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Research Article

Efficacy of Neem Leaf (*Azadirachta indica*), Bitter Leaf (*Vernonia amygdalina*) and Pawpaw Leaf (*Carica papaya*) Powder in the Control of *Callosobruchus maculatus* in Stored Cowpea

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ABSTRACT

A laboratory experiment was carried out to investigate the insecticidal properties *of Azadirachta indica* (Neem), *Vernonia amygdalina* (Bitter Leaf) and *Carica papaya* (Pawpaw) leaf powders against *Callosobruchus maculatus*in stored cowpea. The experiment was carried out using a completely randomized design. The treatments were applied as single and mixed applications at the following rates-3g, 6g and 9g. Each treatment were replicated 4 times making a total of 92 experimental units. Each treatment was applied to 100g. Data collected include adult mortality, larval and pupa emergence, grain damage, weight loss and F_1 progeny emergence. The data collected were subjected to analysis of variance (AVONA) at 5% probability level. The results indicated that, there were significantly differences between plant products treatments and the synthetic treatment over the control throughout the period of the experiment (1 to 8 weeks). However, the various treatment of the plant products used for the experiment proved to be effective in controlling *C. maculatus* of stored cowpea. However, Cypermethrin dust at 0.6g/100g of cowpea was the most effective in controlling grain damage while pawpaw leaf powder and Bitter Leaf powder proved to be most effective in controlling grain damage among the natural botanicals, adult mortality and number of eggs laid by *C. maculatus* on the stored grains. The result clearly indicated the potential values of using plants extracts as complimentary to chemicals pesticides in controlling *C. maculatus* on cowpea grains.

1. Introduction

Cowpea (Vignaunguiculata L. Walp) is one of the most ancient crops known to humankind. Its origin and subsequent domestication is associated with pearl millet and sorghum in West Africa Musa et al. (2009). The cowpea was first domesticated in Africa between 1700 to 1500 before the Current Era (Singh, 2014) and all cultivated varieties grown in the world today originated from East and West Africa Xiong et al. (2016). Cowpea seed pods and leaves are consumed in fresh form as green vegetables in some African Countries (Ghaly and Alkoaik, 2010), while the rest of the cowpea plant after the pods have been harvested serves as a nutritious fodder for livestock (Abebe et al., 2005) and also a source of cash income (Dugje et al., 2009). The nutritive value of cowpea makes it an extremely important protein source to vegetarian and people who cannot afford animal protein (Adeyemi et al., 2012). For human consumption, the cowpea is mainly grown for grain (dry and fresh) and sometimes for fresh pods in West Africa, India, and South America, while also grown for leaves in East Africa. It is an under used legume crop with a high potential for food and nutritional security in South Africa and produced for grain, immature green pods and fresh leaves due to its nutritional composition (Gerrano et al., 2015a; 2017a). The cowpea can be used to produce a large range of dishes and snacks (Uzogara and Ofuya, 1992; Asif et al., 2013). The consumption of the cowpea as a dietary staple in West Africa over millennia has produced extensive and varied culinary practices and many individual foods and dishes. Cowpea consumption in West Africa has led to a culinary practice that requires seed coat removal (also called decortication or dehulling). For example, the popular West African cowpea-based foods, such as *Akara* and *Moin-moin*, are decorticated (Phillips, 2012). The production and storage of cowpea have faced so many constraints, throughout West Africa

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such as diseases and the limited use of fertilizers and irrigation inputs (Brisibe et al., 2011) but major constraints is the insect pest known as *Callosobruchus maculatus* (Musa et al 2009), which infests it before and after harvest consequently leading to loss of economic value (Baidoo et al., 2010). Infestations on stored grains may reach 50% within 3-4 months of storage (Oparekeand Dike, 2005).

2. Materials and Methods

This study was conducted in the Department of Crop Protection laboratory and Ir. Leo Vande Mierop Biotechnology laboratory of the University of Ilorin, Ilorin, Nigeria.

2.1. Source of cowpea seeds and plant materials

The cowpea seeds used for the experiment were purchased from a local market (Oja-Oba) Ilorin, and the natural plant materials, *Azadirachta indica* (neem leaf), *Vernonia amygdalina (Bitter leaf)* and *Carica papaya* (pawpaw leaf) were sourced from the University of Ilorin premises.

