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EVALUATION OF POWDER MIXTURES OF SELECTED PLANTS AS PROTECTANTS OF COWPEA (*VIGNA UNGUICULATA* [L.] WALP.) AGAINST *CALLOSOBRUCHUS MACULATUS* (F.)

JODA Abiodun Oladipupo[1], SANNI Arewa Yisa [2]

[1]Department of Crop Production, Olabisi Onabanjo University, Ago-Iwoye, Nigeria
[2]Plant Quarantine Services, Federal Department of Agriculture, Abuja, Nigeria

Abstract – *Callosobruchus maculatus* (F.) is an important storage pest of cowpea seeds in Nigeria. Although synthetic pesticides are effective at controlling the pest, environmental and health hazards of these chemicals are of increasing concern. Thus, efficacy of leaf powders of *Carica papaya* (CP), *Tithonia diversifolia* (TD), *Acalypha ciliata* (AC), *Azadirachta indica* (AI) and *Vernonia amygdalina* (VA) in control of *C. maculatus* on cowpea was investigated. Cowpea seeds (200 each) were infested separately with 20 newly emerged adults (10 males and females each) of *C. maculatus* respectively in 1 litre kilner jars. The jar contents were mixed separately with 5g and 10g of each of CP, TD, AI, AC or VA per 100g seeds to determine both the toxicity on the adult insects and production of F_1 progenies for the first 28 days and percentage grain weight loss were assessed after 28 days. In the storage material experiments, 5kg of each cowpea seeds were separately weighed into polyethylene bags and plastic containers. A 500g of the five botanicals and their different mixtures (CP+TD+AI+AC+VA; TA+AI+VA; CP+TD+AI+AC; CP+TD+AI+VA; CP+TD+AC; TD+AI+AC; CP+AI+AC; CP+VA+AC) were added separately into cowpea seeds in the storage materials. Untreated cowpea grains and Actellic ® dust treatments served as positive and standard controls respectively. The trials were laid in a completely randomized design (CRD) in three replicates for 5 months in the laboratory at ambient temperature (25±3ºC). Data were collected at one month intervals on seed germination, seed weight loss and insect infestations and analysed using ANOVA and significant means separated with DMRT at $\alpha_{0.05.}$ There were significant differences ($p<0.05$) in mortality rate of the *C. maculatus* among the leaf powders. *Acalypha ciliata* at both 5g and 10g/100g seeds compared to other botanicals resulted in the highest mortality at day 21, with 47.5% - 77.5% mortality recorded for *C. mac-*

ulatus. All the plant powders, irrespective of concentration reduced the F_1 progeny emergence of *C. maculatus* (14% - 78.2%) significantly (P<0.05) compared to the control. Efficacy of *Acalypha ciliata* and its mixtures were significantly different to synthetic insecticide from second month of storage. Leaf powders effectively improved seed germination and reduced weight loss and insects damaged more grains in the polyethylene bags than in plastic containers. Results show that Actellic dust completely inhibited progeny emergence while leaf powders only reduced the F1 progeny emergence. Therefore *A. ciliata* could be used as an alternative control option to synthetic pesticides.

KEY WORDS: *Callosobruchus maculatus,* cowpea, botanicals, Bruchinae, crop protection, pesticides, Actelic dust

Izvleček – VREDNOTENJE MEŠANIC PRAHU IZBRANIH RASTLIN ZA ZAŠČITO KITAJSKEGA FIŽOLA (*VIGNA UNGUICULATA* [L.] WALP.) PRED HROŠČEM *CALLOSOBRUCHUS MACULATUS* (F.)

