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Potency and Repellence Effects of *Jatropha curcas* L. Extracts With and Without Pirimiphos-methyl against *Callosobruchus subinnotatus* (Pic.) in Stored Bambara Groundnut, *Vigna subterranea* (L.) Verdcourt

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Abstract

The study evaluated the potency and repellence effects of *Jatropha curcas* L. leaf and seed powders and their combinations with and without reduced rate of Pirimiphos-methyl, on *Callosobruchus subinnotatus* (Pic.) a major pest of Bambara groundnut, *Vigna subterranea* (L.) Verdcourt. Factorial experiments (4×4×2) and (2×2×2) for potency and repellence assessments, respectively, were laid out in a Complete Randomized Design (CRD) and replicated three (3) times to assess the impact of the different treatment combinations. Potency was assessed by counting emerged adults 60 days after treatment (DAT), while repellence was monitored during the first 5 hours post-application. The results showed that the treatment with 1.5g leaf powder + 0.0g seed powder + 0.00g Pirimiphos-methyl exhibited 100% potency effect ($P \leq 0.001$), while the treatment with 0.5g leaf powder + 0.5g seed powder + 0.01g Pirimiphos-methyl showed up to 90% repellence effect ($P \leq 0.01$). The study highlighted that *J. curcas* have the potential to serve as a botanical insecticide for the sustainable management of *C. subinnotatus* in stored Bambara groundnut.

Keywords: *Jatropha curcas, Callosobruchus subinnotatus*, Botanical insecticide, Potency, Repellence, Bambara groundnut, sustainable pest management

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INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdcourt) is an indigenous African nutrient-rich legume valued for its adaptability to harsh environments where other crops often fail (Massawe *et al.*, 2005). Bambara groundnut also serves as a vital source of protein, carbohydrates and micronutrients as well as income for many smallholder farmers in sub-Saharan Africa (SSA) (Hillocks *et al.*, 2012; Oyeyinka and Oyeyinka, 2018). However, its production is often hampered by pests, including the *Callosobruchus subinnotatus* (Pic.), a major storage insect pest that can cause significant damage to the crop (Adedire and Lajide, 2000). To mitigate this challenge, farmers often resort to synthetic chemical insecticides, which can have adverse effects on human health and the environment (Isman, 2006).

In recent years, there has been a growing interest in the use of botanical insecticides as a sustainable and eco-friendly alternative to synthetic chemical insecticides (Koul *et al.*, 2008). This shift in attention towards botanical insecticides is because they are known to be biodegradable, safer for non-target organisms, and locally available (Regnault-Roger, Vincent and Arnason, 2012). One such plant-based insecticide is *Jatropha curcas* (L.), which has been shown to exhibit potent insecticidal activity against a wide range of pests (Devappa *et al.*, 2010). The use of *J. curcas* as a botanical insecticide offers several benefits, including reduced environmental pollution, toxicity to humans, and development of pesticide resistance (Isman, 2006).

J. curcas, a multipurpose plant, contains secondary metabolites with insecticidal, antifeedant and repellent properties, including phorbol esters, diterpenoids, and saponins (Kumar and Sharma, 2008). Although the pesticidal activity of *J. curcas* extracts against several storage pests is documented (Samuel, *et al.*, 2017), limited information exists on their effects against *C. subinnotatus* on Bambara groundnut, particularly in conjunction with reduced doses of synthetic chemicals. Combining botanicals with low concentrations of conventional insecticides has been proposed to improve efficacy while reducing chemical usage (Shaaya and Kostyukovsky, 2006).

This study aims to investigate the potency and repellence effects of *J. curcas* leaf and seed powders, with and without synthetic chemical application (Pirimiphos-methyl), on *C. subinnotatus*, a major storage insect pest of Bambara groundnut. By exploring the potential of *J. curcas* as a botanical insecticide, this study seeks to contribute to the development of sustainable pest management strategies that minimize the use of synthetic chemical insecticides and promote environmentally friendly practices.