2.2. Insect Culture

C. maculatus used for the experiment was obtained from Nigerian Stored Product Research Institute (NSPRI) Ilorin and this was used to establish a culture in the laboratory of the Department of Crop Protection. Freshly emerged dults of *C. maculatus* were used for the experiment

2.3. Preparation of the Botanicals

Fresh leaves of Neem, Bitter leaf and Pawpaw were collected and air dried for 7 days. The dried leaves were ground with mortar and pestle and sieved using 3mm sieve to obtain a fine powder. The leaf powders were separately packed into air tight containers until required for use.

2.4. Experimental Procedure

The purchased cowpea seeds were disinfected by storing in a deep freezer for 72 hours at 4^{o}C to kill any hidden *C. maculatus* in the seeds. 100g of cowpea seeds were weighed and put into transparent plastic containers.

The plant powders were weighed and applied at the following rates; 3g, 6g and 9g respectively. The containers were shaken to ensure uniform covering of the seeds with the treatments. 6 unsexed freshly emerged adult *C. maculatus* were introduced into each container and the container covered with muslin cloth held in place with the aid of a rubber band.

The experiment was carried out using a completely randomized design. There were 23 treatments and each treatment was replicated 4 times giving a total of 92 experimental units. In the mixed treatments, the powders were mixed in equal ratios. Cypermethrin was used as the positive controls at recommended dose (0.6g/100g of cowpea seeds).

2.5 Data Collection

Data were collected on the following parameters: adult mortality of the *C. maculatus* were carried out at 24hours, 3day, day 5, day 7 and day 9 after the treatment and then recorded (A beetle was assumed dead if there is no movement of its legs and antenna and also if it did not respond to a pin probe at its abdomen), larval emergence was taken at 15^{th} day and 17^{th} day post treatment, pupa emergence was taken at 17^{th} day and 19^{th} day post treatment. The larvae and pupae are normally only found in cells bored within the seeds of pulses. For descriptions and a key including *C. maculatus* larvae. The weight loss of the seeds was taken after the whole experiment

2.6. Data Analysis

The data collected were subjected to analysis of variance (ANOVA) and treatment means that were significantly different were separated using the New Duncan Multiple Range Test at P=0.05 level of probability.

Table 1
Effect of the treatments on percentage (%) adult mortality of *Callosobruchus maculatus*

Treatment	Rate(g)	Days after treatment(DAT)				
		1	3	5	7	9
BL	3	8.34±9.62abc	25±9.62bcde	20.83±15.96ab	21.08±15.52ab	4.17±8.34bc
BL	6	12.5±15.96abc	33.33±0abcd	16.67±13.61ab	12.5±8.34ab	16.67±13.61abc
BL	9	25±9.62a	20.83±15.96cde	12.5±8.34ab	20.83±15.96ab	12.5±8.34abc
NL	3	12.5±15.96abc	29.17±8.33abcde	16.67±23.57ab	25±16.67ab	12.5±15.96abc
NL	6	12.5±15.96abc	29.17±15.96 abcde	20.84±8.33ab	16.67±13.61ab	20.84±8.33ab
NL	9	16.67±0abc	20.84±8.33cde	12.5±15.96ab	25±21.52ab	16.67±13.61abc
PL	3	16.67±0abc	20.83±15.96cde	33.33±13.61a	16.67±13.61ab	12.5±8.34abc
PL	6	16.67±13.61abc	37.5±15.96abcd	29.17±28.46a	8.34±9.62b	4.17±8.34bc
PL	9	20.83±15.96ab	41.67±9.62abcd	16.67±0ab	12.5±15.96ab	4.17±8.34bc
BLNL	3	4.17±8.34bc	29.17±25abcde	12.5±15.96ab	33.34±23.57a	12.5±15.96abc
BLNL	6	12.5±8.34abc	50±0a	16.67±0ab	12.5±8.34ab	4.17±8.34bc
BLNL	9	4.17±8.34bc	33.34±19.24abcd	25±9.62ab	16.67±0ab	8.34±9.62abc
NLPL	3	4.17±8.34bc	29.17±15.96abcde	16.67±13.61ab	20.83±15.96ab	25±9.62a
NLPL	6	12.5±15.96abc	16.67±0de	33.33±23.57a	25±9.62ab	12.5±15.96abc
NLPL	9	4.17±8.34bc	29.17±15.96abcde	16.67±0ab	25±9.62ab	8.34±9.62abc
BLPL	3	8.34±9.62abc	33.33±0abcd	20.83±15.96ab	20.84±8.33ab	4.17±8.34bc
BLPL	6	8.34±9.62abc	45.83±8.34ab	29.17±8.33a	8.34±9.62b	0±0c
BLPL	9	0±0c	37.5±8.33abcd	29.17±8.33a	25±9.62ab	8.34±9.62abc
BLNLPL	3	16.67±0abc	29.17±8.33abcde	20.84±8.33ab	16.67±0ab	12.5±8.34abc
BLNLPL	6	12.5±8.34abc	29.17±15.96abcde	16.67±13.61ab	20.84±8.33ab	20.83±15.96ab
BLNLPL	9	20.84±8.33ab	25±16.67bcde	12.5±8.34ab	25±9.62ab	16.67±13.61abc
Cypermethrin		16.67±13.61abc	33.34±19.24abcd	33.33±13.61a	16.67±0ab	12.5±15.96abc
Control		8.34±9.62abc	8.34±9.62e	4.17±8.34b	4.17±8.34b	8.34±9.62b
S.E.M		5.31	6.64	6.95	6.23	5.63