Callosobruchus maculatus (F.) je pomemben skladiščni škodljivec semen kitajskega fižola v Nigeriji. Čeprav so sintetični pesticidi učinkoviti pri zatiranju škodljivca, povzročajo okoljska in zdravstvena tveganja teh kemikalij vedno večjo zaskrbljenost. Zato je bila preverjena učinkovitost prahu iz listov rastlin *Carica papaya* (CP), *Tithonia diversifolia* (TD), *Acalypha ciliata* (AC), *Azadirachta indica* (AI) in *Vernonia amygdalina* (VA) za zatiranje hrošča *C. maculatus* na kitajskem fižolu. Semena fižola (po 200) so bila ločeno okužena s po 20 sveže izleženimi odraslimi hrošči (po 10 samcev in samic) v litrskih kilnerjevih posodah. Vsebina posod je bila ločeno zmešana s 5g in 10g vsakega od praškov, CP, TD, AI, AC ali VA na 100g semen za ugotavljanje strupenosti za odrasle žuželke in proizvodnje potomcev generacije F_1 v prvih 28 dneh, po tem času je bilo ocenjeno zmanjšanje teže zrnja v odstotkih. V poskusih s skladiščnim materialom je bilo po 5 kg kitajskega fižola ločeno odtehtanega v polietilenske vreče in plastične vsebnike. 500g petih rastlinskih pripravkov in njihovih različnih mešanic (CP+TD+AI+AC+VA; TA+AI+VA; CP+TD+AI+AC; CP+TD+AI+VA; CP+TD+AC; TD+AI+AC; CP+AI+AC; CP+VA+AC) je bilo ločeno dodanih semenom fižola v skladiščnem materialu. Netretirana zrnja fižola in tretiranje s praškom Actellic ® sta služila kot pozitivna in standardna kontrola. Poskusi so potekali po povsem naključni zasnovi v treh 5-mesečnih ponovitvah v laboratoriju pri sobni temperaturi (25±3°C). Na en mesec smo beležili podatke o kaljivosti semen, izgubi teže semen in napadi žuželk, analizirali z uporabo ANOVA in z DMRT pri $\alpha_{0.05}$ ločili statistično pomembne srednje vrednosti. Bile so statistično pomembne razlike (p<0.05) v smrtnosti hroščev *C. maculatus* med različnimi listnimi praški. *Acalypha ciliata* je tako s 5g kot 10g/100g semen povzročala najvišjo smrtnost na 21. dan, 47.5% - 77.5% smrtnost pri *C. maculatus*. Vsi rastlinski praški, ne glede na koncentracijo, so zmanjšali izleganje potomcev generacije F_1 hroščev *C. maculatus* (14% - 78.2%), statistično pomembno (P<0.05) glede na kontrolo. Učinkovitost praška *Acalypha ciliata* in njegovih mešanic je bila pomembno drugačna od sintetičnega insekticida po drugem mesecu skladiščenja. Listni praški so učinkovito izboljšali

kaljivost semen in zmanjšali izgubo teže, žuželke pa so poškodovale več semen v polietilenskih vrečah kot v plastičnih vsebnikih. Rezultati kažejo, da je prah Actellic popolnoma zaustavil izleganje potomcev, medtem ko so listni praški le zmanjšali izleganje generacije F_1. Torej bi lahko prašek *A. ciliata* uporabljali kot zamenjavo za zatiranje s sintetičnimi pesticidi.

KLJUČNE BESEDE: *Callosobruchus maculatus,* kitajski fižol, rastlinski praški, Bruchinae, zaščita pridelkov, pesticidi, prah Actelic

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a staple food crop of significant economic and nutritional importance worldwide (Emeasor *et al*., 2007; Akunne *et al*., 2013). Cowpea seeds, 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). Nigeria is the largest producer of cowpea grain with approximately 3.80 million hectares under cultivation and an annual production estimate of 2.95 million MT (FAO, 2015).

Food grain losses due to insect infestation during storage are a serious problem, particularly in the developing countries with a pre- and post-harvest loss ranging from 10 to 100% (Talukder *et al.,* 2004). These substantial losses are caused by *Callosobruchus* on legumes. *Callosobruchus maculatus* is a major constraint to cowpea storage often leading to loss of economic value (Musa *et al*., 2010; Baidoo *et al*., 2010).

Callosobruchus maculatus is a field-to-store pest of cowpea as its infestation starts in the field when the mature pod dries. It multiplies very rapidly in storage where it causes losses from 10 to 90% on the grains (Caswell, 1981; Boateng and Kusi, 2008; IITA, 2015) and also reduces seed quality, market value and seed viability to 2% after 3 months of storage (Caswell, 1981; Ofuya and Credland, 1995; Boateng and Kusi, 2008).

Chemical method such as the use of phosphine, methyl bromide to control these storage pests has been the most efficient and effective means of protecting grains (Rajendran and Sriranjini, 2008; Mesele *et al*., 2013). However, with the increasing cost of synthetic pesticide, development of resistance such as the case of phosphine and their hazards to the environment such as ozone depletion by methyl bromide which is being phased out completely, alternative controls measure needs to be investigated (Price, 1984; Banwo and Adamu, 2003).

One viable alternative option is the use of botanical pesticides, despite having different active constituents, their modes of action are target-specific, relatively safe, af-

fordable and readily available (Chebet *et al*., 2013). Insecticidal activity of several plant essential oils, powders and other extracts has been evaluated against several insect pests of legumes (Chebet *et al*., 2013; Bohinc *et al*, 2013) and found to have contact toxicity (Asawalam *et al*., 2006; Ogendo *et al.,* 2008), repellence (Kéita *et. al*., 2001; Rosman *et al.,* 2007), fumigant toxicity (Lee *et al*., 2003; Rajendran and Muralidharan, 2005) and anti-feedant (Saxena *et al.,* 1992a, b) effects.

Researches have shown that botanicals have been extensively used on agricultural pests and to very limited extent on insect pests of stored products (Ijeh and Ejike, 2011; Akunne *et al.,* 2013). Moreover, *Carica papaya, Tithonia diversifolia*, *Acalypha ciliata*, *Azadirachta indica*and *Vernonia amygdalina* have been screened *in vivo* and *in vitro* and found to be effective as fungicide and insecticide though with different degree of success compared to the synthetic pesticides (Owolade *et al*., 2004; Moses and Dorathy 2011; Brisibe *et al.*, 2011; Onyeani *et al.,* 2012; Akunne *et al.,* 2013; Chebet *et al*., 2013). Although Akunne *et al.* (2013) reported no improvement in the efficacy of *V*. *amygdalina* and *A. indica* mixtures compared to single application of each of the botanicals against *C. maculatus*, he recommended further research using higher concentration of the botanicals and on a wide range of other common insect pest of stored products in order to increase the efficacy since the plant materials are cheap and readily available.

