

**EFFICACY OF *PIPTADENIASTRUM AFRICANUM* STEM BARK POWDERS AND OIL EXTRACT AS A LONG-TERM STORAGE PROTECTANTS AGAINST COWPEA BRUCHID, *CALLOSBRUCHUS MACULATUS* (FAB.) (COLEOPTERA; CHRYSOMELIDAE) INFESTING COWPEA SEEDS DURING STORAGE**

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**SUMMARY**

This study examined the effectiveness of various *Piptadeniastrum africanum* powder and oil extract concentrations against *Callosobruchus maculatus* in cowpea seeds that had been kept. The treated cowpea seeds were placed in the container together with an adult *C. maculatus*. Cowpea bruchids were subjected to contact and fumigant toxicity tests at 0.1g, 0.2g, 0.5g, 1.0g, and 2.0g/20g of cowpea seeds. Mortality was measured at exposure times of 24, 48, 72, and 96 hours after treatment. The findings demonstrated that within 96 hours of treatment, the insects subjected to *Piptadeniastrum africanum* stem resulted in 100% adult mortality of the cowpea beetles at a dosage of 2.0 g. After being exposed for 72 hours, the adulticidal effects of 2.0 g of *Piptadeniastrum africanum* differ considerably ( $p < 0.05$ ). The same trends of results were observed with oil extracts of the plant; where *Piptadeniastrum africanum* stem oil extract evoked 100%. The fumigant toxicity effects of the plants significantly reduced the number of adult emergence especially at the highest experimental dosage. The experiments on the seeds viability shows that the stem bark powders of the *P. africanum* effectively reduced percentage adult emergence of *C. maculatus*. In cowpea seeds treated with plant component powders at all concentration levels, the percentage of adult emergence was considerably ( $P > 0.05$ ) higher than in untreated seeds. This study showed that the powder and oil extracts of *P. africanum* stem would provide alternative to synthetic insecticides in the management of *C. maculatus* infesting cowpea seeds in Nigeria.

**KEYWORDS:** *Piptadeniastrum africanum*, mortality, *Callosobruchus maculatus*, Toxicity viability and Adulticidal.

**INTRODUCTION**

Post harvest pest problems may begin Once the crop has reached physiological maturity and is experiencing natural drying in the field. For example, *Callosobruchus maculatus*, the cowpea beetle, is an important commercial pest of stored foodstuffs and is distributed throughout all continents (Rees, 2004). In Nigeria, it is a major and widespread pest of healthy, sound cowpea seeds. (Nwana, 1993; NRI, 1996). Since food grain losses during storage have been attributed mostly to insect and pest infestation in the majority of poor nations (Adedire et al., 2011). There are numerous drawbacks to using traditional synthetic pesticides to eradicate cowpea bruchids and other pests associated with stored food. notable risks to workers' safety, environmental persistence, significant mammalian toxicity, insect

resistance, and health (Adedire and Ajayi, 1996). These have stimulated a search for alternative means of storage pests control. Due to their eco-friendliness, low toxicity to humans, ease of use, specificity, and lack of insect pest resistance, botanical insecticides have gained attention from researchers and farmers as a means of controlling pest insects in stored products (Ileke and Oni, 2011). (Isman, 1997; Odeyemi, 1998; Adedire and Lajide, 2003; Arannilewa et al., 2006). Researchers and small-scale farmers have frequently reported the effective use of plant products in insect pest management. There have been reports of the insecticidal effectiveness of plant materials such as spices, vegetable oils, extracts, powders, and inert dust (Keita et al., 2001; Akinkurolere et al., 2006; Adedire et al., 2011). Therefore, the purpose of this study is to assess the effectiveness of oil extracts,

stem bark powder, and *Piptadeniastrum africanum* against adult *Callosobruchus maculatus*.

#### MATERIALS AND METHODS

The plant portion, i.e *Piptadeniastrum Africanum* stem bark, was gathered at the Ago Aduloju camp, which is located along Ado Ikare road in Ado Ekiti, Ekiti State, Africa.