Values with the same letter(s) in the same column are not significantly different 5% level of significance using Duncan's multiple range test

KEY: DAT = Days after Treatment, SEM=Standard error of mean, BL= Bitter leaf, NL=Neem leaf, PL=Pawpaw Leaf

Table 2
Effects of treatments on Larva and Pupa emergence

Treatment	Rate (g)	Days after treatment (DAT)			
		Larva emergence		Pupa emergence	
		15	17	17	19
BL	3	0±0c	0±0b	0±0b	0.75±0.5abc
BL	6	0±0c	0±0b	0.25±0.5b	1±1.15abc
BL	9	0±0c	0±0b	0.5±0.58ab	0.75±0.96abc
NL	3	0±0c	0±0b	0±0b	0.25±0.5c
NL	6	0±0abc	0±0b	0.5±0.58ab	0±0c
NL	9	0±0abc	0±0b	0.5±0.58ab	1±0.82abc
PL	3	0±0abc	0±0b	0±0b	1±0.82abc
PL	6	0±0abc	0.5±0.58ab	0±0b	0.75±0.5abc
PL	9	0.25±0.5ab	0.75±0.5a	0±0b	0.75±0.5abc
BLNL	3	0±0c	0±0b	0±0b	0±0c

Table 2
Effects of treatments on Larva and Pupa emergence

BLNL	6	0±0c	0±0b	0±0b	0.5±1bc
BLNL	9	0±0c	0±0b	0±0b	0.75±0.96abc
NLPL	3	0±0abc	0.25±0.5ab	0.25±0.5b	0.75±0.96abc
NLPL	6	0±0abc	0.5±0.58ab	0±0b	1.75±2.22abc
NLPL	9	0±0abc	0±0b	0±0b	1±1.15abc
BLPL	3	0±0c	0±0b	0±0b	1±0abc
BLPL	6	0±0c	0±0b	0±0b	0.5±0.58bc
BLPL	9	0±0c	0±0b	0.5±0.58ab	1±0.82abc
BLNLPL	3	0±0c	0±0b	0±0b	0.25±0.5c
BLNLPL	6	0±0c	0.25±0.5ab	0.25±0.5b	0.25±0.5c
BLNLPL	9	0±0c	0.25±0.5ab	0.5±0.58ab	0.5±1bc
Cypermethrin		0±0abc	0±0b	0±0b	2±0a
Control		0.25±0.5a	0.75±0.96a	1±0.82a	0.5±0.58bc
S.E.M		0.0737	0.1676	0.1831	0.4235

Values with the same letter(s) in the same column are not significantly different 5% level of significance using Duncan's multiple range test

KEY: DAT = Days after Treatment, SEM=Standard error of mean, BL= Bitter leaf, NL=Neem leaf, PL=Pawpaw Leaf