Also in Nigeria, information on the mixture of two or more botanicals against insect pest of stored products and the impact of different storage materials on the effectiveness of the botanicals is limited. Therefore, the objectives of this study were to

1. evaluate the effects of *Carica papaya, Tithonia diversifolia*, *Acalypha ciliata*, *Azadirachta indica* and *Vernonia amygdalina* at different concentrations in the control of *Callosobruchus maculatus;*
2. evaluate these plant extract combinations for control of F_1 progeny of *Callosobruchus maculatus* and;
3. investigate the influence of the storage materials on effectiveness of the plant extracts.

Materials and methods

Experimental sites

Experiment was carried out in the Entomology laboratory of the Nigeria Agricultural Quarantine Service (NAQS), Moor Plantation, Ibadan located on latitunde 7. 38N and longitude 3·83E. The studies were conducted between the months of August, 2013 through September, 2014.

Collection of plant materials and seeds

Fresh leaves of *Carica papaya* (Pawpaw), *Tithonia diversifolia* (America sunflower), *Acalypha ciliata* (Red Acalypha)*, Vernonia amygdalina* (Bitter leaf) and *Azadirachta indica* (Neem) were collected from Ibadan. Seeds of cowpea (Ife Brown)

were obtained from the seed store of Institute of Agricultural Research & Training (IAR&T), Ibadan while polyethylene bags and plastic containers were purchased from the market.

Preparation of plant powders

Fresh leaves of *Carica papaya* (Pawpaw), *Tithonia diversifolia* (America sunflower), *Acalypha ciliata* (Red Acalypha) *Azadirachta indica* and *Vernonia amygdalina* were washed with sterile distilled water and drained on the laboratory table to remove the water. The plant materials were then air-dried on the laboratory table at 25±3ºC. The dried botanicals were ground using the hammer mill and sieved to obtain fine powders. The plant powders were put in air tight containers separately to ensure that the active ingredients are not lost. The powders were stored in a cool dry place until needed.

Culture of the experimental insects

Adult *Callosobruchus maculatus* used for the experiment were reared in plastic containers under ambient laboratory temperature of 30±3ºC and relative humidity of 75±3%. Weevil-infested cowpea were purchased from Bodija Market Ibadan, Oyo State and were put in culture vial (19cm in diameter) before incubating in the laboratory cupboard so that the old insects will mate and oviposit. This was left undisturbed for two months and the newly emerged adults were used for the experiment.

Cowpea seeds treatment and introduction of test insects

Cowpea seeds with 12-13% moisture content were separately cleaned using 1mm sieve-mesh screen before disinfested in a freezer at -4°C for two weeks. The cowpea seeds were counted (200 seeds) separately into 1L plastic jars, replicated thrice for each of the five plant powders at two different concentrations (5g/100g and 10g/100g of seeds). The seeds were then separately admixed with the plant powders and the contents of each jar were mixed thoroughly to allow even distribution of the powder in the whole grain mass. Actellic super® (0.175g /jar) and untreated cowpea grain treatments served as standard and control respectively.

Twenty, 24hr old *Callosobruchus maculatus* and *Sitophilus zeamais* (ten males and ten females each) were introduced into the plastic jars containing cowpea and maize respectively using camel hair brush. The jars were placed in the shelves at a temperature of 28±2°C and relative humidity of 70±5% for 28 days in a completely randomized design (CRD).

Mortality was assessed for the first 28 days of exposure using the technique of Ceruti and Lazzari (2005) and Nukenine *et al*. (2011). Insects were considered dead on failure to respond by moving to three probings with small paint brush. Percent mortality was determined as follows:

$$\text{Mortality} = \frac{\text{number of dead insects}}{\text{Total number of insects introduced}} \times 100$$

Determination of control efficacy

Toxicity efficacy on F_1 progeny emergence:

After 28 days of exposing the beetles to the treatments, 100 seeds of maize and cowpea were randomly removed from each jar containing the treatments and the number of eggs oviposited on them were counted. These were placed in another equal volume plastic jar separately until progeny emergence. Progeny emergence in each treatments and replicates were taken for F_1 after 28 days of observation, the newly emerged progenies were sieved out, counted and recorded.

Control efficacy on mortality of F_1 progeny:

For each of the five plant extracts powder and their different mixtures, observations were made and recorded for effect on mortality rates of F_1 progeny in all the jars at 24, 48, and 72 hours. The mortality rate was expressed as percentage progeny reduction.