#### PREPARATION OF PLANT MATERIALS

Freshly harvested stem parts of *P. africanum* were taken to the laboratory, for botanical identification and authenticated by Prof. Akinneye J. O. of the Department of Biology, Federal University of Technology Akure. The stem were washed thoroughly with water and air-dried in the laboratory for 30 days. The stem bark were then pulverized into fine powder using Binatone Electric Blender (Model 373). The powders were further sieved to pass through 1mm perforation. Prior to usage, the fine powders were stored in individual plastic bottles with screw-cap lids at room temperature ( $28 \pm 2$  °C) and  $75 \pm 5\%$  relative humidity.

#### EXTRACTION OF PLANT OIL

Methanol served as the extraction solvent. The 600g of the pulverized plant stem bark were then weighed separately into a beaker. The mixture was then sealed in a thimble with muslin cloth and extracted in a soxhlet extractor using 500ml of methanol as the solvent. The extraction process took three hours at  $50^{\circ}\text{C}$  in each case. When the solvent in the thimble turned transparent, the extraction was determined. Then the thimble was moved from the unit and the solvent recovered by redistilling the content obtained from soxhlet using rotary evaporator. The resulting extract contained both the solvent and the oil. After which the oil was exposed to air, so that traces of the volatile solvent evaporated, leaving the oil extract. The resulting oil was kept in glass bottles and used for subsequent experiment.(Adegbe *et al*, 2016).

#### Insect Culture

The *C. maculatus* used for this study were obtained from field-infested cowpea seeds from Federal University of Technology, Akure (FUTA) farm, Ondo State, Nigeria. 400 g of uncontaminated cowpea seeds were placed in two-liter plastic containers to raise adult insects. The powder that was consumed was replaced frequently, and fragments and frass were sieved away to preserve the culture. The plastic container was wrapped in muslin fabric, secured with rubber bands, and set inside a 75 cm x 50 cm x 60 cm wire mesh cage. To keep predatory ants out of the cage, the four legs of the container were dipped in a water-kerosine mixture that was already inside the plastic container. New generation of *C. maculatus* obtained from this stock were reared by infesting 200g clean and uninfested grains with 10 pairs of adult *C. maculatus* to generate the required insect population for the study. The set-up was placed in the Postgraduate Research Laboratory in the Department of Biology, The Federal University of Technology Akure.

#### Insects Bioassay

Contact toxicity of plants part powders on adult of *C. maculatus*: Fine powders of the *P. africanum* stem bark, were admixed with cowpea seeds at the rate of 0.1, 0.2, 0.5 1.0 and 2.0g/ 20g in 250ml plastic containers (8cm diameter and 4cm depth). The container cover was punched with hot rod and lined with muslin on the inside to prevent insect escape and allows for aeration. Ten pairs of adult *C. maculatus* was introduced into plastic containers containing the treated cowpea and untreated samples was also infested to serve as control with adult *C. maculatus* and all treatment were replicated three times. Adult mortality at 24, 48, 72 and 96 hours after treatment were counted and recorded. At the end of 96 hours post treatment, data, on percentage adult mortality was corrected using Abbot (1925) formula thus, Where  $P = \frac{T}{T + C} \times 100$  Corrected Adult mortality  $P_o = \frac{P}{1 - P}$  Percentage mortality of treated insects  $P_c = \frac{P_o}{1 - P_o}$  Percentage mortality on treated insects.

**Fumigant effect of *P. africanum* powders on adult *C. maculatus*.** Fine powder of the *P. africanum*, stem bark powder were sealed in muslin clothes (2cm by 2cm) and hanged on the lid of plastic containers of dimension 8cm depth x 4cm width. Twenty pairs of newly emerged adult *C. maculatus* were introduced into plastic container containing 20g of cowpea seeds and covered with lid. Plant powder were hanged at equal distance between the lid and the base of the container. Untreated cowpea seeds with adult of *C. maculatus* were also set up as control. The treatments were replicated three times. Adult mortality at 24, 48,72 and 96 hours after treatment were determined and recorded. At the end of the 96 hours post treatment period, data on percentage adult mortality were corrected using Abbot (1925) formula.

#### Statistical analysis

A one-way analysis of variance (ANOVA) ( $P < 0.05$ ) was performed on the adult mortality data, and Duncan's New Multiple Range Test was used to differentiate the treatment means. Probit and log transformation were applied to contact and fumigant mortality data as well as stem bark dosages of *P. africanum* in order to ascertain the dosage deadly to 50% and 95% of *C. maculatus* (LD 50 and LD 95). The software programme SPSS 20.0 was used to determine all analyses.

