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USE OF SELECTED PLANT-DERIVED POWDERS AND THEIR COMBINATIONS TO PROTECT STORED COWPEA GRAINS AGAINST DAMAGE BY *Callosobruchus maculatus*

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ABSTRACT

In search for low-cost plant-based post-harvest insecticides against stored product insects, dried seed powders of *Syzygium aromaticum*, *Piper guineense*, *Ocimum basilicum*, *Aframomum citratrum* and leaf powders of *Cyperus aequalis* and *Eucalyptus camaldulensis* were tested against *Callosobruchus maculatus* in cowpea grains. For each, 100g of grains were mixed with 1, 2, 3, or 4g of powder and mortality recorded daily for 5 days. The potency and persistence of various combinations of potent powders were also studied. Regardless of concentration, *S. aromaticum* or *P. guineense* powder treated grains caused 100% and 98.75% adult mortality, respectively, within 5 days after infestation (DAI) compared to 100% 2 DAI for Actellic powder, the standard protectant in Cameroon. *Ocimum basilicum* or *A. citratum* powder were less efficacious compared to *S. aromaticum* and *P. guineense*; each caused significantly lower grain weight loss after three months of storage. *S. aromaticum* powders had longer residual effects by preventing weevil emergence for ≥ 8 weeks compared to 6 weeks for *P. guineense*. Adult weevils introduced weekly on seeds treated with *S. aromaticum* powder were continuously killed for ≥ 8 weeks after treatment compared to 6 and 4 weeks for *P. guineense* and Actellic, respectively. Application of 50:50% combinations of *S. aromaticum* and *C. aequalis* powders caused 100% adult mortality at 5 DAI, earlier than similar mixtures with *E. camaldulensis* or *P. guineense* and *C. aequalis* or *E. camaldulensis*. Equal mixtures of powders of *S. aromaticum* and *C. aequalis* clearly have potentials for long-term and large-scale protection of stored grains.

Keywords: grain storage, cowpea, *Callosobruchus maculatus*, plant powder, grain damage, mortality, protection.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp, also known as the Southern pea or black-eyed pea in the USA, is a highly nutritious grain legume well adapted to the stressful growing conditions of tropical Africa, Asia, Latin America and Southern USA (Singh and Rachie, 1985). Due to the low availability and consumption of animal protein in tropical and subtropical regions of the world, cowpea serves as a major source of dietary protein (Ofuya, 1986; Oparake *et al.*, 1998). Cowpea provides about half of the dietary proteins of the poor in sub-Saharan Africa (Rachie, 1985). In Cameroon, the dry seeds are prepared as porridge and also as traditional steamed cake (called 'khoki') wrapped in leaves (Ntonifor *et al.*, 2006). The tender green pods and leaves serve as a vegetable while the crop residue serves as fodder for animals (Duke, 1990).

In storage, cowpea grains suffer great qualitative and quantitative losses caused by various insect pest species, especially *Callosobruchus sp.* (Rajapakse and Van Enden, 1997; Abdullahi and Muhammad, 2004; Umeozor, 2004). Throughout tropical Africa, *C. maculatus* causes substantial losses of cowpea in storage annually (IITA, 1989). Caswell (1981) reported a loss of *ca* 50% of cowpeas in storage for 3 or 4 months due to infestation by *C. maculatus*. Farm storage for six months can lead to *ca* 70% grain infestation and 30% weight loss which renders the grains virtually unfit for consumption (Singh and Jackai, 1985).