Storage Bioassay with plant extracts and different mixtures:

Bioassay was conducted based on the method described by Talukder and Howse (1994) and modified by Owolade *et al*. (2003). One concentration of 100g/1kg of seeds (1g/10seeds) of each extracts from the leaves of *Carica papaya*, *Tithonia diversifolia*, *Azadirachta indica,Vernonia amygdalina* and *Acalypha ciliata* including the different extracts combination was mixed properly into 5kg of each of the cleaned maize and cowpea seeds separately inside polyethylene bags and plastic containers. Twenty 24hr old *Callosobruchus maculatus* (ten male and ten female each) were introduced into the storage materials containing cowpea using camel hair brush The storage materials containing the seeds were placed on the laboratory table at ambient temperature of 25±3ºC for 5 months.

The experiment was laid up in complete randomized design with three replicates.

Data collection and Statistical Analysis

Data were collected at monthly intervals for five months of storage. The data collected include the weight of seeds, percentage seed infestations, total insect counts and weight of damaged seeds. Data collected were analysed using the Statistical Analysis Software (SAS, 2010). Means were separated using the New Duncan's Multiple Range Test at $P \leq 0.05$.

Results

Effect of the botanical leaf powders on the mortality rate of *Callosobruchus maculatus* at 28 days is shown in Table 1. The efficacy of the plant extracts increased with increase in concentrations irrespective of the botanicals. *Acalypha ciliata* at 5 g and 10g/100 seeds was the most effective compared to other leaf powders at day 28 with 47.5- 77.5% mortality recorded for *C. maculatus* which was not significantly

different at 10g/100 seeds from the standard control using 2% Actellic dust. This was closely followed by powdered extracts from *Azadirachta indica* at 10g/100seeds which recorded 41.0% in *C. maculatus*. Powders of *C.a papaya* and *V. amygdalina* recorded the least number of dead adult insects. The highest mortality was recorded at 0.175 of Actelic dust.

Table 1: Effect of the botanical leaf powders on the mortality rate of *Callosobruchus maculatus* at 28 days.

Treatment	Concentration (g/100g of seed)	Number of dead adults
Control	0.0	1.2e
Actellic	0.175	20.0a
C. Papaya	5	1.5e
	10	2.5de
T. diversifolia	5	3.7d
	10	7.3c
A. indica	5	4.8d
	10	8.2c
V. amygdalina	5	1.2e
	10	2.5de
A. ciliata	5	9.5c
	10	15.5a

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

Mean number of adult *C. maculatus* emergence after 42-day incubation period is shown in Table 2. The F_1 progeny emergence differed significantly with plant extracts and concentrations ($p<0.05$). All the plants extract irrespective of concentration reduced the F_1 progeny emergence of *C. maculatus.* However, the higher the concentrations of the extracts, the higher the percentage reduction in adult emergence of both insects. The percentage reduction in F_1 progeny emergence was significantly higher with the 10g/100g of seeds of *A. ciliata* among the plant extracts relative to the control. The results also indicated that the powdered extract of *A. indica* also significantly reduced the number of adult emergence at a higher concentration when compared with other botanicals. Similar trend was observed for the extracts in the control of *C.maculatus* on cowpea seeds. However, Actellic dust completely inhibited progeny emergence in *C. maculatus*.

Table 2: Effect of botanical leaf powders on adult F_1 progeny emergence of *Callosobruchus maculatus*

Treatment	Concentration (g/100g of seed)	No of live Adults	% PRD
Control	0.0	25.7a	0.0
Actellic	0.175	0.0f	100.0
C. Papaya	5	22.1b	14.0
	10	10.3d	59.9
T. diversifolia,	5	15.2c	40.8
	10	10.5d	59.2
A. indica	5	12.0d	53.1
	10	9.5d	63.1
V.amygdalina	5	18.7bc	27.3
	10	15.8c	38.5
A.ciliata	5	6.5e	74.7
	10	5.6e	78.2

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

% PRD= Percentage progeny reduction relative to control

Table 3 shows that the seed weight loss increased with storage period irrespective of the treatments in the cowpea seeds stored in polyethylene bags. There was a significant difference ($p<0.05$) in weight loss of cowpea seeds among the different treatments from the first to fifth month of storage with the mixtures (except for CP+TD+AI+AC+VA; TD+AI+VA and CP+TD+AI+AC) performing better than their single application. All the mixtures involving *A ciliata* also significantly resulted in the least weight loss due to weevil damage followed by the single application of *A. ciliata* which was not significantly different from the effects of all its mixtures. The effects of other plants extracts in reducing weight loss during storage were not significantly different from the untreated control. However, the application of *A ciliata* was comparable to Actellic dust in the reduction of weight loss during the storage trial. The weight at 5 months of storage ranged between 2.3g (Single application of *Tithonia diversifolia* and 11.2g (Actellic Dust) using polyethylene bags as storage material.

Result in Table 4 revealed that the germination of cowpea seeds was significantly influenced by the type of protectant applied during storage. Using polyethylene bags as storage material for cowpea seeds the percentage of germination reduced to 69.3%

from 96% in the control after a month of storage, which was significantly different ($p<0.05$) from other treaments except *Carica papaya, Vernonia amygdalina* and combinations of *Carica papaya* + *Tithonia diversifolia*,+ *Azadirachta indica* + *Acalypha ciliata* + *Vernonia amygdalina*. Although there were significant differences among the treatments after 2 months of storage, the germination percentage was reduced to zero from the 4th month of storage irrespective of the plant extracts.