#### RESULTS

**Contact toxicity of *P. africanum* stem bark powder on adult mortality of *C. maculatus*:** Table 1 presents the adult mortality rate of *C. maculatus* after exposure to powdered *Piptadeniastrum africanum*. Within 96 hours of application, the stem bark of *P. africanum* resulted in 90% mortality in adult *C. maculatus* at a dosage level of 2.0 g. Table 1 shows that mature *C. maculatus* on treated cowpea seeds was significantly ( $P < 0.05$ ) reduced by the tested plant part powders. After 72 hours of application, the stem bark powder killed over 40% of the adult *C. maculatus* at all concentrations; there was a significant

difference ( $P < 0.05$ ) in the adult insect mortality between the treatments.

**Table 1: Contact toxicity of *P. africanum* stem bark powder on adult mortality of *C. maculatus*.**

Rate(g/20g) Cowpea (seeds)	%Mortality (Mean±S.E.)			
	Day 1	Day 2	Day 3	Day 4
0.1	23.33±3.33 <sup>bc</sup>	33.33±3.33 <sup>b</sup>	44.33±3.33 <sup>bcd</sup>	50.67±3.33 <sup>b</sup>
0.2	36.67±3.33 <sup>de</sup>	43.33±3.33 <sup>def</sup>	44.33±3.33 <sup>defg</sup>	56.67±3.33 <sup>bcd</sup>
0.5	30.00±0.00 <sup>bcd</sup>	40.00±0.00 <sup>cdef</sup>	40.00±0.00 <sup>cdef</sup>	53.33±3.33 <sup>bcd</sup>
1.0	46.67±3.33 <sup>fg</sup>	50.00±0.00 <sup>fg</sup>	50.00±0.00 <sup>fg</sup>	63.33±3.33 <sup>de</sup>
2.0	56.67±3.33 <sup>gh</sup>	70.00±5.77 <sup>i</sup>	70.00±5.77 <sup>i</sup>	90.00±5.77 <sup>fg</sup>
Control	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Mean follow by the same alphabet in column are not significantly different from one another ( $p > 0.05$ ) using Duncan New Multiple Range Test (DNMRT).

**Fumigant toxicity of *P. africanum* stem bark powder on adult mortality of *C. maculatus***

Table 2 highlights the fumigant effects of the plant part powders on adult mortality of *C. maculatus*. It shows

gradual increase in adult mortality as the powder dosage increased from 0.1 – 2.0g and length of exposure from 24 hours to 96 hours. An increase in dosage from 0.1 – 2.0g powder under 24 hours exposure shows significant difference ( $P < 0.05$ ) from the control sample. There is no significant difference ( $P < 0.05$ ) in mortality of adult cowpea beetles among the treatments.

**Table 2: Fumigant toxicity of *P. africanum* stem bark powder on adult mortality of *C. maculatus*.**

Rate (g/20g) Cowpea (seeds)	%Mortality (Mean±S.E.)	
	Day 1	Day 4
0.1	20.00±0.00 <sup>bc</sup>	80.00±5.77 <sup>de</sup>
0.2	30.00±5.77 <sup>def</sup>	83.33±3.33 <sup>e</sup>
0.5	33.33±3.33 <sup>efg</sup>	100.00±0.00 <sup>f</sup>
1.0	40.00±0.00 <sup>gh</sup>	100.00±0.00 <sup>f</sup>
2.0	40.00±0.00 <sup>gh</sup>	100.00±0.00 <sup>f</sup>
Control	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

