EVALUATION OF THE EFFICACY OF THREE PARSLEY EXTRACTS (AQUEOUS, CHLOROFORM AND METHANOL) AGAINST *Callosobruchus maculatus* Fab. (COLEOPTERA: BRUCHIDAE) ON STORED BAMBARA NUT *Vigna subterranea* (L.) Verdc.

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Abstract
Laboratory experiment was carried out to evaluate the efficacy of three parsley extracts (Aqueous, Chloroform and Methanol) against Callosobruchus maculatus on stored Bambara nut (Farin ngangala). The experiment was conducted at Entomology Laboratory, Mohamet Lawan College of Agriculture, under ambient laboratory conditions of 36-40ºC temperature and 12-16% relative humidity. Thirty unsexed 0-1 day old adult insects were placed on 50g grain samples of Farin ngangala treated with the plant extracts at four application rate: 2.0, 4.0, 6.0 and 2.0 ml/kg, replicated three times. Adult mortality, oviposition, number of progeny emerged; percentage damage and percentage weight loss were assessed. On Bambara nut seeds treated with aqueous extract of parsley, significant differences were recorded between application rate and exposure periods on adult mortality ranging from 23.8±2.3 at 2ml/kg after 7 days of exposure to 91.3±0.9 at 8ml/kg after 21days of exposure. Mortality was also recorded at 2ml/kg after 7 days of exposing the beetles to Bambara nut treated with chloroform and methanol parsley extracts ranging from 22.3±6.2 and 23.3±3.3 to 100 and 83.3±2.0 after 21 days of exposure respectively. The oviposition, number of progeny emerged, and the percentage damage and weight loss were also recorded, indicating least oviposition (44.0+3.2), progeny emerged (1.2±0.9), percentage damage (0.9+0.1) and percentage weight loss (0.0) in the chloroform parsley extract. Most of application rates significantly varies with control and all the three extracts works followed the same trend i.e. mortality increases with increase in application rate and exposure period; and oviposition, progeny emergence, percentage damage and weight loss decreases with increase in application rate.
Keywords: *Botanical Insecticides, Stored Product Protection, Bruchid Management*

Introduction

Bambara nut (*Vigna subterranea* (L.) Verdc is an underutilized legume of African origin. The fruits are subterranean and the legume is widely cultivated by smallholders' farmers in semi-arid zones of Africa (Akpalu *et al*., 2013). It represents the third most important legume crop after cowpea and groundnut (Somta *et al*., 2011). *Vigna subterranea* is very rich in protein and calories with 16 to 25% of protein and 42 to 65% of carbohydrate on a dry weight basis (Linnemann and Azam-Ali 1993). Bambara nut is considered to be originated from West and Central Africa, widely grown in sub-Saharan Africa and also grown in low levels in Thailand, Malaysia, and Indonesia (Temegne *et al*., 2018; Mayes *et al*., 2019).

Nigeria is the largest producer of Bambara nut with a mean production of 0.1 million tons, followed by Burkina Faso 44,712 tons, and Niger 30,000 tons (Hillocks *et al*., 2012). The grains of Bambara nut are usually ground into four which are used to prepare different dishes such as 'alele, ganye, danwake, gauda, kosai, and waine' in Northern Nigeria (Linnemann 1988). The flour is also used for making a local delicacy called 'igba or okpa' in Igbo land and Idoma land, Eastern Nigeria (Echezona *et al*., 2013). The fresh seeds can be roasted or boiled and eaten as snacks and the taste is quite pleasant and relishing. Bambara groundnut are also grown for poultry and livestock feeds (Hillocks *et al*., 2012).

Bambara nut is susceptible to *C. maculatus* beetle infestation, a field-to-store insect pest. It is most often infested simultaneously by two species of Bruchids, *Callosobruchus subinnotatus* (Pic.) and *Callosobruchus maculatus* (F.) (Lale and Vidal, 2001). However, most often they are infested by *C. maculatus* (Coleoptera: Bruchidae) (Ouédraogo *et al*., 1996; Kosini and Nukenine, 2017). Despite the importance of Bambara nut in Nigeria, availability of their seeds to farmers for planting and grains for consumption are constrained by *C. maculatus* attack. An estimated 40% of the loss occurs in countries lacking adequate storage facilities. When available, pesticides are primarily utilized to control these pests. These synthetic pesticides have several downsides, i.e., they are applied indiscriminately, eliminate natural adversaries, alter the ecology, and damage the environment (Warra *et al*., 2020).