MATERIALS AND METHODS

Collection and preparation of the Bambara groundnut seeds

Fifty Kilogrammes of unshelled Bambara nut, cream/brown eye variety seeds were purchased at a local market in Dambatta Local Government Area, Kano State. The seeds were decorticated manually. Shrivelled (shrunken) and damaged seeds as well as all other debris were removed. To disinfest the cleaned whole seeds, they were put in a polythene bag together with two phostoxin tablets (in an envelope) for 24 hours. The top of the bag was tied securely to ensure that any insect pest present within the seeds was killed according to the method of Ogunwolu *et al.,* (2002). Thereafter, the seeds were opened and spread in a shaded well-ventilated place for 48 hours to ensure that the seeds were free from the phostoxin residue. To avoid subsequent re-infestation and to ensure that any insect pest that might still remain within the seeds was killed the previously fumigated seeds were transferred into a fresh different polythene bag and kept at -4˚C inside a fridge for four days (Ahmed, 2007).

Collection and preparation of the plant parts

The leaves and seeds of the physic nut *J. curcas* were collected at the orchards of Audu Bako College of Agriculture, Dambatta, Kano State, while the Pirimiphos-methyl (Actellic dust) was purchased from a pesticide store at Abubakar Rimi market, Sabon-gari, Kano, Kano State, Nigeria.

The plant parts were shade-dried to crispy condition. Thereafter, it was pounded in a mortar with pestle and then passed through a sieve 40 µmm to give a very fine powder as described by Youdeowei (2004) and Yusuf and Ahmed (2005). The fine powdered plant materials were kept in plastic bags until needed.

Source and rearing of the insect culture

The initial culture of the Bambara nut bruchids were obtained from naturally infested Bambara nut seeds at Kurmi market, Kano city, Kano State, Nigeria. A sample of the insects on the infested seeds were taken to the Department of Crop Protection, Ahmadu Bello University, Zaria, Kaduna State, Nigeria for proper taxonomic identification as *Callosobruchus subinnotatus* (Pic.). These were then massively reared in transparent plastic buckets measuring 30 cm in height and 15 cm top diameter. The top end of the plastic buckets were covered with white muslin cloth, secured firmly into place with rubber bands, hence allowing for ventilation. These were incubated in Kliner jar at an ambient temperature and relative humidity (32±3°C and 57±3% respectively) with alternating light and dark cycle for 12 hours as described by Abduljalal *et al.* (2011).

One kilogramme of the clean disinfested Bambara nuts were transferred into ten plastic buckets each. In addition, 100 adults of *C. subinnotatus* from the initial culture were introduced into the buckets. To ensure that the introduced bruchids did not escape away, the top ends of the buckets were covered with white muslin cloth and secured firmly with rubber bands. The insects were allowed to mate and oviposit for 10 days after which they were removed.

Experimental Design

The potency of *J. curcas* leaf and seed powders was assessed in a 4×4×2 factorial experiment. The first and the second factors, (leaf and seed, respectively) had 4 levels each (0.0, 0.5, 1.0 and 1.5 g/20 g Bambara groundnut seeds) while the third factor; Pirimiphos-methyl, which served as the check and control had two levels (with and without). The Pirimiphos-methyl was applied at a reduced rate of 0.01 g/20 g Bambara groundnut seeds (Gwinner, *et al.,* 1996). There were 32 treatments replicated 3 times in a completely randomized design. A total of 96 transparent plastic cups measuring 10 cm in depth and 9 cm top diameter, containing the treatment combinations and the test-insects, were kept in the laboratory at ambient temperature and relative humidity of 32±3°C and 57±3%, respectively.