The fact that cowpea grains are used as human food and feed for livestock renders the use of toxic synthetic insecticides unacceptable since this may lead to serious problems of toxic residues, health and environmental hazards (Aslam *et al.*, 2002). Given the disadvantages of synthetic insecticides, small-scale farmers are more inclined to use traditional approaches to protect their grains. This underscores the importance of seeking alternative anti-weevil measures such as the use of plant-derived natural pesticides for grain storage since these would be readily available, affordable, relatively less toxic and detrimental to humans and the environment (Jacobson, 1989; Niber, 1994; Hassanali and Bekele, 1997; Aslam *et al.*, 2002; Echezona, 2006). Furthermore, the current renewed interest in reducing environmental contamination and global warming have served as an added impetus for the re-evaluation and intensification of environmentally friendly and cost-effective pest management technologies such as the use of traditional botanical pest control agents. Therefore the objectives of our study were to evaluate powders derived from cloves (*Syzygium aromaticum* [L.] Merr. ET Perry *(*Myrtaceae)), basil (*Ocimum basilicum (*Lamiaceae)), 'mbongo" *(Aframomum citratum* (Pareira) K. Schum (Zingiberaceae)), pepper *(Piper guineense* Schum et Thonn *(*Pipericeae)), cypress *(Cyperus aequalis.*Vahl (Cyperaceae)), and eucalyptus *(Eucalyptus camaldulensis* Dehnh. *(*Myrtaceae)) for insecticidal and/or repellent activities against *Callosobruchus maculatus* Fab. On stored cowpea as well as testing the potency of various combinations of the plant powders for possible large-scale use.

MATERIALS AND METHODS

Sources of insects and cowpea

C. maculatus adults were collected from infested cowpea grains (unknown cultivar) bought from a local market in Cameroon. The weevils were identified with the aid of taxonomic keys and by comparing with laboratory voucher specimens at the University of Buea. The weevils were sexed based on their elytral patterns since the females are dark coloured and have four elytral spots in contrast to the pale brown and less distinctly spotted males (Halstead, 1963). A stock culture of the weevils was maintained on a known bruchid susceptible cowpea variety in a 500ml capacity glass jar. The jar was covered with nylon mesh held in place with a rubber band and kept in the laboratory at ambient conditions of 25±5^{0}C and 70±10% r.h. wholesome cowpea grains were used in all the experiments. The grains were procured and selected to remove damaged grains with obvious emergence "windows". The grains were then kept in an oven at 60^{0}C for 2 weeks to kill any hidden infestation with eggs, larvae or adults.

Plant powders

Matured fresh leaves of *O. basilicum*, *C. aequalis* and *E. camaldulensis* were harvested at their flowering stages, thoroughly washed then dried in an oven at 40^{0}C for 5 days and ground separately in a blender into a fine powder. Similarly, dried fruits of *P. guineense*, *A. citratum* and flower buds of *S. aromaticum* were procured and sun-dried for 4hrs and also ground. The powdered samples were preserved in sealed containers for subsequent use. Pirimiphos-methyl (Actellic® dust) bought from a commercial store was used as the positive control.

Oviposition and egg hatch

Powders of either *P. guineense*, *S. aromaticum*, *A. citratum* or *O. basilicum* were added to each of 4 replicates cowpea seeds in 500ml capacity glass jars at the doses 1, 2, 3, 4g per 250 seeds (*ca* 100g). *C. aequalis* and *E. camaldulensis* powders were not tested singly because these plants are currently being used traditionally as grain protectants by farmers in Cameroon. The negative and positive controls were four replicates each without plant powder or having 0.05g Actellic® dust, respectively. Ten pairs of newly emerged *C. maculatus* adults from the stock culture were added to each jar and then covered with a nylon mesh held firmly with a rubber band. The jars were kept at ambient conditions of 25 ± 5°C and 70±10% relative humidity in a completely randomized experimental design. At 5 and 15 days after infestation (DAI), the grains and jars were observed under a stereomicroscope and the number of eggs laid on the seeds and on walls of the jars as well as the number of hatched eggs recorded.

Assessment of adult mortality and grain damage

To test the effect of each plant powder on survival of adults of *C. maculatus*, ten pairs of adult weevils were placed in 500ml glass jars each with 150 cowpea seeds. The same powder treatments and controls as in the previous experiment were applied with four replicates. The jars were then kept as in the previous experiment and the numbers of dead insects counted daily. Insects were considered dead if they did not respond to a third touch with a blunt object. After ten days all surviving adults in the jars were removed and the seeds incubated, and 90 days later, 100 grains were randomly collected from each jar to assess the level of damage, by counting the number of insect-damaged (grains with obvious emergent 'windows') and undamaged grains from the lot and then the weights of the grains also taken. The percentage weight loss (percent insect damage) of the grains was computed following the method described by Haines (1991) as follows:

$$\%\text{Weight loss} = \frac{UNd - DNu}{U\,(Nd + Nu)} \times 100$$

Where U and D are the weights of undamaged and damaged grains respectively while
Nu and ND are the numbers of undamaged and insect-damaged grains, respectively.