To minimize the menace of the use of synthetic chemicals in pest control and to assist farmers in reducing losses due to beetles, efforts should be positioned on the development of alternative control methods, such as use of botanical insecticides for insect management. Several plant parts have been evaluated for their insecticidal properties, many of them have been reported to act as natural protectants of cereals and legumes against insect pests by inhibiting reproduction while some acts as insect growth inhibitors, toxicants, antifeedant and repellent (Alabi and Adewole 2017; Trivedi *et al*., 2018; Ileke *et al*., 2020). The use of plant products in form powders and extracts to manage weevils and beetles is more convenient to farmers due to ease of application, affordability, and neatness of the produce after treatments and they are attractive to buyers (Ojo and Ogunleye 2013).

The objectives of study were to;

i. evaluate the mortality of the insects treated with three extracts (aqueous, methanol and chloroform) of parsley on stored Bambara nut;
ii. evaluate oviposition and progeny production of the beetles on Bambara nut treated with the extracts;
iii. determine the damage and weight loss caused by the beetles on Bambara nut treated with the extracts.

Materials and Methods

Experimental Site

The study was conducted at the Entomology Laboratory of the Department of Agricultural Technology, Mohamet Lawan College of Agriculture, Maiduguri, Borno State. All experiments were conducted under ambient laboratory conditions. Temperature between 36 – 40 ºC and relative humidity ranging from 12-16%.

Source and Culturing of Insects

Callosobruchus maculatus adults were collected from cultures maintained on cowpea in the laboratory and were used to establish new cultures on Bambara nut. The insects were then reared for two generations for the experiments. Culturing entails placing a hundred unsexed adult insects into 300 g of clean Bambara nut seeds placed in 1-liter capacity jar for seven days and then sieved out and discarded. The resulting F_1 generation aged 0 – 1 day old were used for the experiments.

Source and Preparation of Bambara Nut Seeds

The seeds (*Farin ngangala*) were obtained from certified Bambara nut marketers at Gamboru market in Maiduguri, Borno state. The seeds, after acquisition, were cleaned and placed under the sun for about one (1) week to kill all life stages of the insect that might be present. The seeds were conditioned to room temperature for at least 10 days before commencement of the experiment.

Source and Preparation of Plant Material (Parsley)

The plant material was obtained from the vegetable vendors at Gidan madara in Maiduguri, Borno state. The plant material after collection was washed to remove debris and then dried under shade. The plant material was pounded using pestle and mortar and sieved using 1mm sieve.

Method of Extraction

Extraction was done using the maceration method. Hundred gram (100 g) powder of the plant material was soaked in 1000 ml methanol, chloroform and distilled water solvents each and macerated for 72 h with regular shaking and stirring thrice daily. The mixture of solvent and powder was filtered through cheesecloth and Whatman No. 1 filter paper. The extract was left to evaporate in an open plastic bowl to dryness.

Bioassay Procedure

All experiments were laid out in completely randomized design (CRD), with four treatments of each extract, replicated three times. The seeds were treated at the following application rate of 2, 4, 6 and 8ml/kg. For each treatment, 50g of clean grains were placed in 250 liter capacity bottles and the appropriate amount of plant extracts were added to each samples. The jars were capped and shaken vigorously, manually to achieve uniform distribution of insecticide on the entire grain mass after which thirty (30) unsexed 0- 1 day old adult *C. maculatus* were infested to the treated grains, then covered and left for observation. Nine (9) bottles (three (3) for each solvent extract) of 50g replicates were prepared and left untreated which serves as the control.