Repellence effect of the treatments was also assessed in a 2×2×2 factorial experiment. The leaf and seed powders were applied at two levels each (0 and 1.5 g/20 g Bambara groundnut seeds) while the third factor; Pirimiphos-methyl, which also served as the check and control had two levels (with and without). The Pirimiphos-methyl was also applied at the reduced rate of 0.01 g/20 g Bambara groundnut seeds (Gwinner, *et al.,* 1996). There were eight treatments, which were replicated 3 times in a completely randomized design. A total of 24 treatment combinations together with the test-insects were placed in Petri dishes (9 cm diameter) lined with two halves (treated and untreated sides) Whatman size filter paper (9 cm diameter) and kept in the laboratory at ambient temperature and relative humidity of 32±3ºC and 57±3%, respectively.

Assessment of Potency (Residual toxicity)

Data on potency (residual toxicity) were collected by counting the number of dead insects out of the first generation (F_1) progeny reproduced by the parents which were introduced into the treated seeds 54 days after treatment when all the introduced parents were dead and the off springs from the eggs they laid had emerged (Abduljalal *et al.,* 2011).

Assessment of Repellence Effect

Half Whatman size filter paper (9 cm diameter) discs were prepared by cutting the filter paper into two halves and placed inside Petri dishes. One half of the disc contained extracts of the treatments (leaf and seed powders of *J. curcas* and their combinations (with and without Pirimiphos-methyl) diluted in 2 ml of distilled water (w/v). Similarly, in the control treatment 2 ml of distilled water only was added to the other half of the disc. Both the treated and the untreated control halves were air-dried to evaporate the moisture completely. Each treated half was attached lengthwise, edge to edge with an untreated control using thin strip of adhesive tape then placed in the Petri dish. The orientation of the seams was changed in the replicates to avoid the effect of any external directional stimulus affecting the distribution of the insects. Adult bruchids were released by the use of an aspirator onto the middle of each filter paper circle. For ventilation and visibility, transparent netted cloth was placed on top of the Petri dishes and held in place with the aid of rubber bands. Number of insects that moved and settled on each half of the filter paper were counted after one hour, then at hourly intervals for five consecutive hours as described by Liu and Ho (1999); Juntarajumnong and Chandrapatya (2009). Percentage repellency was calculated according to Sighamony *et al.* (1984).

$$\% \, repellency = \frac{C - T}{C} \times 100 \quad \text{\_\_\_\_\_\_[1]}$$

Where: C = number of insects on untreated surface

T = number of insects on treated surface

The repellency was then classified according to McGovern (1977) as follows:

Class 0 = negative repellency;
Class I = 0.1-20% repellency;
Class II = 20.1-40% repellency;
Class III = 40.1-60% repellency;
Class IV = 60.1-80% repellency and
Class V = 80.1-100% repellency.

Phytochemical Screening

Phytochemical analyses of the crude leaf and seed powder collected were conducted according to standard procedures to identify their chemical components (Sofowora, 1993; Trease and Evans, 1989; Kokate *et al.,* 2008 and Harborne, 1988). This was conducted at the National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria. Ferric chloride (FeCl3) test for tannins, organic acid (OAT) test for oxalates, foam test for saponins, Keller-Killiani test for glycosides, ferric chloride (FeCl3) test for flavonoids and Wagner's Reagent as well as Meyer's Reagent tests for alkaloids were carried out to identify the chemical constituents present in the leaf and seed products of the plant.

Tannin test
Five milliliter (5ml) of each of the plant part (leaf and seed) extract was treated with 2ml of Ferric chloride ($FeCL_3$) and boiled for 5 minutes. Formation of red precipitate confirmed the presence of tannins.

Oxalate test
To three millilitre (3ml) portion of the extracts, a few drops of ethanoic acid glacial were added. Greenish black colouration indicated presence of oxalates.

Saponin test
To one milliliter (1ml) of each of the plant part extract, four milliliter (4ml) of distilled water was added and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously; and then for the formation of emulsion as evidence for presence of saponins.

Glycoside test
The plant part extracts were treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, formation of two layers was observed. Lower reddish-brown layer and upper acetic acid layer which turns bluish green indicated a presence of glycosides.

Flavonoid test
Test plant parts were treated with few drops of Ferric chloride ($FeCl_3$) solution which resulted in the formation of blackish red colour indicating the presence of flavonoids.

