectranthus kirbii* leaf extracts on adult *Callosobruchus maculatus*.

Extracts	R ²	Slope ± SE	LC ₅₀ (95% FL)	LC ₉₅ (95% FL)	χ ²
3 days					
Leaf powder	0.58	0.59 ± 0.11	9.48 (7.10; 14.73)	-	9.96 ^{ns}
Aqueous extract	0.67	0.54 ± 0.11	33.42 (18.92; 117.74)	-	8.92 ^{ns}
5 days					
Leaf powder	0.57	0.58 ± 0.11	8.74 (6.53; 13.37)	-	9.72 ^{ns}
Aqueous extract	0.48	0.56 ± 0.11	1.31 (0.15; 2.51)	-	17.95 ^{ns}
6 days					
Leaf powder	0.59	0.74 ± 0.13	0.32 (0.02; 0.83)	54.38 (23.57; 534.60)	15.44 ^{ns}
Aqueous extract	0.34	0.42 ± 0.11	0.52 (0.00; 1.66)	-	18.98*

^{ns}: P > 0.05; *: P < 0.05; LC: Lethal content; -: LC₉₅ value was not possible to be calculated or too large due to inadequate mortality.

Suppression of *C. maculatus* population growth and reduction of cowpea damage

P. kirbii powder and aqueous extract significantly suppressed *C. maculatus* population growth (Figure 2) and reduced grain damage (Table 3). This extracts' activity was dose dependent, the insecticidal effect improved as the content increased. In negative control, more than 400 adults were recorded in grain cowpea, with 94.88% perforated grain and 17.38% weight loss. The two treatments applied at 2 g/kg; the population increase was considerably suppressed by both extracts; less than 30% of insects. At 2 g/kg, 18.52% and 9% perforated and 2.86% and 1.36% weight loss were recorded in cowpea treated by leaf powder and aqueous extract respectively. Almost complete suppression of population

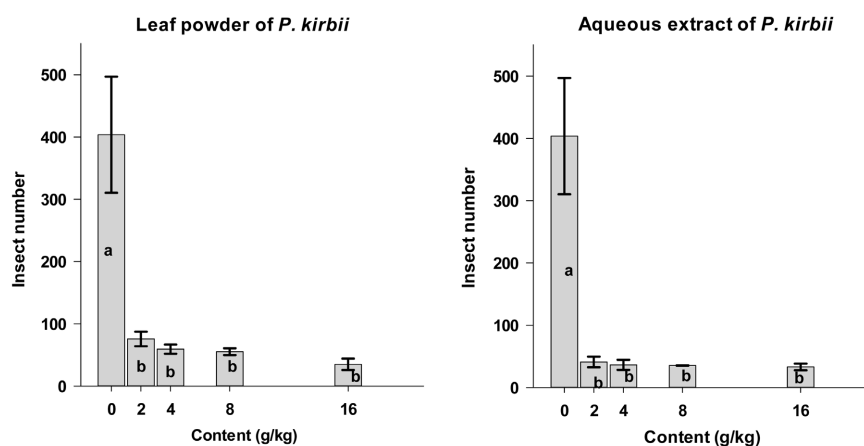


Figure 2. Population growth of *Callosobruchus maculatus* recorded in cowpea grain treated with leaf powder and aqueous extract of *Plectranthus kirbii* for three months of storage under fluctuating laboratory conditions. The bands containing the same letter do not differ significantly according to Tukey's test at $P < 0.05$.

Table 3. Damage recorded after three months on cowpea grain treated with the *Plectranthus kirbii* extracts under ambient laboratory conditions.