Evaluation of persistency of powders

Given the potency of powders derived from *P. guineense* and *S. aromatium*, their persistency was also assessed by treating 100g of cowpea seeds with 1, 2, 3g or 4g of each plant powder and the positive and negative controls with 0.05g Actellic® powder and nothing respectively. Twenty newly emerged *C. maculatus* adults from the stock culture were then added to each jar weekly and numbers that died each day counted. The experiment stopped when subsequent insects added did not die.

Bioassay with mixtures of plant powders

Previous tests showed that 2g and 3g of *S. aromaticum* and *P. guineense* powders (two most potent powders) respectively caused 100% of weevil mortality. Therefore powdered mixtures of 0:2, 0.5:1.5, 1:1, 1.5:0.5, or 2:0g of *S. aromaticum* and 0:3, 0.5:2.5, 1.5:1.5, 2.5:0.5 and 3:0g of *P. guineense* and those of the traditionally used either *C. aequalis* or *E. camaldulensis* were also tested. Each of the above treatment was applied to 100g of cowpea seeds in the test jars and then 10 pairs of weevils also added; each treatment was replicated 4 times. The experiment was then kept as in the previous experiments and the number of dead insects in each jar counted daily for five days.

Statistical analysis

Data were analysed by analysis of variance (ANOVA) and the differences between means assessed using the LSD test.

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RESULTS

The number of eggs laid and hatched

Egg counts taken at 5 and 15 DAI showed that at 4g, all the plant powders variedly reduced oviposition. Each of *S. aromaticum* and *P. guineense* significantly (P<0.05) reduced oviposition at 1-4g more than the untreated control, though not as much as Actellic® powder. Grains treated with *S. aromaticum* powder had the least number of eggs followed by those treated with *P. guineense* powder and these were not significantly different from the number laid on grains treated with 0.05g Actellic® powder (Table-1).

Table-1. Mean number (±S.E) of eggs laid by *Callosobruchus maculatus* at 5 and 15 days on cowpea grains treated or untreated with various concentrations of either *Syzygium aromaticum, Piper guineense, Aframomum citratum, Ocimum basilicum* or acteliic® powder.

Plant powder	**Concentration** (g)						
	0	**1**	**2**	**3**	**4**	**0.05**	**L.S.D** (P=0.05)
Day 5							
S. aromaticum	154.0±0.7	4.0±0.4	3.3±0.7	2.0±0.5	4.8±0.6	NA	6.3
P. guineense	169.0±0.7	3.0±0.71	13.3±2.3	4.3±1.10	5.0±0.7	NA	13.7
A. citratum	240.0±1.1	33.8±2.4	67.3±2.9	12.5±1.5	6.5±1.0	NA	26.1
O. basilicum	225.0±0.9	175.0±1.5	48.5±1.8	110.0±2.4	11.8±1.7	NA	17.2
Actellic	NA	NA	NA	NA	NA	13.5±1.4	
Day 15							
S. aromaticum	355.0±0.9	5.3±0.6	4.5±0.6	2.5±0.5	3.5±0.6	NA	6.1
P. guineense	220.0±0.7	8.8±0.9	19.8±2.3	12.5±0.8	7.0±0.8	NA	12.6
A. citratum	312.0±1.3	70.5±2.6	40.5±3.1	48.3±3.1	15.3±1.8	NA	32.4
O. basilicum	238.0±0.9	182.0±1.6	155.0±1.7	135.2±2.2	120.3±1.6	NA	15.4
Actellic	NA	NA	NA	NA	NA	14.7±1.5	

Similarly, all the plant powders tested significantly reduced (P<0.05) the number of eggs that hatched. Again, with the exception of grains treated with 0.05g Actellic® powder, the least number of eggs hatched on *S. aromaticum* treated grains followed by those of *P. guineense* (Table-2).

Table-2. Cumulative mean number (±S.E) of hatched eggs of *Callosobruchus maculatus* at 15 days on cowpea grains treated or untreated with various doses of either *Syzygium aromaticum, Piper guineense, Aframomum citratum,Ocimum basilicum* or 0.05acteliic® powder.

