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Evaluation of binary combination of Rice Husk Ash with some other Insecticidal Plant Powders in Grain Protection against Damage by two Storage Beetles

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Abstract

Evaluation of protectant ability of Jemila rice variety husk ash (JRHA) in binary combination with some other insecticidal plant powders against grain damage by the cowpea seed beetle, *Callosobruchus maculatus* (F) and the maize weevil, *Sitophilus zeamais* (Mots), was conducted at the Research Laboratory of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria, under ambient temperature of 28 ± 2 °C and 70 ± 5% relative humidity. Each binary combination was in the 1:1 ratio and was applied and evaluated at different dosages of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 g/20 g of grain, against ten paired adult beetles for *C. maculatus* and ten unpaired adult beetle for *S. zeamais*. Irrespective of beetle or and weevil species, dosage and time of observation, all the binary combinations protected grain from damage significantly in comparison with the control. The JRHA and *E. aromatic* combination proved generally superior to the other combinations in protecting grain from damage by the two beetles with respect to lethality to adults, inhibition of oviposition, reduction in F1 adult emergence and grain weight loss. The mixture had the lowest LD_{50} for the two beetles. The JRHA and *P. guineense* combination closely followed the JRHA and *E. aromatica* combination in superiority for grain protective ability against damage by the beetles. They may be recommended for use by grain handlers to mitigate damage by *C. maculatus* and *S. zeamais*. They could also be included as a component of integrated grain protection strategies.

Keywords: Ash, plant powder, binary combination, *Callosobruchus maculatus*, *Sitophilus zeamais*, grain protection

Introduction

Cowpeas, *Vigna unguiculata* (L.) Walpers and maize, *Zea mays* L. are important food grains in many countries in Africa and other tropical and sub-tropical areas of the world, supplying the much needed vegetable protein and carbohydrates in diets of different peoples. Their sufficient production and utilization can enhance food security in these different countries. However, these food grains are frequently devastated in storage by beetles when the activities of these insects are not checked, leading to food insecurity in many countries. Stored cowpeas are damaged mainly by the seed beetle, *Callosobruchus maculatus* Fabricius while stored maize is depredated by the weevil, *Sitophilus zeamais* Motschulsky. In Nigeria, without protection, cowpea grain could be damaged by *C. maculatus* that it becomes unfit for human or animal consumption within six months of storage (Ogunkoya and Ofuya, 2001). It has been estimated that *S. zeamais* accounts for about 10 – 40% of total damage to stored maize grains worldwide (Medudu *et al*., 2020).

It is therefore imperative that stored cowpeas and maize need protection from insect damage, and this has principally and successfully been achieved through the use of synthetic insecticides. However, concern for human health and the environment is discouraging the continued use of these hazardous synthetic chemicals. Traditionally, many grain producers in Africa use insecticidal plants to ward off storage insect pests (Ewete *et al*., 2007; Obeng-Ofori, 2010) but appreciable damage continues to occur (Ofuya, 2018). Plant-derived insecticidal materials have been the focus of research by scientists in many countries over many decades in trying to obtain possible and suitable replacements for the synthetic chemical compounds. Botanicals are generally assumed to be cheaper, readily available, and more biodegradable, leading to less environmental problems (Gouboungou *et al*., 2018).The insecticidal plants whose products have been observed to be effective in stored grain protection against insect damage include *Azadirachta indica* A. Juss., *Eugenia aromatica* (L.) Baill., *Piper guineense* Schumach., *Ocimum gratissimum* L., *Oryza sativa* L., *Dennettia tripetala* G. Baker, but effective rates of application are rather high and impracticable (Ashamo, 2019). Combination of different botanicals could enhance biological activity against insect pests and minimize the quantity used (Goudoungou *et al*. 2018). In practice, some African farmers use a mixture of herbs for stored grain protection against insects (Obeng-Ofori, 2010), but in this present study it is basically mixture of rice husk ash and insecticidal plant powders. This paper presents results of studies on effect of binary combinations of rice husk ash with some other insecticidal plant powders on abilities of *C. maculatus* and *S. zeamais* to damage stored grains. Ofuya and Adler (2018) had reported the lethality of binary combinations of rice husk ash with some insecticidal plant powders to adults of *C. maculatus* and *Lasiodermas erricorne* L., but the full protection of the infested grain was not determined.