Observation and Data Collection

The parameters observed were;

i. Adult mortality, was evaluated after 7, 14 and 21 days of exposure. Adult insect that do not respond to touch was considered dead.
ii. Oviposition; the number of eggs produced were counted after 8 days of infestation. Twenty (20) Bambara nut seeds were randomly selected and the number of eggs were counted using a hand lens.
iii. After the mortality is recorded, all insects were discarded, and jars containing treated and untreated Bambara nut were maintained in the same laboratory conditions until progeny emergence (1^{st} generations). The counting of F_1 progeny was carried out once a week, for four consecutive weeks commencing fourth week after infestation. After each counting session, the insects were removed from the jars and recorded.
iv. The percentage damage and percentage weight loss was determined by randomly taking 20 grains from each jar of all treatments, and separated into holed (damaged) and whole (undamaged) grains. The grains in each category was counted and the numbers used to calculate the percentage damage:

Damage (%) = damaged grains/ total grains × 100 -- (1)

For percentage weight loss, the grains in each of the above categories was weighed, and the weights used to calculate the percentage weight loss: (Golob *et al*., 1982).

Weight loss (%) = (Und) – (Dnu)/ U (nd + nu) × 100 -- (2)

where:

U – weight of undamaged grains

D – weight of damaged grains

nd – number of damaged grains
nu – number of undamaged grains

Data Analysis

All data were subjected to analysis of variance (ANOVA) using statistical software (Statistix 8.0). Differences in mean values of treatment were separated using Tukey Krammer's HSD test at 5% level of probability.

Results and Discussion

Effect Three (3) Extracts of Parsley on Mortality of *C. maculatus* on Stored Bambara Nut

The results in table 1 shows the effects of aqueous parley extract on mortality of C. maculatus on stored Bambara nut. Mortality of the beetles increased significantly with increase in dose rate and exposure period from 23.8 ± 2.3 to 44.5 ± 3.8 at 7 days after treatment, 28.1± 3.1 to 70.0 ± 4.0 at 14 days after treatment and 64.3 ± 4.3 to 91. 3 ± 0.9 at 21 days after treatment respectively.

Similarly, in table (2) which shows the effect of chloroform extract of parsley on mortality of *C. maculatus* exhibited effectiveness in toxicity as mortality rate increase with increase in dose rate from 22.3 ± 6.2 to 50.0 ± 5.7 after 7 days of treatment and maximum mortality was recorded (100%) at the highest dose of 8ml/kg after 21 days of treatment.

In the same trend, table (3), showing the effect of methanol extract of parsley on the mortality of *C. maculatus*, indicated increase in mortality with increase in dose rate and exposure period. Mortality significantly increased from 23.3 ± 3.3 at lowest dose ate of 2ml/kg at 7 days after treatment to 83.3 ± 2.0 after 21 days of treatment at 8ml/kg. At all the dose rate and exposure periods, treatments varies significantly with control.

The present study was conducted to determine the most effective parsley extracts from three different solvents (distilled water, chloroform and methanol).The harmful effects of the compounds in extracts of the studied plant observed in this study might have contributed to the mortality of *C. maculatus*, which is in close agreement with other similar works reported by Muhammad *et al*. (2018). Even though all of the extracts exhibited promise as insecticides, their toxic potencies against *C. maculatus* differed, most likely due to differences in solvent composition. Currently, chloroform extract of parsley exhibited the greatest mortality of *C. maculatus* at application rate of 8ml/kg after 21 days of exposure.

Table 1: Effect of Parsley (Aqueous Extracts) on Mortality of *C. maculatus* on Stored Bambara Nut

Application Rate	Mortality (Exposure Period)		
(ml/kg)	7(DAT)	14(DAT)	21(DAT)
0.0	1.0 ± 1.0^{c}	2.0 ± 1.0^{d}	3.0 ± 0.2^{d}
2.0	23.8 ± 2.3^{b}	28.1 ± 3.1^{c}	64.3 ± 4.3^{c}
4.0	30.9 ± 1.5^{ab}	49.6 ± 3.6^{b}	76.3 ± 2.0^{b}
6.0	44.1 ± 5.8^{a}	62.0 ± 2.5^{ab}	86.0 ± 1.5^{ab}
8.0	$44.5+3.8^{a}$	$70.0+4.0^{a}$	$91.3+0.9^{a}$
F	28.2	81.8	25.2
P	< 0.0001	<0.0001	<0.0001

*Means within a column followed by same letters are not significantly different from one another (Turkey Kramer's HSD Test, P< 0.005).