Plant powder	**Concentration** (g)					
	0	**1**	**2**	**3**	**4**	**0.05**
S. aromaticum	355.0±0.9	2.0±0.5	1.5±0.6	0.8±0.49	1.0±0.5	NA
P. guineense	200.0±0.9	8.0±1.5	4.3±1.5	4.3±0.7	2.5±0.6	NA
A. citratum	292.0±1.1	25.8±2.1	14.0±1.6	18.0±1.3	7.0±1.3	NA
O. basilicum	227.0±1.04	143.3±1.4	117.0±1.8	58.3±2.2	55.8±1.3	NA
Actellic	NA	NA	NA	NA	NA	13.5 ±1.4

Unhatched eggs and those with dead embryos or larvae appeared transparent compared to the eggs identified as having hatched (Rajapakse and Van Emden, 1997).

Adult mortality

The percent mortality of weevils after 5 days of exposure to the various plant powders varied with plant species and concentrations used. All plant powders killed more insects than the negative control. Using a dose of 2g/100g of grain *S. aromaticum* caused 100% and *P. guineense* 97.5% mortality at 3 and 5 DAI, respectively (Figure-1).

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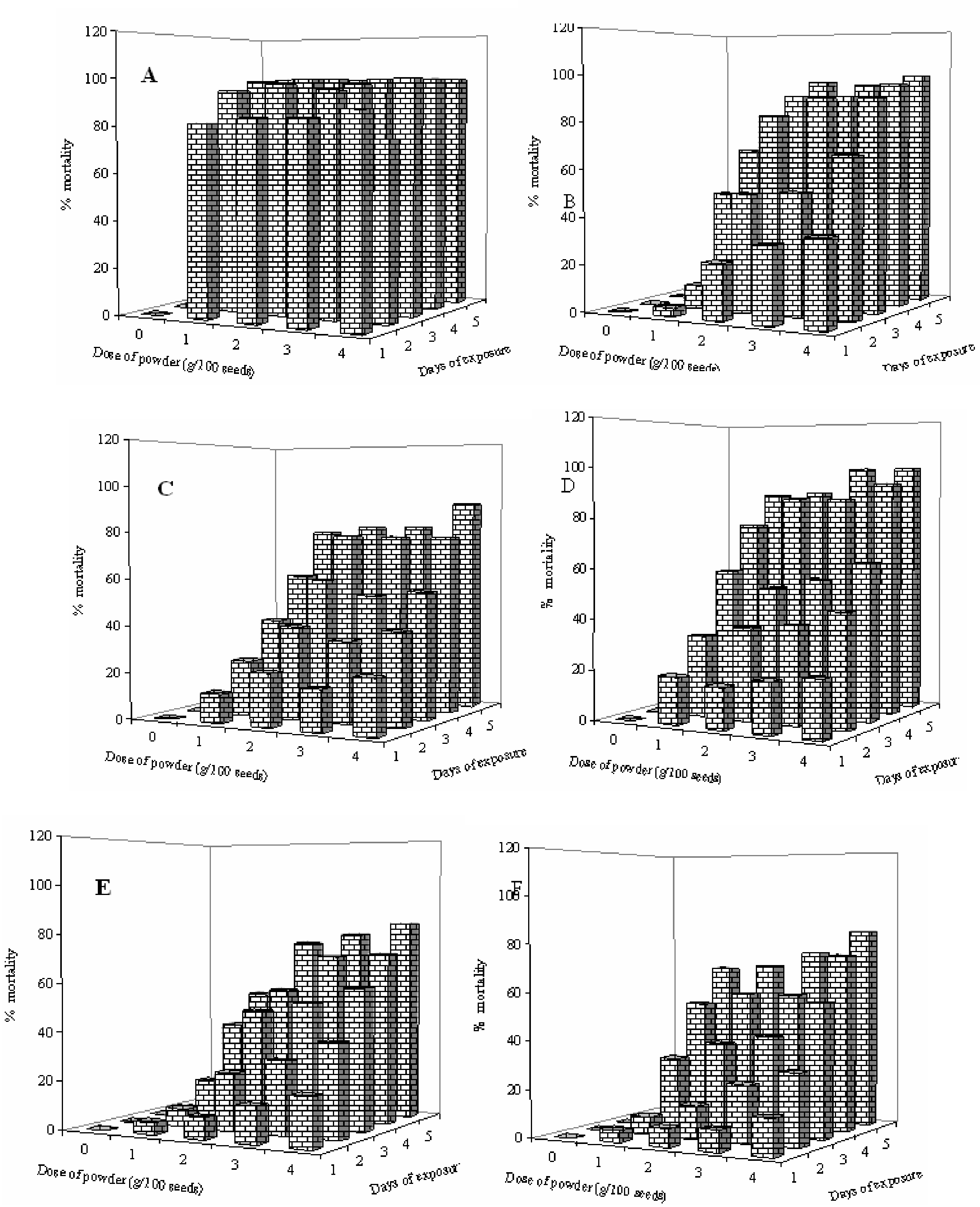