Alkaloid test
Five milliliter (5ml) of 2% of hydrogen chloride (hydrochloric acid HCL) was added to 2ml of each of the plant part extract in a test tube placed on a steam bath and warmed. It was filtered and divided into two parts for the following tests:
Few drops of Wagner's Reagent (Potassium-iodine solution) were added to one part of the filtrate in a test tube. A reddish-brown precipitate was observed.
Few drops of Meyer's Reagent (Potassium mercuric iodine solution) were added to the other filtrate of the solution in a test tube. A cream coloured precipitate was observed.
In both cases, formation of precipitation indicated presence of alkaloids.

Data Analysis
Data in percentages were transformed into arc sine percentages, while numerically count data were transformed using $\sqrt{n+1}$ before analysis, as described by Little (1978). All the data were subjected to analysis of variance using computer software (SAS for windows) statistical package. Treatments with significant differences were compared and separated at 0.05% level of probability using Duncan's multiple range test (DMRT).

RESULTS

The potency effects of different combinations of the *Jatropha curcas* leaf and seed powders with and without Pirimiphos-methyl on *C. subinnotatus* 60 days after treatment (DAT) were presented in Table 1. The untreated control 0.0g leaf powder + 0.0g seed powder + 0.00g Pirimiphos-methyl recorded the highest proportion of emerged adults (84.67), which indicated severe second-generation infestation. All other treatment combinations containing *J. curcas* leaf or seed powders, whether applied singly or in combination with Pirimiphos-methyl, significantly ($P \leq 0.001$) reduced second-generation emergence (0–12 insects) and no significant differences existed among the treated groups (all designated "b"), suggesting comparable effectiveness across all botanical and combination treatments

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Table 1: Potency effect of *Jatropha curcas* extracts with and without synthetic chemical application on *Callosobruchus subinnotatus* at 60 DAT

Treatment			**Potency**⌂
Leaf powder(g)	**Seed powder(g)**	**Pirimiphos-methyl(g)**	
0.0	0.0	0.00	84.67$^{a\Omega}$ (66.89)
0.0	0.0	0.01	0.00^{b} (0.00)
0.0	0.5	0.00	1.00^{b} (5.74)
0.0	0.5	0.01	0.00^{b} (0.00)
0.0	1.0	0.00	0.33^{b} (0.99)
0.0	1.0	0.01	0.33^{b} (0.99)
0.0	1.5	0.00	0.00^{b} (0.00)
0.0	1.5	0.01	0.00^{b} (0.00)
0.5	0.0	0.00	0.33^{b} (0.99)
0.5	0.0	0.01	0.00^{b} (0.00)
0.5	0.5	0.00	0.33^{b} (0.99)
0.5	0.5	0.01	0.67^{b} (1.40)
0.5	1.0	0.00	0.00^{b} (0.00)
0.5	1.0	0.01	0.00^{b} (0.00)
0.5	1.5	0.00	4.67^{b} (12.39)
0.5	1.5	0.01	4.00^{b} (11.54)
1.0	0.0	0.00	0.33^{b} (0.99)
1.0	0.0	0.01	0.00^{b} (0.00)
1.0	0.5	0.00	0.33^{b} (0.99)
1.0	0.5	0.01	0.00^{b} (0.00)
1.0	1.0	0.00	0.00^{b} (0.00)
1.0	1.0	0.01	12.00^{b} (20.27)
1.0	1.5	0.00	0.00^{b} (0.00)
1.0	1.5	0.01	0.00^{b} (0.00)
1.5	0.0	0.00	0.00^{b} (0.00)
1.5	0.0	0.01	1.33^{b} (6.55)
1.5	0.5	0.00	0.00^{b} (0.00)
1.5	0.5	0.01	0.00^{b} (0.00)
1.5	1.0	0.00	0.00^{b} (0.00)
1.5	1.0	0.01	0.00^{b} (0.00)
1.5	1.5	0.00	5.00^{b} (12.92)
1.5	1.5	0.01	0.00^{b} (0.00)
L.S.[*]			***
SE±			4.047

$^{\Omega}$Means within a column followed by different letters are statistically significantly different at *** = P ≤ 0.001, Duncan's multiple range test. ⌂Figures in parentheses are Arcsine √percentage transformations. [*]L.S = level of significance.