Materials and Methods

Laboratory, Grains and Insect Cultures

The study was conducted at the Research Laboratory Department of Crop, Soil and Pest Management, the Federal University of Technology, Akure, Nigeria, under ambient laboratory conditions of 28 ± 2ºC and 75±5% temperature and relative humidity, respectively. The grains, cowpea (Ife-brown variety) and maize (SUWAN -1-yellow variety) used in the study were obtained from the Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria. The maize and cowpea were first disinfested by deep freezing for two weeks, and acclimated to ambient laboratory conditions, before use. The cultures of *C. maculatus* and *S. zeamais* were derived from already infested cowpea and maize grains from IAR &T.*C. maculatus* and *S. zeamais* was reared on clean Ife Brown variety of cowpea and SUWAN -1 yellow variety of maize, respectively, in 5 l plastic containers holding 500 g of grain. The insect cultures were recycled monthly by sieving out adults from damaged grain and introducing them into clean grain in other plastic containers under 12:12 light and dark regime.

Rice husk ash, insecticidal plant powders and combinations

Rice husk ash (RHA) was produced from husk of Jemila rice variety obtained from a rice milling plant in Kaduna State, Nigeria. The husk was purified by removing all extraneous objects and subsequently pre-ashed by setting on fire to burn completely. After cooling, the pre-ash material was transferred into a Muffle furnace to produce ash at temperature of 550°C (Monti *et al*., 2008). The Jemila rice husk ash (JRHA) was kept separately in a plastic container with firm cover and stored in the laboratory until when needed.

Plant powders were produced from dry flower buds of *E. aromatica*, dry seeds of *P. guineense*, dry fruits of *X. aethiopica*, rhizomes of *Z. officinale* and leaves of *O. gratissimum*. (base on literature all the parts show potency and insecticidal in nature). All the plant materials were purchased from local herbal sellers in Oba market, Akure, Nigeria, except *O. gratissimum* obtained from a home garden in Akure, Nigeria. The plant materials were air dried separately until a moisture content of 10-12% was achieved and they were ground in 1.5 HP kitchen mill (model - KOAHLBACH). The resulting powders were sieved to a particle size of 300 µm with a British laboratory test standard sieve (serial number 133032) and were separately kept in air-tight plastic containers for subsequent use. JRHA was similarly sieved. JRHA was mixed with the different insecticidal plant powders in binary combinations at a 1:1 ratio which were also kept in separate plastic containers until needed.

Efficacy Tests

Twenty grams of disinfested grain (cowpea for *C. maculatus* and maize for *S. zeamais*), was separately weighed into 250 ml plastic containers to which was added a dosage of JRHA-plant powder combination and ten adult insects (unsexed with respect to *S. zeamais* (because oviposition of *S. zeamais* was not recorded in this study) but 5 males and 5 females of *C. maculatus*). The dosages of each combination applied were 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 g, respectively. The

0.0 dosage constituted the control. All treatments were replicated thrice. The experimental design was the completely randomized design. For *C. maculatus*, 5 days post infestation, all introduced insects were removed and number of eggs laid by the female beetles on the grain was counted and recorded. The treatments were observed for F1 adult emergence from 20 days' post infestation and the number found, recorded daily (it was done daily for accuracy to know exert number of F1 adult emergence each day) until no insects were found for five consecutive days from the day adult emergence started. The emerged insects were removed and the grain reweighed. The percentage adult emergence, seed damage, weight loss and percentage weevil perforation index (WPI) were calculated using the formulae below:

$$\%\ \text{Adult emerged} = \frac{\text{Number of emerged adult}}{\text{Total number of egg laid}} \text{x} \frac{100}{1}$$

$$\%\ \text{Damaged grains} = \frac{\text{Number of seeds with holes}}{\text{Total number of seeds}} \text{x} \frac{100}{1}$$

$$\%\ \text{weight loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \text{x} \frac{100}{1}$$

$$\%\ \text{WPI} = \frac{\%\ \text{treated cowpea seed perforated}}{\%\ \text{control cowpea grain perforated}} \text{x} \frac{100}{1}$$