Figure-1. Cumulative percent mortality of adult *Callosobruchus maculatus* caused by treating stored cowpea grains with different concentrations of plant powders A-F. A, *Syzygium aromaticum*; B, *Piper guineense*; C, *Aframomum citratum*; D, *Ocimum basilicum*; E, *Cyperus aequalis*; F, *Eucalyptus camaldulensis*.

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At a lower dose of 1g/100g of grain, *S. aromaticum* powder caused 100% at 4 DAI. However, none of the plant powders killed more insects than Actellic® which caused 100% mortality on day 2. Mortality of adult weevils in all treated grains increased over time (depending on the strength of the powders in the order, Actellic® dust > *S. aromaticum* > *P. guineense* > *O. basilicum* > *A. citratum* > *E. camaldulensi* **>** *C.* *aequalis***,** at 5 DAI irrespective of the concentration used. Each of the spices killed significantly more weevils than *E. camaldulensi* leaves or *C.* *aequalis* leaves singly.

Regardless of the plant powder used, weevil's mortality also increased with the concentration of the plant powders used and more remarkably with each of *S. aromaticum* or *P. guineense* compared to *E. camaldulensi* and *C. aequalis* leaves, but less remarkably with the positive control (Actellic® powder).

Grain damage

The difference amongst the powders in reducing damage caused by *C. maculatus* on cowpea grains was remarkable (P<0.05) (Table-3).

Table-3. Mean (±SE) percent cowpea grain weight loss due to *Callosobruchus maculatus* damage at 90 days after treatment with various concentrations of plant powder or Actellic® powder.

Dosage (g/100g grains)	*Syzygium aromaticum*	*Piper guineense*	*Aframomum citratum*	*Ocimum basilicum*	0.05Actellic® powder
0	41.7±0.8	42.6±1.2	40.9±0.6	42.3±0.9	NA
0.05	NA	NA	NA	NA	8.1±1.3
1	0.2±0.06	0.5±0.02	24.1±1.1	29.7±0.8	NA
2	0.2±0.03	0.5±0.06	20.5±0.9	29.3±1.3	NA
3	0.1±0.02	0.5±0.03	16.7±0.6	28.9±1.1	NA
4	0.1±0.03	0.5±0.07	11.7±1.0	18.6±0.9	NA
L.SD (P = 0.05)	2.3	8.1	11.9	31.5	NA

NA = Not applicable

At 4g, all plant materials caused less than 20% of cumulative grain damage, while the untreated control recorded over 100% damage. However, Actellic® powder was more effective than *A. citratum* and *O. basilicum* after 90 days of grain storage. *S. aromaticum* significantly reduced damage (P<0.05) at all concentrations, resulting in less than one percent damage regardless of concentration tested. Though none of the spices offered complete protection, there was not much difference (P>0.05) amongst the various concentrations of *S. aromaticum* and *P. guineense*, from which the level of damage was quite low compared to the untreated control.

Potency of combinations of plant powders

Combinations of 1:1g *S. aromaticum* (clove) and *C. aequalis* killed 100% of the weevils 5 DAI, while a similar 1:1g of clove: *E. camaldulensi* caused 98.8% mortality (Table-4).