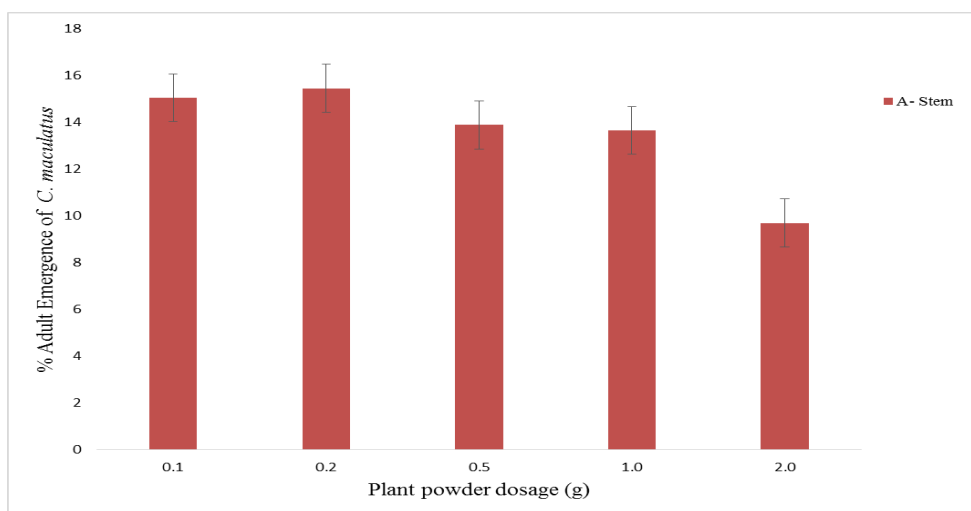
Mean follow by the same alphabet in column are not significantly different from one another ( $p > 0.05$ ) using Duncan New Multiple Range Test (DNMRT).

**Percentage adult emergence of *C. maculatus***

The stem bark powders of the *P. africanum* effectively reduced percentage adult emergence of *C. maculatus*. There was significant difference ( $P > 0.05$ ) in the percentage adult emergence. The percentage adult

emergence in the untreated seeds was significantly different ( $P > 0.05$ ) from emergence in the cowpea seeds treated with plant part powders at all concentration levels.

It shows an instant and continuing level of effectiveness as the dosage increased from 0.1 to 2.0g across the four exposure periods ranging from 24 to 96 hours.



**Figure 1: Percentage adult emergence of *C. maculatus*.**

### Contact toxicity of *P. africanum* oil extract on adult mortality of *C. maculatus*

Table 3 below depicts percentage mortality of adult *C. maculatus* on treated cowpea seeds with different concentration of methanolic oil extracts of the *P. africanum* stem bark. Mortality increases as the concentration increases and exposure periods. Methanolic oil extracts of the plant part was able to cause

70% mortality of adult bruchids after 48 hours post application period, However, *P. africanum* stem bark at 20% concentration caused over 93% mortality of adult *C. maculatus* after 72 hours post application.

The percentage mortalities across the plant extracts were significantly different ( $P < 0.05$ ) at each concentration. And was significantly different from the control.

**Table 3: Contact toxicity of *P. africanum* oil extract on adult mortality of *C. maculatus*.**

Plant Extract	Concentration (%)	%Mortality (Mean±S.E.)			
		24 hours	48 hours	72 hours	96 hours
A – Stem	1	23.33±3.33 <sup>bc</sup>	30.00±0.00 <sup>bc</sup>	46.67±3.33 <sup>bcd</sup>	46.67±3.33 <sup>bc</sup>
	2	33.33±3.33 <sup>cde</sup>	40.00±5.77 <sup>cde</sup>	48.67±3.33 <sup>def</sup>	56.67±3.33 <sup>def</sup>
	5	33.33±3.33 <sup>cde</sup>	43.33±3.33 <sup>def</sup>	53.33±3.33 <sup>ef</sup>	63.33±3.33 <sup>fg</sup>
	10	43.33±3.33 <sup>def</sup>	56.67±3.33 <sup>ghi</sup>	76.67±3.33 <sup>h</sup>	96.67±3.33 <sup>i</sup>
	20	56.67±3.33 <sup>hi</sup>	70.00±5.77 <sup>jk</sup>	93.33±6.67 <sup>i</sup>	100.00±0.00 <sup>i</sup>
Control	00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Mean follow by the same alphabet in column are not significantly different from one another ( $p > 0.05$ ) using Duncan New Multiple Range Test (DNMRT).

KEY:

A = *P. africanum*

Toxicity of *P. africanum* stem bark oil extract on adult mortality of *C. maculatus* indicating gradual increase in mortality as the dosage increased from 0.1 – 2.0g oil dosage.