Table 3: Effects of plant powders on weight of 100 cowpea seeds stored in polyethylene bags during 5 months of storage.

Plant Extract	Cowpea 100-seed weight (g)					
	INW	WA1M	WA2M	WA3M	WA4M	WA5M
CL	22.3	15.6 b	11.2 b	8.1 b	4.7 b	4.6 b
CP	23	15.5 b	12.0 b	8.0 b	4.2 b	3.2 bc
TD	22.5	15.0 b	12.2 b	8.3 b	4.4 b	2.3 c
AI	23.5	14.8 b	11.5 b	7.4 b	4.1 b	2.4 c
VA	22	15.2 b	10.8 b	8.1 b	3.8 b	3.5 c
CP+TD+AI+AC+VA	23	15.2 b	10.8 b	7.7 b	3.8 b	3.3 c
TD+AI+VA	22	14.6 b	11.2 b	7.8 b	4.4 b	3.1 c
CP+TD+AI+AC	21.4	15.2 b	11.4 b	8.8 b	4.6 b	2.9 c
CP+TD+AI+VA	23.7	20.4 a	17.5 a	17.7 a	14.5 a	8.8 a
CP+TD+AC	22.6	21.2 a	17.9 a	16.0 a	13.2 a	7.9 a
TD+AI+AC	23	21.8 a	18.9 a	17.8 a	13.2 a	9.1 a
CP+AI+AC	22.8	20.3 a	19.3 a	17.5 a	14.7 a	9.2 a
CP+VA+AC	23	20.1 a	19.8 a	17.2 a	15.2 a	8.9 a
AC	22	19.5 a	17.2 a	16.5 a	14.5 a	9.2 a
AD	22	21.8 a	18.6 a	16.3 a	13.5 a	11.2 a

INW = Initial weight, WA1M-WA5M= Seed weight 1 -5 months after storage. Values followed different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control (untreated seed) ; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina*, AC= *Acalypha ciliata;* AD= *Actellic dust*

Table 4: Effect of the powdered plant extracts on the percentage of germination of cowpea seeds stored in polyethylene bags during 5 months of storage.

Plant Extract	Percentage of germination (%)					
	INW	PG1M	PG2M	PG3M	PG4M	PG5M
CL	96.0	69.3 e	42.7 f	19.6 d	0.0 a	0.0 a
CP	97.0	69.7 e	51.7 d	26.3 cd	0.0 a	0.0 a
TD	99.0	73.7 d	48.3 ed	22.3d	0.0 a	0.0 a
AI	98.6	73.3 d	56.0 d	34.3 bc	0.0 a	0.0 a
VA	99.0	70.0 e	50.3 ed	32.7 bc	0.0 a	0.0 a
CP+TD+AI+AC+VA	97.0	71.3 e	44.7 e	24.7 d	0.0 a	0.0 a
TD+AI+VA	99.0	72.7 e	48.0 ed	28.3 c	0.0 a	0.0 a
CP+TD+AI+AC	98.7	71.3 e	48.3 ed	31.7 c	0.0 a	0.0 a
CP+TD+AI+VA	99.7	76.3 ed	49.0 ed	25.0 c	0.0 a	0.0 a
CP+TD+AC	96.8	87.0 b	82.3 b	40.3 a	0.0 a	0.0 a
TD+AI+AC	99.0	97.0 a	95.3 a	43.3 a	0.0 a	0.0 a
CP+AI+AC	98.0	86.7 b	85.3 b	39.0 ab	0.0 a	0.0 a
CP+VA+AC	98.7	79.3 c	74.0 c	42.0 a	0.0 a	0.0 a
AC	99.0	87.0 b	86.0 b	39.3 ab	0.0 a	0.0 a
AD	98.5	84.0 bc	84.0 b	41.0 ab	0.0 a	0.0 a

ING = Initial germination PG1M-PG5M= seed percentage germination 1 -5 months after storage

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina*, AC= *Acalypha ciliata;* AD= *Actellic dust*

The synthetic insecticide (Actellic dust) effectively controlled the infestation of *C. maculatus* (Table 5). The mixtures of *Tithonia diversifolia* + *Azadirachta indica* + *Acalypha ciliata* (1:1:1) and that of *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* (1:1:1) as well as the single application of *Acalypha ciliata* significantly reduced infestation of cowpea seeds stored in polyethylene bags throughout the period of storage. There were however 100% infestations by the cowpea weevil in other treatments as well as the untreated control.