Table-4. Cumulative percent mortality of adult *Callosobruchus maculatus* caused by treating 100g of stored cowpea grains with different combination of *Syzygium aromaticum* and *Cyperus aequalis* or *Eucalyptus camaldulensis* powders or 0.05g actellic® dust.

Treatments	Days expo-sed	Combinations (g:g)							
		0:0	0:2	0.5:1.5	1:1	1.5:1.5	2.0:0	0.05 Actellic®	LSD (P=0.05)
S. aromaticum + C. aegualis	1	0±0.0	11.3±1.9	55.0±1.8	85.1±0.8	95.0±0.4	96.3±0.7	66.3±1.2	16.2
	2	10±0.0	17.5±2.0	85.0±0.3	97.5±0.3	98.8±0.2	100±0.0	95.0±0.5	12.0
	3	10±0.4	36.3±1.5	93.8±0.5	98.8±0.5	100±0.0	100±0.0	100±0.0	17.8
	4	15±0.3	43.8±1.2	98.8±0.2	98.8±0.7	100±0.0	100±0.0	100±0.0	11.6
	5	16±0.8	71.3±0.8	98.8±0.1	100±0.0	100±0.0	100±0.0	100±0.0	14.1
S. aromaticum + E. camaldulensis	1	0±0.0	6.3±1.6	18.8±1.5	35.0±1.4	70.0±0.9	100±0.0	92.5±0.6	12.6
	2	0±0.0	10.0±0.9	31.3±1.1	81.3±0.8	100±0.0	100±0.0	100±0.0	10.8
	3	10±0.6	30.0±1.2	95.0±0.8	95.0±0.4	100±0.0	100±0.0	100 ±0.0	16.4
	4	10±0.3	57.5±1.1	98.8±0.3	98.8±0.7	100±0.0	100±0.0	100±0.0	18.7
	5	15±0.5	63.8±1.3	98.8±0.5	98.8±0.2	100±0.0	100±0.0	100±0.0	16.4

For *P. guineense*, 0.5:2.5g, 1.5:1.5g of *P. guineense*:*C. aequalis* respectively caused 91.3% and 97.5% of weevil mortality 5 DAI. A 1.5:1.5g of *P. guineense* to *E. camaldulensi* killed 88.8% of the weevils compared to the untreated control with *ca* 15% mortality (Table-5).

Table-5. Cumulative percent mortality of adult *Callosobruchus maculatus* caused by treating 100g stored cowpea grains with different combinations of *Piper guineense and Cyperus aequalis* or *Eucalyptus camaldulensis* powder or 0.05g Actellic® dust.

Treatment	Days exposed	Combination (g:g)							
		0:0	0:3	0.5:2.5	1.5:1.5	2.5:0.5	3:0	0.05 Actellic®	LSD (P=0.05)
P. guineense + C. aequalis	1	0±0.0	7.5±2.1	10.0±2.4	17.0±2.3	37.5±1.8	40.0±0.0	98.8±0.6	12.3
	2	0±0.0	17.5±2.0	27.5±1.6	37.5±1.9	66.3±1.3	38.8±1.2	100±0.0	10.8
	3	5±0.7	60.0±0.6	60.0±0.9	86.3±0.8	98.8±0.6	92.5±0.4	100±0.0	14.1
	4	10±0.2	75.0±0.8	81.3±0.6	92.5±0.3	100±0.0	97.5±0.6	100±0.0	12.6
	5	10±0.3	86.3±0.4	91.3±0.8	97.5±0.5	100±0.0	100±0.0	100±0.0	16.2
P. guineense + E. camaldulensis	1	0±0.0	5.0±2.2	6.3±1.3	18.0±2.2	36.2±2.0	38.8±0.9	85±0.9	10.3
	2	0±0.0	8.8±1.5	12.8±1.8	27.5±1.7	48.8±1.3	56.2±1.2	100 ±0.0	13.2
	3	5±0.7	17.5±1.1	21.3±2.0	50.0±0.9	56.2±1.7	86.3±0.7	100±0.0	16.4
	4	5±0.9	35.5±1.4	45.5±0.8	72.5±0.8	72.5±0.8	98.8±0.3	100 ±0.0	12.7
	5	10±1.0	68.8±0.8	70.0±0.6	92.5±0.3	100±0.0	92.5±0.1	100 ±0.0	11.9

Overall, all the plant combinations significantly ($P<0.05$) reduced adult weevil survival on cowpea in all the trials, more than the untreated control (Tables 4 and 5); Actellic® powder caused 100% mortality 2 DAI.