Content g/kg	Leaf powder	Aqueous extract
% perforation $m \pm ES$		
0	94.88 \pm 0.73 ^a	94.88 \pm 0.73 ^a
2	18.52 \pm 3.73 ^b	9.54 \pm 2.51 ^b
4	13.14 \pm 2.15 ^b	5.78 \pm 2.89 ^b
8	11.70 \pm 1.49 ^b	5.68 \pm 0.12 ^b
16	6.77 \pm 3.52 ^b	4.66 \pm 1.86 ^b
$F_{(4; 10)}$	203.80***	419.20***
% weight loss		
0	17.38 \pm 1.30 ^a	17.38 \pm 1.30 ^a
2	2.86 \pm 0.52 ^b	1.36 \pm 0.58 ^b
4	1.92 \pm 0.54 ^b	0.79 \pm 0.28 ^b
8	1.89 \pm 0.49 ^b	0.72 \pm 0.31 ^b
16	1.14 \pm 0.69 ^b	0.70 \pm 0.37 ^b
$F_{(4; 10)}$	80.93***	116.26***

Mean \pm S.E. followed by the same letter in a column do not differ significantly at $P < 0.05$ (Tukey's test); *** $P < 0.0001$.

growth was observed for the two extracts at the highest content (16 g/kg), the same tendency was also observed concerning weight loss; 1.14% and 0.70% weight loss in grain treated with leaf powder and aqueous extract respectively.

Repellency of extracts on *C. maculatus* adult

Aqueous, methanolic and ethyl acetate extracts of *P. kirbii* significantly repelled *C. maculatus* adult (Table 4). The repellency rate increased as the products contents increased, but this variation was not statistically significant in general. Two repellency classes were observed; methanolic and ethyl acetate extracts were repellency class III products, whereas aqueous extract was in class IV. The lowest repellency rate (38.75%) was observed with the lowest content (0.5 mg/cm²) of ethyl acetate within 90 min exposure period, whereas the highest repellency percent (83.75%) was recorded with the highest content of aqueous extract (4 mg/cm²). The effective repellency period varied according to the extract. Statistically, there was not significant difference in general between the periods

Table 4. Repellency induced by the different extracts of *Plectranthus kirbii* on *Callosobruchus maculatus* within different exposure periods.

Concentration (mg/cm ²)	Period (min)				$F_{(3;12)}$
	30	60	90	120	
Aqueous extract					
0.5	68.75 ± 2.39 ^{aA}	68.75 ± 10.68 ^{aA}	70.00 ± 6.77 ^{aA}	61.25 ± 4.73 ^{Ba}	1.40 ^{ns}
1	71.25 ± 8.99 ^{aA}	75.00 ± 4.082 ^{aA}	71.25 ± 7.18 ^{aA}	72.50 ± 7.50 ^{abA}	0.32 ^{ns}
2	78.75 ± 5.54 ^{aA}	78.75 ± 5.15 ^{aA}	73.75 ± 3.75 ^{aA}	77.50 ± 2.50 ^{abA}	1.32 ^{ns}
4	80.00 ± 0.00 ^{aA}	81.25 ± 4.27 ^{aA}	76.25 ± 3.15 ^{aA}	83.75 ± 1.25 ^{aA}	0.37 ^{ns}
$F_{(3;12)}$	1.04 ^{ns}	0.67 ^{ns}	0.86 ^{ns}	4.19*	
Repellency class	RC-IV	RC-IV	RC-IV	RC-IV	
Methanolic extract					
0.5	46.25 ± 4.73 ^{bA}	45.00 ± 8.90 ^{aA}	42.50 ± 8.54 ^{aA}	41.25 ± 8.26 ^{Aa}	0.91 ^{ns}
1	48.75 ± 2.39 ^{bA}	47.50 ± 6.29 ^{aA}	47.50 ± 1.44 ^{aA}	46.25 ± 1.25 ^{Aa}	0.90 ^{ns}
2	62.50 ± 5.20 ^{abA}	47.50 ± 2.50 ^{aA}	56.25 ± 8.26 ^{aA}	51.25 ± 7.18 ^{aA}	0.27 ^{ns}
4	68.75 ± 3.75 ^{aA}	61.25 ± 1.25 ^{aAB}	63.75 ± 3.75 ^{aAB}	51.25 ± 3.15 ^{Aa}	5.47*
$F_{(3;12)}$	6.76*	1.72 ^{ns}	2.25 ^{ns}	0.70 ^{ns}	
Repellency class	RC-III	RC-III	RC-III	RC-III	
Ethyl acetate extract					
0.5	42.50 ± 10.10 ^{aA}	42.50 ± 4.79 ^{aA}	38.75 ± 3.75 ^{aA}	41.25 ± 1.25 ^{Aa}	0.20 ^{ns}
1	50.00 ± 10.21 ^{aA}	45.00 ± 8.66 ^{aA}	50.00 ± 7.36 ^{aA}	51.25 ± 3.75 ^{aA}	0.06 ^{ns}
2	60.00 ± 2.04 ^{aA}	48.75 ± 7.18 ^{aA}	51.25 ± 4.73 ^{aA}	53.75 ± 9.66 ^{Aa}	0.44 ^{ns}
4	63.75 ± 5.54 ^{aA}	61.25 ± 4.73 ^{aA}	58.75 ± 2.39 ^{aA}	57.50 ± 3.23 ^{Aa}	1.32 ^{ns}
$F_{(3;12)}$	1.54 ^{ns}	1.61 ^{ns}	2.83 ^{ns}	1.62 ^{ns}	
Repellency class	RC-III	RC-III	RC-III	RC-III	