The fumigant effect of methanolic oil extract of *P. africanum* stem bark on adult mortality of *C. maculatus* is presented in table 4. 1 - 2.0 % concentration of the

root bark oil extract caused 20 – 50% adult mortality after 24 hours post treatment which were significantly different ( $P > 0.05$ ) from untreated cowpea seeds (control). However, at 96 hours post treatment period *P. africanum* stem bark oil extract, 100% adult mortality was obtained at 1.0% concentration. Therefore, at 96 hour post treatment period, there were significant difference between all the treatments and the control.

**Table 4: Fumigant toxicity of *P. africanum* stem bark oil extract on adult mortality of *C. maculatus*.**

Oil extract	Concentration(%)	%Mortality (Mean±S.E.)	
		Day 1	Day 4
A – Stem	1	23.33±3.33 <sup>bc</sup>	63.33±3.33 <sup>cde</sup>
	2	33.33±3.33 <sup>def</sup>	73.33±3.33 <sup>fgh</sup>
	5	43.33±3.33 <sup>ghi</sup>	90.00±0.00 <sup>k</sup>
	10	46.67±3.33 <sup>hij</sup>	100.00±0.00 <sup>l</sup>
	20	46.67±3.33 <sup>hij</sup>	100.00±0.00 <sup>l</sup>
Control	00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

Mean follow by the same alphabet in column are not significantly different from one another ( $p > 0.05$ ) using Duncan New Multiple Range Test (DNMRT).

KEY:

A = *P. africanum*

### EXPERIMENTS ON SEED VIABILITY

*P. africanum* stem bark powders successfully decreased the percentage of *C. maculatus* adults that emerged. (Table 5). The percentage of adult emergence showed a significant difference ( $P > 0.05$ ). In cowpea seeds treated with plant component powders at all concentration levels, the percentage of adult emergence was considerably ( $P > 0.05$ ) higher than in untreated seeds.

As the dosage was increased from 0.1 to 2.0g over the course of four exposure periods, ranging from 24 to 96

hours, it demonstrated an immediate and sustained degree of effectiveness.

**Table 5: Percentage seeds damaged, weight loss, BPI caused by *C. maculatus* in cowpea seeds treated with 3 plants part powders.**

Plant Powder	Dosage (g)	Number of Seed	% Seed Damage	% Weight Loss	Beetle Perforation Index
A- Stem	0.1	72.00	6.96±0.88 <sup>gh</sup>	7.50±0.87 <sup>gh</sup>	9.28±1.07 <sup>fg</sup>
	0.2	72.00	0.92±0.92 <sup>ab</sup>	1.00±1.00 <sup>ab</sup>	1.22±1.22 <sup>ab</sup>
	0.5	72.67	0.46±0.46 <sup>a</sup>	0.50±0.50 <sup>a</sup>	0.65±0.65 <sup>ab</sup>
	1.0	72.67	0.47±0.47 <sup>a</sup>	0.50±0.50 <sup>a</sup>	0.67±0.67 <sup>ab</sup>
	2.0	73.00	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Control	0.0	71.00	72.70±1.18 <sup>i</sup>	77.50±0.00 <sup>j</sup>	>50 <sup>h</sup>

Mean follow by the same alphabet in column are not significantly different from one another ( $p>0.05$ ) using Duncan New Multiple Range Test (DNMRT).

KEY:

A= *Piptadeniastrum africanum*

## DISCUSSION

The use of powdered ash and oil extracts derived from plants has been shown to mitigate the economic damage caused by insect pests and fumigant repellent toxicity, as well as to have developmental inhibitory properties. (Adedire and others, 2011). According to this study, *P. africanum* has a lot of promise for use as a contact biopesticide agent against cowpea bruchids.