Table 2: Effect of Parsley (Chloroform Extracts) on Mortality of *C. maculatus* on stored Bambara nut

Application Rate	Mortality (Exposure Period)		
(ml/kg)	7(DAT)	14(DAT)	21(DAT)
0.0	2.1±1.1^{c}	2.3±1.2^{c}	2.7±0.4^{c}
2.0	22.3±6.2^{bc}	43.7±.4.1^{b}	47.3+5.9^{b}
4.0	31.0±4.9^{ab}	60.3±3.2^{ab}	74.3+13.1^{ab}
6.0	38.7±5.9^{ab}	57.7±3.2^{ab}	90.0+10.0^{a}
8.0	50.0+5.7^{a}	66.7+3.8^{a}	100+0.0^{a}
F	12.3	50.0	24.9
P	0.0007	<0.0001	<0.0001

*Means within a column followed by same alphabets are not significantly different from each other (Turkey Kramer's HSD Test P<0.005).

Table 3: Effect of Parsley (Methanol Extract) on Mortality of *C. maculatus* on Stored Bambara Nut

Application Rate	Mortality (Exposure Period)		
(ml/kg)	7(DAT)	14(DAT)	21(DAT)
0.0	1.1±1.1^{d}	3.0±0.2^{e}	3.1±0.2^{d}
2.0	23.3±3.3^{c}	29.3±0.9^{d}	45.7±5.2^{c}
4.0	37.0±1.5^{b}	45.7±2.8^{c}	63.7±2.0^{b}
6.0	46.7±1.7^{a}	57.7±1.2^{b}	73.0+1.7^{ab}
8.0	53.0+1.7^{a}	65.3+1.2^{a}	83.3+2.0^{a}
F	10.5	26.1	12.9
P	< 0.0001	<0.0001	<0.0001

*Means within a column followed by same letters are not significantly different from one another (Turkey Kramer's HSD Test, P< 0.005).

Effect of Parsley Extracts on Oviposition, Progeny Emergence, Percentage Damage and Weight Loss Caused by *C. maculatus* on Stored Bambara Nut

The result on table 4, indicates the effect of aqueous extract of parsley on oviposition, progeny emergence, and percentage damage and weight loss caused by *C. maculatus* on stored Bambara nut. Oviposition decreases significantly with increase in dose rate, though there is no significant different between control and application rate of 2m/kg; oviposition reduces significantly from 211.0±41.9 to 89.0±5.9. Similarly, progeny emerged significantly reduces with increase in application rate varying significantly with the control. The least percentage damage and weight loss were recorded at 8ml/kg with 20.7±1.2 and 4.9±0.7 respectively.

In the same trend, the number of eggs produced by *C. maculatus* treated with chloroform and methanol extracts were recorded in table 5 and 6 below, respectively. The result shows that oviposition decreases with increase in application rate from 44.0±3.2 to 19.3±1.5 and 202.3±11.0 to 66.3±13.5 respectively. The progeny emerged and the percentage damage and weight loss also works in a similar manner. It is evident that both extracts applied differ significantly with control.

Amongst the three extracts of parsley, only chloroform extract recorded 0.0 % weight loss (table 5).

The results agreed with several workers who reported similar reports for other plants part as insecticidal and antifeedant (Mostafa *et al*., 2012). All the different extracts in this study, (Mostafa *et al*., 2012) inhibited the number of eggs laid by *C. maculatus*. Oviposition by *C. maculatus* was significantly hindered in extract-treated seeds than untreated seeds. The percentage adult emergence in the untreated seeds was significantly higher than percentage adult emergence in the treated seeds.