Mean ± S.E. followed by the same capital letter in a line and the same lower-case letter in a column do not differ significantly at $P < 0.05$ (Tukey's test); RC: Repellency Class; ^{ns} $P > 0.05$; * $P < 0.05$.

concerning each extract, but the slight variation was observed. At 0.5 mg/cm², the repellency percent of aqueous extract was 68.75% within 30 min but it decreased to 61.25% within 120 min. Methanolic and ethyl acetate had 46.25% and 42.50% respectively within 30 min, the same extracts, in the same order induced 41.25% and 38.75% respectively within 120 and 90 min respectively. The highest content of all extracts induced highest repellency rate, which varied according to the exposure period. At their highest content (4 mg/cm²), the three extracts reached their highest repellency performance; there were 83.75%, 68.75% and 63.75% with aqueous extract within 120 min, methanolic and ethyl acetate within 30 min respectively.

Germination of cowpea seeds conserved by *P. kirbii* extracts

The seed germination rate varied whether the grain was infested by bruchid or not (Table 5). The non-infested cowpea seeds recorded higher germination rate compared to infested ones. The non-infested grains treated with the different extracts had statistically the same germination percentage ($P > 0.05$). But, as concerns the infested grain, the viability varied according to the treatment. The lowest germination was observed with infested and non-treated grains, it was 11.33% at 0 g/kg. The highest germination rate (95.45%) was recorded in non-infested seed treated with aqueous extract of *P. kirbii*; followed by leaf powder (94.45%). In infested cowpea seeds, the highest germination rate was observed with leaf powder (37.78%) followed by aqueous extract (33.33%) whereas lowest rate (11.33%) was recorded in the control.

4. Discussion

The different extracts and powder of *P. kirbii* leaves showed insecticidal properties against cowpea bruchid *C. maculatus*. The leaf powder and aqueous extract induced significant mortality of *C. maculatus* and this effectiveness was concentration-dependent. Then, the mortality increased with ascending period of exposure and extract content. The extension of exposure period increased the contact with insecticidal product and the rise of content increased the quantity

Table 5. Germination recorded in cowpea seeds treated by the different extracts of *Plectranthus kirbii* after three months of storage.