Table 2 showed the repellence effects during the first 5 hours after treatment (HAT). The untreated control (0.0g leaf powder + 0.0g seed powder + 0.00g Pirimiphos-methyl) maintained, consistently, higher number of insect presence (10 insects) with no any repellence effect observed. In contrast, all other treatments significantly ($P \leq 0.001$) repelled the adult bruchids, which markedly showed reduced insect counts (0.33–3 insects) throughout the observation period (1 to 5 HAT). Similar repellence levels were observed among all botanical and mixed treatments and treatment combinations (denoted "b").

Table 2: Repellence effect of *Jatropha curcas* extracts with and without synthetic chemical application on adults of *Callosobruchus subinnotatus*

Treatment			**Repellence effect hours after treatment (HAT)**$^{\triangle}$				
Leaf powder(g)	**Seed powder(g)**	**Pirimiphos-methyl(g)**	**1**	**2**	**3**	**4**	**5**
0.0	0.0	0.00	$10.00^{a\Omega}$ (18.44)	$10.00^{a\Omega}$ (18.44)	$10.00^{a\Omega}$ (18.44)	$10.00^{a\Omega}$ (18.44)	$10.00^{a\Omega}$ (18.44)
0.0	0.0	0.01	1.33^{b} (6.55)	1.33^{b} (6.55)	0.67^{b} (1.40)	2.33^{b} (8.72)	1.33^{b} (6.55)
0.0	0.5	0.00	1.67^{b} (7.27)	2.33^{b} (8.72)	2.67^{b} (9.28)	1.67^{b} (7.27)	1.00^{b} (5.74)
0.0	0.5	0.01	3.00^{b} (9.98)	2.67^{b} (9.28)	2.00^{b} (8.13)	1.33^{b} (6.55)	1.00^{b} (5.74)
0.5	0.0	0.00	3.00^{b} (9.98)	3.00^{b} (9.98)	2.00^{b} (8.13)	0.67^{b} (1.40)	1.00^{b} (5.74)
0.5	0.0	0.01	2.33^{b} (8.72)	1.00^{b} (5.74)	2.00^{b} (8.13)	3.00^{b} (9.98)	2.67^{b} (9.28)
0.5	0.5	0.00	0.67^{b} (1.40)	2.67^{b} (9.28)	3.00^{b} (9.98)	3.00^{b} (9.98)	1.33^{b} (6.55)
0.5	0.5	0.01	1.33^{b} (6.55)	1.33^{b} (6.55)	0.33^{b} (0.99)	0.33^{b} (0.99)	1.67^{b} (7.27)
L.S.*			***	**	***	***	***
SE±			0.711	0.856	0.925	0.939	0.762

$^{\Omega}$Means within a column followed by different letters are statistically significantly different at ** = $P \leq 0.01$, *** = $P \leq 0.001$, Duncan's multiple range test.
$^{\triangle}$Figures in parentheses are Arcsine √percentage transformations.
*L.S = level of significance.

Table 3 showed the presence and/or absence of phytochemical compounds in the leaf and seed of the *J. curcas* plant. The table indicated that some of the compounds are present in both parts of the plant (leaf and seed), while others (like cynogenic glycoside) are only found in the leaves. However, both the leaf and the seed contain tannins, oxalates, saponnins, flavonoids, and alkaloids, which indicated that these compounds are widely distributed in the plant.

Table 3: Phytochemical constituents in the leaf and seed of *Jatropha curcas*

Chemical compound	**Leaf**	**Seed**
Tannin	+$^{\Omega}$	+$^{\Omega}$
Oxolate	+	+
Saponnin	+	+
Cynogenic glycoside	+	-
Flavonoid	+	+
Alkaloid	+	+

$^{\Omega}$ + = indication of presence and - = indication of absence of chemical compound.