Persistency effects of plant powders

The persistency of the two most potent plant materials showed that *S. aromaticum* was more persistent than *P. guineense* as it caused about 100% mortality of *C. maculatus* adult introduced weekly for 8 weeks (Table-6).

Table-6. Mean (±SE) percent mortality of *Callosobruchus maculatus* adults caused by treating 100g stored cowpea grains with varied concentrations of *Syzygium aromaticum* or *Piper guineense* or 0.05g Actellic® dust at different weeks of infestations**.**

Plant species	**Weeks of infestation**	**Concentration** (g/ 100g of grains)					
		0	**1**	**2**	**3**	**0.5Actellic®**	**LSD** (P=0.05)
Syzygium aromaticum	1	0±0.0	100±0.0	100±0.0	100±0.0	100±0.0	0.0
	2	0±0.0	98.8±0.4	100±0.0	100±0.0	100 ±0.0	6.4
	3	0±0.0	97.5±0.7	100±0.0	100±0.0	100 ±0.0	8.7
	4	10±0.3	97.5±0.3	98.7±0.6	100±0.0	100±0.0	4.9
	5	10±0.7	95.0±0.5	98.7±0.4	100±0.0	0.0 ±0.0	10.1
	6	10±0.2	95.0±0.2	98.7±0.2	98.7±0.9	0.0 ±0.0	7.2
	7	15±0.9	77.5±0.8	97.5±0.6	98.7±0.6	0.0 ±0.0	6.7
	8	15±0.6	76.3±0.5	95.0±0.3	98.7±0.3	0.0 ±0.0	9.3
Piper guineense	1	0.0±0.0	100±0.0	100±0.0	100±0.0	100 ±0.0	0.0
	2	0.0±0.0	100±0.0	100±0.0	100±0.0	100±0.0	0.0
	3	0.0±0.0	100±0.0	98.7±0.4	100±0.0	100±0.0	3.4
	4	0.0±0.0	97.5±0.5	98.7±0.8	98.7±0.6	100±0.0	2.8
	5	0.0±0.0	97.5±0.3	98.7±1.0	96.3±0.9	0.0 ±0.0	3.2
	6	5.0±0.4	92.5±0.6	96.3±0.8	95.0±0.7	0.0±0.0	4.7
	7	5.0±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ±0.0	0.0
	8	5.0±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0

P. guineense remained effective for six weeks, after which new generations started emerging showing that the effect of the active ingredient had elapsed. Similarly, after six weeks of treatment, new generations of adults emerged from actellic treated grains, meaning that Actellic® also lost its potency after a period of about six weeks and needs to be re-treated if grains are stored in open bins. Nonetheless, the two spices demonstrated greater persistence than 0.05g Actellic® powder.