Treatments	Without insect	With insect	t value
Control	86.00 ± 2.08 ^a	11.33 ± 1.45 ^b	56.00***
leaf powder	94.45 ± 4.01 ^a	37.78 ± 4.01 ^a	8.17*
Aqueous extract	95.56 ± 4.44 ^a	33.33 ± 3.85 ^{ab}	10.58*
Methanol	86.67 ± 9.82 ^a	24.44 ± 8.68 ^{ab}	28.00**
Ethyl acetate	81.11 ± 9.49 ^a	25.56 ± 5.88 ^{ab}	13.87**
F _(4; 10)	0.90 ^{ns}	3.58*	

Mean ± S.E. followed by the same capital letter do not differ significantly at $P < 0.05$ (Tukey's test); ^{ns} $P > 0.05$; * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$.

of active ingredients. According to Akinkulere *et al.* [31], the accumulation of compounds from *Anchomanes difformis* led to death of *C. maculatus*, thereby reducing the beetle population. The insecticidal activity of these extract could be attributed to the phytochemical compounds contained in the used products, and the increase of mortality according to period and content were due to the quantity of active compounds picked up by the insect. Many plant extracts have proven to cause mortality against *C. maculatus*. Powders of *Lantana camara* [32], *Murraya koenigii* and *Eupatorium cannabinum* [33], *Guirea senegalensis*, *Piliostigma reticulatum* and dried fruit powder of *Piper guineense* [34] caused significant mortality of *C. maculatus* adults. The insecticidal efficacy of plant powders could be attributed to their repellency, digestive poisoning, disturbance of respiratory system through occlusion of spiracles [13]. Generally, in the present study, the plant leaf powder revealed more toxic than the aqueous extract, and this discrepancy could be attributed to difference in their chemical composition. The screening showed that leaf powder contained more phenolic compounds, terpenoids and sterols than the aqueous extract. The findings of the present are in accordance with the report made by some authors, who showed that certain botanicals exercise toxic effect against storage pests including *C. maculatus* [35] [36] [37] [38].

To ensure a good protection, a protectant whether plant extract or any natural substance must be able to suppress pest population then reduce loss. The leaf powder and aqueous extract of *P. kirbii* suppressed population of *C. maculatus*, and reduced grain damage as well as weight losses of cowpea grains. The plant extracts reduced insect population by the modes of action such as antifeedant action, inhibition of moulting, growth reduction, loss of fecundity, respiratory inhibition [39]. The results obtained in this work corroborate certain findings conducted by several authors including Adedire *et al.* [36], Mukanga *et al.* [37], Ileke and Oni [38], in which certain botanicals are effective to control several insect pest species in grain storage. The chemical screening of *P. kirbii* showed different compounds that are responsible to induce insecticidal activities. Terpenoids, alkaloids and phenolic compounds are known for their insecticidal effect [39]. Phenolic compounds exercise antifeedant, toxic and regulatory activity; which affect insect physiological processes, and have ability to the phytophagous insects reducing the contact then damage [40]. The cowpea treated with the ethanol extract of *Anchomanes difformis* at the contents 1% and 3% recorded 8.43% and 0% damage induced by *C. maculatus* within three months of storage [31]. The same tendency was observed in the present work, when the cowpea treated with *P. kirbii* leaf powder and aqueous extract at 2 g/kg recorded 18.52% and 9% damages grain respectively. This reduction of grain damage might be linked to the suppression *C. maculatus* population growth. The reduction of insect pest population could be due to ovicidal or repellency of used botanical, larval mortality or even the disturbance of postembryonic development [41].

The plant extracts due to their chemical constituents may represent excellent repellents and insecticides and could be used in different storage environments.