DISCUSSION

The results of this study suggested strong bioactivity of *J. curcas* leaf and seed powders against *C. subinnotatus* in stored Bambara groundnut. High number of second generation (F_2) adult emergence in the untreated (control) Bambara groundnut highlighted the destructive capacity of *C. subinnotatus* in unprotected stored Bambara groundnut. However, the addition of *Jatropha* powders (leaf and seed) to the Bambara groundnut grains significantly suppressed adult emergence, which suggested strong potent effect (residual toxicity) or growth-inhibitory effects. This agreed with the findings of Kumar and Sharma (2008) which stated that phytochemicals such as diterpenoids and phorbol esters contained in *J. curcas* interfere with insect metabolism.

The comparable potency observed between the botanical-only (the leaf and seed powders) and botanical/synthetic treatment combinations suggested that the *J. curcas* extracts alone could exert sufficient lethal action against the bruchids minimizing the need for the conventional insecticide. Similar findings were reported by Samuel *et al.* (2017) in which *Jatropha* seed oil provided sufficient control against major stored-product insect pests during storage.

In addition, the lack of significant improvement as a result of the addition of reduced rate of Pirimiphos-methyl to the treatment combinations (leaf and seed powders) reinforced the efficacy of the *Jatropha*-based treatments as stand-alone protectants. In the same vein, the presence of various phytochemical compounds in the *J. curcas* leaf and seed might have contributed to its insecticidal and repellence properties as stated in a study conducted by Opuba *et al.* (2018).

The repellence activity in the early post-treatment hours further suggested the potential of the *J. curcas* leaf and seed powders as pre-contact deterrents. This agreed with the findings of Mgbemena *et al.* (2016) which stated that the bioactive effect of *Jatropha* is attributed to the volatile phytochemical constituents of the plant which disrupt oviposition site selection by adult bruchids. The rapid repellence (within 1 to 5 HAT) as observed in this study also suggested added advantage in reducing oviposition rate on the stored Bambara groundnut and subsequent re-infestation and population buildup of the bruchids (Dattijo *et al*., 2016; Opuba *et al*., 2018).

Furthermore, studies conducted by Dattijo *et al.* (2018); Dattijo *et al*. (2021a) had shown that *J. curcas* leaf and seed powders exhibited significant insecticidal activity against *C. subinnotatus*, which resulted in reduced damage on stored Bambara groundnut. Also, the leaf and seed powders had been reported to exhibit fumigant, repellent, and contact effects against *C. subinnotatus*, making it a potential alternative to synthetic insecticides (Dattijo *et al.,* 2021b).

CONCLUSION & RECOMMENDATION

The *Jatropha curcas* leaf and seed powders effectively suppressed re-infestation of second generation (F_2) and repelled *C. subinnotatus* in the stored Bambara groundnut. The standalone performance of the *Jatropha* extracts (leaf and seed powders) was comparable to that achieved when combined with the low doses of Pirimiphos-methyl, suggested that the *J. curcas* extracts could serve as a viable and sustainable alternative to the synthetic insecticide for the control of *C. subinnotatus* in stored Bambara groundnut. The dual action of repellence and insecticidal potency makes the extracts a promising component of sustainable storage pest management. The phytochemical compounds present in *J. curcas*, which included tannins, saponnins, flavonoids, and alkaloids, might have contributed to its insecticidal effects against the bruchid, *C. subinnotatus.*

From the findings of this study, the following recommendations were drawn:

1. The bioactive and insecticidal effects of the phytochemical compounds contained in *Jatropha curcas* plant should be explored further to ascertain their full potential for commercial production of *jatropha*-based botanical insecticide.
2. There is the need for further research to fully understand the mechanisms of action of the phytochemical compounds contained in the leaf and seed of *J. curcas* and to optimize their use as natural insecticides

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