Infestation of *C. maculatus* in cowpea stored in polyethylene bags during 5 months of storage (Table 5) revealed that only single application of *A. ciliata* and its mixtures

significantly reduced (P≤0.05) the percentage of *C. maculatus* infestation throughout the duration of storage. However, effect of *A. ciliata* and its mixtures on *C. maculatus* infestation was not significantly different from actellic dust treatment in the first month of storage but was significantly different from the second month (9%) to 5th month (13%) of storage. The Actellic treated seeds were devoid of insect attack (0%) from the second month of storage onward in polyethylene bag. It was observed that cowpea seeds stored with the untreated control and other extracts were completely (100%) infested by *C. maculatus* from the 4th month of storage.

Table 5: Effect of the plant powders on the percentage of seed infestation by *Callosobruchus maculatus* stored in polyethylene bags during 5 months of storage.

Plant Extracts	Percentage of insect infestation (%)				
	INF1M	INF2M	INF3M	INF4M	INF5M
CL	49.7 a	58.7 a	94.3a	100.0 a	100.0 a
CP	25.3 b	41.7 c	80.3 b	100.0 a	100.0 a
TD	26.0 b	43.3 bc	79.7 b	100.0 a	100.0 a
AI	25.7 b	47.0 b	68.7 bc	100.0 a	100.0 a
VA	26.7 b	43.7 bc	75.0 b	100.0 a	100.0 a
CP+TD+AI+AC+VA	17.3b	37.0 c	60.7 c	100.0 a	100.0 a
TD+AI+VA	22.7b	44.3 bc	80.3 b	100.0 a	100.0 a
CP+TD+AI+AC	24.0 b	37.0 c	70.7 bc	100.0 a	100.0 a
CP+TD+AI+VA	23.3 b	42.0 bc	74.7 b	100.0 a	100.0 a
CP+TD+AC	6.7 c	12.7 d	12.0 d	16.7 b	19.7 b
TD+AI+AC	0.7 c	6.0 d	12.0 d	15.0 b	12.0 c
CP+AI+AC	6.0 c	10.3 d	13.0 d	12.0 c	20.0 b
CP+VA+AC	5.0 c	12.0 d	13.0 d	10.0 d	12.0 c
AC	5.0 c	9.0 d	12.0 d	15.7 b	13.0 c
AD	3.0 c	0.0 e	0.0 e	0.0 e	0.0 d

INF1M – INF5M = Percentage seed infestation 1 -5 months after storage

Values followed by different letter in each column are significantly different *P*≤0.05 according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina;* AC= *Acalypha ciliata;* AD= *Actellic dust*

The result in Table 6 shows that seed weight reduced with increase in storage period irrespective of the treatments in cowpea seeds stored in plastic containers. There were significant differences ($p<0.05$) in weight loss among the different treatments. All the mixtures involving *A ciliata* also significantly resulted in the least weight reduction due to weevil damage followed by the single application of *A. ciliata* which was not significantly different from the effects of all its mixtures. The results also revealed that the percentage decrease in 100-seed weight 5 months after storage was 2g for the untreated control while it was 15.8g for Actellic treated seeds. However, weight reduction for the seeds stored with mixtures of *Carica papaya + Vernonia amygdalina + Acalypha ciliata* and single application of *A. ciliata* was the least with 11g and 11.3g respectively. The weight reduction for other treatments ranged from 5.4g in mixture of *Carica papaya +Tithonia diversifolia +Azadirachta indica + Vernonia amygdalina* to 9.9g for *Carica papaya+ Azadirachta indica + Acalypha ciliata* in cowpea seeds stored in plastic containers after 5 months of storage.

Table 6: Effect of the plant powders on the weight of 100 cowpea seeds stored in plastic container during 5 months of storage.

Plant Extracts	Cowpea 100-seed weight (g)					
	INW	WA1M	WA2M	WA3M	WA4M	WA5M
CL	22.3	12.9 e	9.9 c	8.6 d	4.7 e	2.0 de
CP	23.0	15.4 bc	12.6 b	10.3 c	8.0 c	6.9 d
TD	22.5	15.7 b	12.4b	9.7 c	7.5 cd	7.1 d
AI	23.5	13.7 ed	12.55b	9.5 c	7.6 cd	6.5 d
VA	22.0	13.4 e	12.3 b	9.7 c	8.4 c	6.2 d
CP+TD+AI+AC+VA	23.0	14.8 cd	18.9 b	9.3 c	8.1 c	6.2 d
TD+AI+VA	22.0	14.0 cd	12.1 b	9.7 c	7.9 cd	6 d
CP+TD+AI+AC	21.4	15.6 bc	12.5 b	9.7 c	7.2cd	5.4 d
CP+TD+AI+VA	23.7	15.4 bc	11.2 b	9.2c	6.8 d	6.2 d
CP+TD+AC	22.6	17.3 a	15.2 a	12.5 b	10.9 b	9.2 c
TD+AI+AC	23.0	17.8 a	15.5 a	12.9 b	11.1 b	8.4 c
CP+AI+AC	22.8	16.7 b	15.1 a	12.8 b	11.4 b	9.9 bc
CP+VA+AC	23.0	18.0 a	16.3 a	15.2 a	12.9 b	11.0 b
AC	22.0	17.3 a	15.2 a	13.6 b	12.3 b	11.3 b
AD	22.0	18.2 a	17.5 a	16.2 a	16.0 a	15.8 a

INW = Initial weight, WA1M-WA5M= seed weight at 1 -5 months of storage.