Powder of *P. africanum* was able to achieve high adults mortality of the cowpea bruchids at the various concentration tested. Stem bark of *P. africanum* completely prevented the emergence i.e (100% mortality) of adult *C. maculatus*, Considerable reduction observed in the numbers of adult emerged in *C. maculatus* exposed to both contact and fumigant toxicity test of *P. africanum* powders and ethanolic oil extract especially at the highest experimental dosage and exposure time, shows that this plant possesses insecticidal properties against *C. maculatus*. This concurs with the findings of Ashamo and Akinawonu (2012). Several reports of the insecticidal effectiveness of various plant materials against *S. zeamais* have been made. The physical abrasion of *C. maculatus*'s wings and cuticle by this plant material may be the cause of the high contact toxicity of this plant's root. This might have led to the significant mortality rate of *C. maculatus* seen in this study, as well as fluid loss and dehydration. Because of the strong odor that the plant portion releases, bruchids exposed to fumigant tests exhibited higher mortality rates than those exposed to contact tests. The fact that *P. africanum* did not contaminate the produce or change its colour further implies that it would be preferable to employ it as a fumigant against *C. maculatus*. Since there were no appreciable variations when compared, it may also be utilised as a contact pesticide. This plant's high volatility of the active ingredients—which kill the insect by diffusing in through the spiracles—may be the cause of its high fumigant toxicity against the insect. This may have caused the bruchid spiracles to become blocked, suffocating the insects and resulting in the high death rate seen in insects subjected to fumigant test.

## Contact toxicity of *P. africanum* on adult mortality of *C. maculatus*

The contact toxicity of *P. africanum* depends on concentration, and exposure periods. Mortality varied with rate of application and exposure periods. At 24hrs post treatment, the 0.5g rate of the root bark showed slightly above 30% adult mortality of the insect. This findings was similar to observation of Akinneye (2011) in the control of *E. Cautella* with *C. patens* powder. The root bark powder at the rate of 2.0g and 2.5g concentration was effective against *C. maculatus*, producing 100% mortality within 96 hrs post treatment. This observation also tallies with the findings of Adedire and Lajide (2001) that the pulverized powder of *P. umbrellatum* seed and *Eugenia aromatic* were toxic to *C. maculatus* producing 100% mortality at 24hr post treatment across and concentrations. The toxic effect of the stem bark of *P. africanum* in this study could be attributed to their pungency which evoked suffocating action of the insect. The powder may also bind to the enzyme, cholinesterase thus preventing the removal and resultant accumulation of acetylcholine, the neurotransmitter, restlessness convulsion and paralysis may occur resulting in death of insect (Ashamo, 2000).

## Fumigant toxicity of *P. africanum* Powders on adult mortality and Adult emergence of *C. maculatus*.

The fumigant toxicity of *P. africanum* stem bark powders on adult mortality of *C. maculatus* observed at 96 hours post treatment with different dosage level revealed the insecticidal potency of *P. africanum* stem bark. The percentages mortality varied with rate of application and exposure periods. There was above 30% mortality at 24hrs post treatment, at 0.5g rate of stem bark. At 96hrs post treatment, all rates of the root bark, i.e 0.1-2.0g rate caused greater than 83% adult mortality of the insect. This findings is similar to the findings of Ashamo and Akinneye (2004) that *Eugenia aromatic* caused greater than 43.3% mortality of *E. vapidella*, at 0.05g/15g of yam. The powder of Plant material acted mainly through fumigant modes of action. This powder may cause death through respiratory inhibition, inhibition of oxidative phosphorylation and amine metabolism (Ashamo 2000). Also *P. africanum* stem bark effectively reduced percentage adult emergence of *C. maculatus*. There was



significant difference ( $P > 0.05$ ) in the percentage adult emergence. The percentage adult emergence in the untreated seeds was significantly different ( $P > 0.05$ ) from emergence in the cowpea seeds treated with plant part powders at all concentration levels.

## CONCLUSION

In this study, the stem bark powder and ethanol oil extract of *P. africanum* were found to be the most effective for the control of *C. maculatus* on cowpea seeds since they completely inhibited development of *C. maculatus* from eggs to adult stage. The powder and ethanol oil extract were able to achieve 100% adult mortality after 96 hours post-treatment. Furthermore, the powder and oil extract of *P. africanum* stem bark tested on the liver and kidney of albino rat and were found to be non-toxic, since there was no significant difference between the control and the animal treated with the powder and the ethanolic oil extracts. Therefore, both the powder and the oil extract of *P. africanum* stem bark could be recommended for use to protect stored cowpea seeds and can also be integrated with other pest management procedure. Additionally, the cowpea seeds are safe for consumption after post-harvest treatment since the stem bark and oil were found to be non-toxic to mammals.

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