Aqueous, methanolic and ethyl acetate extracts of *P. kirbii* significantly repelled *C. maculatus* adults, and that repellency increased with the increasing concentration, and varied with the solvents used for the plant extraction even though methanolic and ethyl acetate belonged to the same class (RC III). This repellent ability is conferred to these extracts by their chemical composition. The chemical compounds in terms of diversity and content vary according to the type of solvent used for extraction. That explained the difference in repellency rate among the solvent plant extracts. The repellency permits to improve grain protection keeping it out of the reach of insect pest. Chebet *et al.* [42] reported that the repellency of plant extract may be in part attributed to the presence of volatile compounds such as monoterpenes and sesquiterpenes which are well-known repellents of phytophagous insects. The repellency rate of the different extract decreased as the duration increased for the concentration of certain extracts. In the present work, the repellency rate induced by aqueous and methanolic extracts increased within 60 and 90 mins respectively, then decreased after these periods. These findings are in conformity with the observations made by Shoukat *et al.* [43]. They observed that *Sophora alopecuroides* extract at concentrations of 5 mg/cm² repelled 93.11% of *Aedes albopictus* within 90 min, this repellency started decreasing with extension of exposure period, and within 240 mins the repellency rate went down to 53.14%. The decrease of repellency with time raised can be explained by the fact that the efficacy of chemical compounds with low molecular weight and high volatility decreased rapidly [44]. The different extracts contain phenolic compounds, terpenoids. These compounds are constituted by several volatile molecules endowed with repellent and fumigant properties [45] [46] [47].

The attack of stored cowpea by bruchid *C. maculatus* lowers seed quality, reduces market value, and at same time reduce viability of infested grains. Therefore, it is necessary even imperative for a grain protection especially in sub-Saharan Africa to preserve grains with their viability. Because of their low income, the peasants and smallholders use their stored grains not only for meal but also as seeds for cropping. The non-perforated grains selected from infested ones even looked undamaged recorded the weak germination rate, it may due to the development of insect larvae which consumes the reserve of grain and the part of embryo then destroying at the same occasion the viability. The non-infested grains by insect statistically had the same germination capacity but little difference was noticed among the different treatments. Masagwa *et al.* [48] reported that the acetone and water crude extracts of *Syzygium cordatum*, *Agapanthus caulescens*, *Allium sativum*, *Carica papaya* tested on seeds of cowpea and bean did exhibit any adverse effects on their viability. But the same authors also noticed that the germination of cowpea seed was improved when *Allium* (15 mg/ml), *Agapanthus* (5 mg/ml) and *Carica* (15 mg/ml) water extracts and *Agapanthus* (5 mg/ml) acetone extracts were applied. Certain studies revealed that the plant extracts can affect the germination of grain positively or negatively according to type or con-

centration of chemical compounds involved. Phenolic compounds at low concentration did not inhibit germination of six weed species (*Chenopodium album*, *Plantago lanceolata*, *Amaranthus retroflexus*, *Solanum nigrum*, *Cirsium* sp. and *Rumex crispus*), but the highest concentration of the same compounds inhibited the germination of all these weeds [49]. The lowest concentrations had no effect or stimulated germination of the six weed species. Shimada *et al.* [50] reported that the excess sterols impair proper seed coat formation, thereby inhibiting germination of seed. Coumarins also inhibit seed germination at high concentration and inhibition occurs during the early phase of seed imbibition's by inhibition of water intake [50]. Abenavoli *et al.* [51] showed that coumarin was able to rapidly and significantly inhibit the germination of durum wheat seed during the early stages of imbibition from concentrations above 200 μ M. In the present work, the seeds treated with leaf powder and aqueous extract of *P. kirbii* had better germination rate compared to the non-infested and non-treated ones, this may suggest a stimulating effect of extracts on seed viability.

5. Conclusion

The leaf extracts of *P. kirbii* were able to control infestation of cowpea grain by inducing mortality of adult *C. maculatus*, suppressing population growth and reducing grain damage. The extracts did have any negative effect on seed viability, and significantly improved germination capacity when cowpea grains were treated with the leaf powder and aqueous extract of *P. kirbii*. The findings encouraged the use of *P. kirbii* extract in the protection of cowpea grain against *C. maculatus* during storage. The extracts of *P. kirbii* thanks to their chemical composition made up of several compounds could reduce the chance of the insect pest developing resistance. However, some studies need to be carried out concerning optimisation of their use, remanence and mammalian toxicity.

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Conflicts of Interest

Authors have declared no relevant conflict of interest that may have influenced the study.

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