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; *AI=Azadirachta indica*; VA= *Vernonia amygdalina*; AC= *Acalypha ciliata;* AD= *Actellic dust*

Table 7 shows the effect of the different plant powders on the percentage of germination of cowpea seeds stored in plastic container for 5 month. The percentage of germination of the cowpea seeds was significantly influenced by the type of plant extracts and mixtures. The result revealed that there was drastic reduction in seed germination at 3 months of storage with the untreated control recorded 0% germination while the Actellic treated control recorded 98%. *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* and single application of *A. ciliata* recorded significantly higher ($P \leq 0.05$) percent of germination of 78.7% and 77.7% respectively compared to treatments with other extracts after three months of storage.

All the plant extract treated seeds except *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* and single application of *A. ciliata* recorded between 0% and 25.4% of germination after four months of storage. The same trend was observed after five months of storage.

Table 7: Effect of the plant powders on the percentage of germination of cowpea seeds stored in plastic containers during 5 months of storage.

Plant Extract	Percentage of germination (%)					
	ING	PG1M	PG2M	PG3M	PG4M	PG5M
CL	98	85.0cd	57.0 e	0.0 e	0.0 d	0.0 d
CP	97.9	84.0cd	80.7 c	25.7cd	0.0 d	0.0 d
TD	99.2	87.7cd	81.0 c	33.0 c	0.0 d	0.0 d
AI	98.7	85.7cd	81.7 c	25.0cd	0.0 d	0.0 d
VA	97.5	85.0 d	81.0 c	22.6 d	0.0 d	0.0 d
CP+TD+AI+AC+VA	99	85.7cd	83.0 c	30.0 c	0.0 d	0.0 d
TD+AI+VA	98.5	84.5cd	79.0 c	31.0 c	0.0 d	0.0 d
CP+TD+AI+AC	97.5	80.0 d	73.0 d	30.0 c	0.0 d	0.0 d
CP+TD+AI+VA	96.8	82.0 d	76.7 d	28.6cd	0.0 d	0.0 d
CP+TD+AC	99	98.0 a	90.3 b	20.5 b	0.0 d	0.0 d
TD+AI+AC	98.3	95.2 b	92.7ab	31.0 b	20.5 c	6.2 c
CP+AI+AC	98.7	93.7 b	91.3 b	49.0 b	25.4 c	5.2 c
CP+VA+AC	99	95.3 b	90.3 b	78.7 b	62.7 b	40.5 b
AC	97.2	97.0ab	91.4 b	77.7 b	66.0 b	45.4 b
AD	100	99.2 a	98.0 a	98.0 a	90.0 a	85.2 a

ING= Initial germination

PG1M-PG5M= seed percentage of germination after 1 -5 months of storage

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina;* AC= *Acalypha ciliata;* AD= *Actellic dust*

Infestation by *C. maculatus* as affected by the efficacy of the plant extracts is shown in Table 8. The results revealed that only the extracts from *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* and single application of *A. ciliata* among the extract mixtures caused significant ($p<0.05$) reduction (100%) in the percentage of insect infestation in seeds stored in plastic containers for the period of five months. It was observed that cowpea seeds stored with the untreated control and other extracts/mixtures were completely infested by *C. maculatus.* The actellic treated control was devoid of insect attack throughout the period of storage.

Table 8: Effect of the plant powders on the percentage of cowpea seed infestation by *Callosobruchus maculatus* stored in plastic containers during 5 months of storage.

Plant Extract	Percentage of insect infestation				
	INF1M	INF2M	INF3M	INF4M	INF5M
CL	34.3 a	56.0 a	100.0 a	100.0 a	100.0 a
CP	33.0a	48.7 ab	100.0 a	100.0 a	100.0 a
TD	30.7 a	48.3 ab	100.0 a	100.0 a	100.0 a
AI	30.7 a	47.7 b	100.0 a	100.0 a	100.0 a
VA	31.7 a	48.0 ab	100.0 a	100.0 a	100.0 a
CP+TD+AI+AC+VA	27.3 b	49.7 ab	100.0 a	100.0 a	100.0 a
TD+AI+VA	30.7 a	45.7 b	100.0 a	100.0 a	100.0 a
CP+TD+AI+AC	27.3 b	48.7 ab	100.0 a	100.0 a	100.0 a
CP+TD+AI+VA	34.6 a	56.0 a	100.0 a	100.0 a	100.0 a
CP+TD+AC	4.2 c	12.3 d	32.0 b	100.0 a	100.0 a
TD+AI+AC	4.6 c	10.5 d	33.0 b	100.0 a	100.0 a
CP+AI+AC	5.2 c	9.7 d	24.0 c	100.0 a	100.0 a
CP+VA+AC	3.7 c	11.2 cd	22.7 c	48.7 b	56.7 ab
AC	2.3 c	9.6 d	11.2 d	25.3 c	54.7 c
AD	0.0 c	0.0 e	0.0 e	0.0 d	0.0 d