DISCUSSIONS

The results indicate that *S. aromaticum* and *P. guineense* used singly deterred oviposition and also had considerable action on adult weevils. This combined action of inhibition of oviposition and reduced adult longevity will therefore offer greater protection of stored grains against weevil damage. *A. citratum* and *O. basilicum* were not very effective in protecting cowpea grains against the weevil attack although it was shown that oils from *Ocimum spp* might have effect on some insect pests (Bekele *et al.*, 1997). As expected, Actellic® dust (0.05g) was more effective than all the plant powders. Actellic® is a conventional insecticide specifically formulated with high insecticidal activity on stored pests (Anon, 1993). However, its continuous use raises questions regarding the potential risk involved in handling and use. The insecticidal activity of the plant powders corroborates earlier observations that *S. aromaticum* (Aslam *et al*., 2002) and *P. guineense* (Ivbijaro, 1990, Ntonifor and Monah, 2001; Udo, 2005) caused early mortality of weevils thus interfering with their ability to commence a fresh cycle of oviposition. Gwinner *et al*, (1996), Ntonifor and Monah (2001) also reported similar findings that the entire or powdered fruits of *Piper spp.* have insecticidal and/or repulsive effects against many pests. Miyakato *et al.* (1989) had identified several insecticidal unsaturated isobutylamides in *P. nigrum* including guineensine, pipericide and piperine, which are also present in *P. guineense* (Parmar *et al*., 1997). Similarly, the essential oil of *S. aromaticum* contains 85-92% eugenol (Dorman and Deans, 2000) which is known to completely inhibit the development of eggs, larvae and pupae of *Sitophilus granaries, Sitophilus zeamais, tribolium castaneum and Prostephanus truncatus* inside grains (Obeng-Ofori and Reichmuth, 1997). Therefore, eugenol presumably was also responsible for the mortality of the adults and juveniles of *C. maculatus*. At lower concentrations, all the plant powders are less effective; however, increasing their concentration to 2-4g resulted in increased adult weevil mortality approaching 100 percent. This corroborates the findings of Jilani and Hassan (1984) that different plant materials at varied concentrations caused 100% mortality in Coleopterans. The higher weevil mortality in cowpeas treated with either *S. aromaticum* or *P. guineense* powder was translated into better protection of the grains against weevil damage after 90 days of storage, resulting in reduced weight loss.

The proportional combination of 50:50% (1:1g) *S. aromaticum* and *C. aequalis* caused unusually high adult weevil mortality; similar combinations of *P. guineense* and *C. aequalis* or *E camaldulensis* were also very effective. This is the first of such combination "therapy" for storage protection with plant derived powders. This result may have been due to additive and/or synergistic toxicity on the bruchids (Echezona, 2006). The fresh and dry leaves of *C. aequalis* emit strong persistent aromatic odours that are repellent to weevils (Parh *et al*., 1998). Similarly, 1,8-cineole, a primary compound of *Eucalyptus globulus* oil toxic to the rice weevil (Lee *et al*., 2001) may also be present in *E camaldulensis* which is also frequently used in Sub-Saharan Africa to protect stored grains. The combination drastically reduces the quantity of the relatively more expensive *S. aromaticum* or *P. guineense* used in the mixture i.e significantly reducing the cost of the preparations, while maintaining their potency. These combinations caused increased adult mortality as would occur when the grains are treated with either *S. aromaticum* or *P. guineense* alone. These results underscore the importance of evaluating plants in combinations to elucidate their full potency in a given bioactivity (Bekele and Hassanali, 2001). The combinations elucidated in this study potentially offer a cheaper and easy control method for farmers since tropical farmers are more receptive to methods of stored produce protection that are within their technical and financial means (Taylor, 1974). Using combinations of plant-derived powders is also advantageous since this may prevent or retard the development of resistance to these powders due to their chemical complexities.

S. aromaticum had a longer residual effect than *P. guineense*. Grains treated with *S. aromaticum* powder continued to cause high mortality of adult weevils after re-infestation on a weekly basis, for 8 weeks, while, *P. guineense* lost its effects after 6 weeks of infestation. This is useful for traditional smallholder grain storage for short duration. The spices did not offer complete protection probably as a result of decreasing activity due to evaporation of volatile oils, as the containers were constantly opened each week and contents poured out for counting. Also, the inability of the spices to offer complete protection could have been as a result of the powder settling to the bottom of the containers (Niber, 1994).

The proportional combinations of *S. aromaticum* or *P. guineense* with either *C. aequalis* or *E camaldulensis* offer greater potential for large-scale traditional storage since such combinations are relatively cheaper than using either of the spices singly. These therefore, represent important potential for integrated post-harvest pest management programs in sub-Saharan Africa given that these are locally sourced materials which can be produced by trational farmers themselves. Furhteremore, they can provide adequate and acceptable protection to cowpea against *C. maculatus* during storage since they do not impart much odour or taste to the grains nor do they affect seed viability after long-term storage. The use of easily available and affordable plant-based post-harvest protectants could become important supplements or alternatives to imported often more toxic and non-sustainable synthetic insecticides.

Author contribution

N.N. Ntonifor developed the concept and designed the experiments. E.O. Oben performed the experiments supervised by C.B. Nsuh-Konje

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