Table 3a
Effects of treatments on F_1 progeny emergence of *C. maculatus*

Treatment	Rate(g)	Days after treatment (DAT)				
		28	30	32	34	36
BL	3	0.75±0.5abc	1±1.15ac	1±1.15ab	2.5±1.91a	0.5±1b
BL	6	1.25±0.5abc	0.25±0.5c	1±2ab	2.5±3.7a	0.5±0.58b
BL	9	2±1.83a	0.25±0.5c	0.25±0.5b	1.75±2.06a	0±0b
NL	3	0.75±0.96abc	0.5±1c	1.25±2.5ab	5.25±10.5a	1.5±3b
NL	6	0.5±0.58abc	0±0c	0.25±0.5b	0±0a	0±0b
NL	9	0.25±0.5bc	2.5±1ab	1.25±0.5ab	4.25±3.1a	0.25±0.5b
PL	3	0.5±0.58abc	0.5±0.58c	0.75±0.96ab	2±2.71a	0.75±0.96b
PL	6	0.75±0.5abc	0.75±0.5c	3.25±5.19a	10.75±21.5a	3.25±5.25b
PL	9	1.5±1.29abc	1±0.82abc	0.5±0.58b	1.75±1.26a	0±0b
BLNL	3	1.5±1.29abc	1±2abc	0.75±0.96ab	2±3.37a	1.25±1.89b
BLNL	6	0.5±0.58abc	0±0c	0.5±0.58b	0±0a	0.25±0.5b
BLNL	9	1.75±1.5ab	0.25±0.5c	0.25±0.5b	1.25±1.26a	0.75±0.96b
NLPL	3	0.5±0.58abc	0.5±1c	0±0b	2.75±2.87a	1.5±1.73b
NLPL	6	1.5±1.73abc	1±2abc	1±2ab	5.25±10.5a	2.75±3.77b
NLPL	9	1.75±1.5ab	0.5±0.58c	1±0ab	0.5±0.58a	0±0b
BLPL	3	1.5±1abc	0±0c	0.25±0.5b	0±0a	0.75±1.5b
BLPL	6	0.5±0.58abc	0.5±1c	1.5±1.73ab	2.25±4.5a	0±0b
BLPL	9	1±0abc	1±1.15abc	0±0b	2.5±2.89a	0.75±1.5b
BLNLPL	3	0.5±0.58abc	0.25±0.5c	1.25±1.26ab	1±0a	1.25±2.5b
BLNLPL	6	1.75±0.5ab	0±0c	0±0b	0.75±0.96a	0.25±0.5b
BLNLPL	9	0±0c	2.5±2.38a	0.5±0.58b	5±4.55a	1.25±2.5b
Cypermethrin		0±0c	0.75±0.5c	2.5±1ab	7.25±7.23a	0.75±0.5b
Control		0.75±0.96abc	1±0.82abc	2±1.63ab	8±6.63a	7.25±2.99a
S.E.M		0.4692	0.509	0.772	3.112	0.975

Values with the same letter(s) in the same column are not significantly different 5% level of significance using Duncan's multiple range test

KEY: DAT = Days after Treatment, SEM=Standard error of mean, BL= Bitter leaf, NL=Neem leaf, PL=Pawpaw Leaf

Table 3b
Effects of treatments on F_1 progeny emergence of *C. maculatus* (continuation)

Treatment	Rate(g)	Days after treatment (DAT)				
		38	40	42	44	46
BL	3	0.25±0.5bc	0.25±0.5b	0±0a	0±0b	0±0b
BL	6	0.5±0.58bc	1.25±2.5ab	0±0a	0±0b	0±0b
BL	9	0±0c	0±0b	0±0a	0±0b	0±0b
NL	3	1±2bc	0.75±1.5b	0.25±0.5a	0.25±0.5b	0±0b
NL	6	0±0bc	0±0b	0±0a	0±0b	0±0b
NL	9	0.25±0.5bc	0.5±0.58b	0±0a	0±0b	0±0b
PL	3	0.75±0.96bc	0.5±0.58b	0±0a	1.5±3b	0±0b
PL	6	2.25±3.86b	1.25±2.5ab	0.75±1.5a	0.5±1b	0±0b
PL	9	0±0bc	0.25±0.5b	0±0a	0±0b	0±0b
BLNL	3	1±0.82bc	0±0b	0±0a	0±0b	0±0b
BLNL	6	0.25±0.5bc	0.25±0.5b	0±0a	0±0b	0±0b
BLNL	9	0±0c	0±0b	0±0a	0±0b	0±0b
NLPL	3	1.5±1bc	0±0b	0±0a	0±0b	0±0b
NLPL	6	1.5±3bc	0.5±1b	1.25±2.5a	1.25±2.5b	0.5±1b
NLPL	9	0±0bc	0±0b	0±0a	0±0b	0±0b
BLPL	3	0.25±0.5bc	0±0b	0±0a	0±0b	0±0b
BLPL	6	0.25±0.5bc	0±0b	0±0a	0±0b	0±0b
BLPL	9	0.25±0.5bc	0±0b	0±0a	0±0b	0±0b
BLNLPL	3	0.5±1bc	0.5±1b	0±0a	0±0b	0±0b
BLNLPL	6	0±0bc	0±0b	0±0a	0±0b	0±0b
BLNLPL	9	0.75±1.5bc	1.25±2.5ab	1.25±2.5a	1.25±2.5b	2.75±5.5a
Cypermethrin		0.5±1bc	3±3.46a	0±0a	0±0b	0±0b
Control		6±1.63a	2.25±2.87ab	1.25±1.26a	5.75±4.65a	0±0b
S.E.M		0.653	0.697	0.4246	0.694	0.583