Table 4: Effect of Parsley (Aqueous Extracts) on Oviposition, Progeny Emergence, Percentage Damage and Weight Loss Caused by *C. maculatus* on Stored Bambara Nut

A.R(ml/kg)	Oviposition	Progeny(F_1)	% Damage	%Weight Loss
0.0	275.0±41.9^{a}	244.7+15.8^{a}	74.9±5.5^{a}	74.3±8.9^{a}
2.0	211.0+13.2^{ab}	86.3±8.7^{b}	60.9+4.9^{ab}	59.1±9.3^{b}
4.0	117.3+50.5^{b}	45.0±8.6^{c}	46.6+3.4^{bc}	30.2±4.3^{c}
6.0	114.0+6.2^{bc}	36.3±5.2^{d}	29.1±2.9^{cd}	7.7±1.5^{d}
8.0	89.0+5.9^{c}	20.7+1.2^{e}	19.1+0.9^{d}	4.9+0.7^{e}
F	28.3	7.27	34.3	25.6
P	< 0.0001	0.0052	<0.0001	<0.0001

*Means within a column followed by same letters are not significantly different from one another (Turkey Kramer's HSD Test, P< 0.005).
*A.R means Application Rate

Table 5: Effect of Parsley (Chloroform Extracts) on Oviposition, Progeny Emergence, Percentage Damage and Weight Loss Caused by *C. maculatus* on Stored Bambara Nut

A.R(ml/kg)	Oviposition	Progeny(F_1)	% Damage	%Weight Loss
0.0	256.3±57.8^{a}	248.6+48.9^{a}	83.1±3.4^{a}	53.2±6.2^{a}
2.0	44.0±3.2^{b}	23.2 +4.3^{b}	6.0±0.5^{b}	2.9±0.7^{b}
4.0	35.3±2.3^{bc}	11.5+2.9^{c}	2.7±0.2^{c}	1.8±0.3^{b}
6.0	31.0±5.5^{c}	3.2+0.8^{d}	2.6±0.3^{c}	0.6±0.2^{c}
8.0	19.3+1.5^{c}	1.2+0.9^{d}	0.9+0.1^{d}	0.0+0.0^{d}
F	14.8	12.4	52.9	67.7
P	0.0003	<0.0001	<0.0001	<0.0001

*Means within a column followed by same letters are not significantly different from one another (Turkey Kramer's HSD Test, P< 0.005).
*A.R means Application Rate

Table 6: Effect of Parsley (Methanol Extracts) on Oviposition, Progeny Emergence, Percentage Damage and Weight Loss Caused by *C. maculatus* on Stored Bambara Nut

A.R(ml/kg)	Oviposition	Progeny(F_1)	%Damage	%Weight Loss
0.0	397.3±25.1^{a}	267.3±9.8^{a}	84.3±3.7^{a}	56.9±8.6^{a}
2.0	202.3±11.0^{b}	126.3±10.9^{b}	53.4+4.4^{b}	28.6±4.3^{b}
4.0	137.6±7.2^{bc}	99.7±8.5^{b}	39.7+5.7^{b}	6.6±1.9^{c}
6.0	95.7±12.8^{cd}	61.7±3.5^{c}	7.6+1.0^{c}	3.7±0.9^{c}
8.0	66.3+13.5^{d}	16.3+2.2^{d}	6.8+4.8^{c}	2.6+0.5^{c}
F	75.4	14.8	58.5	28.3
P	< 0.0001	<0.0001	<0.0001	<0.0001

*Means within a column followed by same letters are not significantly different from one another (Turkey Kramer's HSD Test, P< 0.005).
*AR means Application Rate

According to Mobolade and Elizabeth (2016), plant extracts can considerably, significantly reduce infection and weight loss, increasing the mortality rate of *C. maculatus*, and reducing grain emergence. The reduction in beetle seed damage is concentration-dependent, attributed to higher adult mortality and less mature beetle emergence in treated grains (Wagner and Bakare, 2017). The results of the current trial showed that parsley extracts had discernible impact on the management of *C. maculatus* on stored Bambara nut.

Conclusion

The use of plant extracts with insecticidal properties has the potential of reducing the effects of insect pests of agricultural crops. These can be of importance to the resource-poor farmers in many areas of the developing world who store small quantities of the seeds for their consumption, sales and planting. The significant reduction in pests' numbers on the treated seeds was an indication that they can be used as alternatives to chemical insecticides. The extracts of parsley can be used for developing natural pest control products that may replace the synthetic bio-pesticides that are currently used against *C. maculatus*. In conclusion, parsley extracts (Aqueous, Methanol and Chloroform) can be used in managing insect pests of stored products, though chloroform extract presented the best result with 100% mortality after 21 days of exposure and 0.0 % weight loss at 8ml/kg.

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