INF1M – INF5M = percentage of seed infestation after 1 -5 months of storage

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina;* AC= *Acalypha ciliata;* AD= *Actellic dust*

Discussion

Effects of five leaf powders of *Carica papaya*, *Tithonia diversifolia*, *Acalypha ciliata*, *Azadirachta indica* and *Vernonia amygdalina* in control of *C. maculatus* on Ife Brown cowpea was studied. Adult mortality of *C. maculatus* was found to increase with increase in concentration levels, although in some cases the mortality with 5g treatments was the same with 10g concentration, and time of exposure to treatments. This trend of results compared favourably with the reports given by Gupta and Tandon (2004) with the use of *Withania somnifera* (leaf, stem, fruit and root extracts) applied at 5% and 10% against *Callosobruchus chinensis* L. infesting green gram (*Vigna radiata*). High mortality of storage beetles have been recorded in treatments of lemon grass products and Eucalyptus products (Dike and Mbah, 1992; Oparaeke, 1997; Ojiako and Adesiyun, 2013). Higher concentration levels in the treatments of this study as well as duration of exposure of the pest to treatments caused higher mortality of *C. maculatus*.

Higher concentrations of the treatments recorded lower oviposition in all the plants under trials which was similar to the findings of Ofuya *et al., (*2010) who reported that 2.0 g plant powder added to 500 cowpea seeds reduced oviposition and egg hatch in *C. maculatus.* Oviposition rates between and within treatment were significantly lower than the control, which confirms Ivbijaro (1983a) work that neem seed powder drastically reduced egg laying in female *S. oryzae* from 154 in the untreated control to only 9 and 3 at neem powder doses of 0.5 and 1.0/20 g maize grains, respectively. Dike and Mbah (1992) reported similar conclusions on cowpea treated with lemon.

Results from the present study indicate that botanical leaf powders can be used to control *C. maculatus* in storage. The utilisation of different plant products as stored crop grain protectants has been reported by Swella and Mushobozy (2007); Araya and Emana (2009) and Mulungu *et al.* (2010).

It was also observed that the type of storage materials used to store cowpea seeds had significant effect on the germination capacity. Seeds stored in plastic containers had higher percentage of germination than polyethylene bags, which is in consonance with data obtained by Kamara *et al.* (2014).

In Nigeria, some of the plant materials used in this study have been found individually effective in storage pest's control (Oparaeke, 1997). Information on the use of plant extracts for field pests control is limited. However, Oparaeke (2004) and Olaifa *et al.* (1987) have shown that these plant extracts exhibited varying degrees of efficacy on pests of field cowpea. This is the first time a mixture of plant extracts are employed to effectively checkmate the nuisance of pests on cowpea and maize seeds.

The present study showed that the effectiveness of botanical pesticides decreases with time and they therefore need constant reapplications. The effectiveness of botanical pesticides was better with *A. ciliata* leaf powder and its mixtures (CP+VA+AC, CP+AI+AC; TD+AI+AC and CP+TD+AC) used to treat maize grain at 10g. There was a low mortality rate of maize weevils at application rates of 5 g and 10 g for all the botanicals though there was a significant mortality rate for *C. papaya* at 5 g after

3 months. The toxic and repellent action of the leaf powders of *A. ciliata*, *T. diversifolia, A. indica* and *C. papaya* on *C. maculatus* indicates that these botanical products can be utilised as good alternatives to synthetic pesticides in the control of the pest in stored maize grain. Where synthetic grain protectants like Actellic dust are unavailable or unaffordable, small holder farmers can use traditional techniques to confer some measure of protection of their maize grain against weevils.

Results obtained from this study also suggest that plastic containers are better packaging materials than polyethylene bags. The applications of *Tithonia diversifolia + Azadirachta indica + Acalypha ciliata, Carica papaya + Azadirachta indica + Acalypha ciliata, Carica papaya + Vernonia amygdalina + Acalypha ciliata*, as well as single application of *Acalypha ciliata* in airtight plastic containers reduced *C. maculatus* infestation of cowpea seeds significantly. The results of this investigation showed that botanical mixtures could form the basis for a successful formulation and commercialization of biopesticides in developing countries, where low input agriculture is in vogue. In Nigeria, these plants are readily available in the local markets all the year round for farmers' use to protect their crops. Since the materials are used in ethno-botany for the treatment of various ailments, they are safe, cheap, easily biodegradable, and technologically and environmentally friendly. They could provide valuable alternatives to synthetic chemicals which have been proven to be hazardous to man and environment.

Further work should be done to identify and isolate active compounds contained in these plant powders to determine the efficacy and methods of formulations. This may involve chemists, biochemists and environmental scientists. These botanical powders should be incorporated into grain protection practice of resource-poor farmer. In addition, there is the need to investigate the shelf life of the powders to find out if repeated application is needed after a given period and also the health implication to man, if any.

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