Values with the same letter(s) in the same column are not significantly different 5% level of significance using Duncan's multiple range test

KEY: DAT = Days after Treatment, SEM=Standard error of mean, BL= Bitter leaf, NL=Neem leaf, PL=Pawpaw Leaf

Table 4
Effects of treatments on mean seed weight loss

Treatment	Rate	Weight Loss (g)
BL	3	0.48±0.43c
BL	6	2.68±0.63ab
BL	9	1.7±0.54abc
NL	3	2.33±2.24ab
NL	6	1.93±0.48abc
NL	9	2.43±0.5ab
PL	3	1.98±1.09abc
PL	6	2±0.66abc
PL	9	2.45±1.57ab
BLNL	3	1.48±0.43abc
BLNL	6	2.75±0.64ab
BLNL	9	1.88±0.87abc
NLPL	3	2.05±1.52abc
NLPL	6	1.03±0.62abc
NLPL	9	2.73±0.79ab
BLPL	3	1.8±1.37abc
BLPL	6	1.95±0.57abc
BLPL	9	2.7±0.37ab
BLNLPL	3	1.83±0.88abc
BLNLPL	6	1.55±1.99abc
BLNLPL	9	1.55±0.26abc
Cypermethrin		1.48±1.13abc
Control		2.93±0.38a
S.E.M		0.508

Values with the same letter(s) in the same column are not significantly different 5% level of significance using Duncan's multiple range test

KEY: DAT = Days after Treatment, SEM=Standard error of mean, BL= Bitter leaf, NL=Neem leaf, PL=Pawpaw Leaf

3. Results and Discussion

The results of the study revealed that the various treatments used in the experiment had significant effects, mortality increased with increase in level of treatment. The plants leaf powder caused adult mortality of *C. maculatus* at the high and low rates when compared to the control, which was indicative of bioactive characteristics of the plant part. This is in agreement with the report of (Malungu et al., 2007) that the use of plant powders has been reported to produce higher death of insects because of physical barrier with the tendency of blocking the spiracles of the insects, thus impairing respiration leading to death of the insects. BLNL have the highest mortality followed by NLPL. Please cross check to confirm this claim by me. So with the mortality known that will affect the other experiment such as pupa emergence and weight loss.

The insecticidal activity of powders of *Vernonia amygdalina, Carica papaya* and *Azadirachta indica* on larva and pupa emergence of adult *Callosobruchus maculatus at* different Days After Treatment (DAT) shows that there was a significant difference between the treatments and the control for larva and pupa emergence (Table 2 and 3).This could be attributed to the adult mortality already observed (Table 1) and the inhibition of oviposition as well as the remarkably high reduction in survival to adulthood of mature stages of *C. maculatus* compared to the control. This result corroborates that of (Okonkwo and Ewete 1999) in pepper fruit, (Babatunde and Musa, 2020) in *Eucalyptus globulus* leaf extract on cowpea beetle

The plants leaf powder was also observed to have effects in reducing the damage on cowpea seeds by *C. maculatus* (Table 4). Damage on cowpea seeds may have been reduced as a result of the extracts acting as a deterrent to *C. maculatus*, keeping them from infesting and damaging the seeds.

The study reveals that *Vernonia amygdalina, Carica papaya* and *Azadirachta indica* leaf powder could be very effective for use as bio-pesticides for protecting cowpea seeds from *C. maculatus* infestation and damage. It has been reported by the pest management specialists that botanicals are not known to leave any residue in any crop they are used to protect and the protective ability of essential oils could be attributed to interspecific insect responses to oil constituents (Enan, 2001).

The use of natural toxicants from plants as insecticides had been inexistent since the ancient times (Adebayo and Gbolade 1994 and Ismam, 2008, Babatunde et al 2020). The natural insecticides which require low cost to prepare, are readily available, environmentally and ecologically friendly are best suited for use in the storage of produce. (Babatund and Musa, 2020).

4. Conclusion

Plant extracts can be another source of pesticides against stored grain pests. It is recommended that the active molecule in *Vernonia amygdalina, Carica papaya* and *Azadirachta indica* responsible for their activities be isolated for the development of bio pesticides to protect grains in storage. For more effectiveness of plant extracts, a large amount proportional to the quantity of grains is required for post-harvest control of *C. maculatus* in stored cowpea for planting. This study has revealed that *Vernonia amygdalina, Carica papaya* and *Azadirachta indica* extract can be used to protect cowpea grains under small scale storage.

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