For *S. zeamais* all introduced adults were removed 5 days post infestation and the treatments observed for F1 adult emergence from the 25th day post infestation until no insects were found for five consecutive days from when adult emergence began. The adults were then removed and the grain reweighed. The percentage adult inhibition rate (IR), and weight loss were calculated using the formulae below:

$$\%\ \text{IR} = \frac{\text{Number of insect in the treatment} - \text{number of insect in the control}}{\text{Number of insect in the control}} x \frac{100}{1}$$

$$\%\ \text{weight loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \text{x} \frac{100}{1}$$

Data Analysis

Data were subjected to analysis of variance consistent with the completely randomized experimental design using SPSS version 17. Prior to analysis all data obtained by counts and percentages were square root and arcsine transformed, respectively. Significant means were separated using Tukey's (Honestly Significant Difference) Test at 5%level of significance. Mortality data were further subjected to regression analysis to calculate the LD_{50} of the mixtures (Finney, 1971).

Results

Irrespective of insect species, dosage used and time of observation, grain protected by the JRHA-plant powder combination produced significantly ($p < 0.05$) higher mortality of exposed *C. maculatus* and *S. zeamais* adults, when compared with the control (Tables 1-5; 7-11). There were significant differences amongst the mixtures in their lethality to the adult beetles irrespective of beetle species and dosage. The JRHA combination with *E. aromatica* or *P. guineense* were more lethal to the adult beetles than others at all dosages and during 24 and 48 h post treatment. At the highest dosage of 1.0 g the JRHA combination with *E. aromatica* had produced 100% *C. maculatus* mortality within 24 h (Table 1), but with *S. zeamais* it was100% mortality 48 h post treatment (Table 7).

Oviposition, F1 adult emergence, and weight loss of cowpea grain in treatments involving introducing *C. maculatus* to grain treated with various dosages of JRHA in combination with different plant powders is presented in Figures 1-5. Irrespective of dosage, the binary combinations of ash and another plant powder significantly ($p < 0.05$) reduced oviposition, F1 adult emergence and grain weight loss with *C. maculatus*, in comparison with the control. Reduced oviposition, F1 adult emergence, and grain weight loss was significantly more pronounced in treatment involving combination of JRHA and *E. aromatica* amongst the different combinations. F1 adult emergence, adult inhibition rate (IR) and weight loss of maize grain in treatments involving introducing *S. zeamais* to grain treated with different dosages of JRHA in combination with different plant powders is presented in Figures 6-10). Irrespective of dosage, the binary combinations of ash and another plant powder significantly ($p < 0.05$) reduced F1 adult emergence and grain weight loss with *S. zeamais*, in comparison with the control. Reduced F1 adult emergence, and grain weight loss was significantly more pronounced in treatment involving combination of JRHA and *E. aromatica* amongst the different combinations. IR was significantly higher in treatments involving combination of JRHA and *E. aromatica* or *P. guineense*, than other combinations, irrespective of dosage.

Summary of regression analysis of mortality data on *C. maculatus* and *S. zeamais* exposed to JRHA in combination with different plant powders within 24 h of observation is presented in Tables 6 and 12 respectively. LD_{50} was significantly ($p < 0.0001$) lower in treatments involving combination of JRHA and *E. aromatica* (0.02 g) and combination of JRHA and *P. guineense* (0.05 g) than other combinations, with *C. maculatus* (Table 6). LD_{50} was significantly ($p < 0.0001$) lower in treatments involving combination of JRHA and *E. aromatic* (0.33 g) and combination of JRHA and *P. guineense* (0.56 g) than other combinations, with *S. zeamais* (Table 12). The Chi-square values of the treatments were above 3.81. The slope and intercept values of the treatments are very low.

Table 1: Mortality of *C. maculatus* exposed to 0.2 g JRHA in combination with different plant powders

JHRA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	26.67 ± 3.33^{b}	50.00 ± 5.77^{b}	70.00 ± 5.77^{b}	90.00 ± 5.77^{b}
Z. officinale	43.33 ± 3.33^{bc}	60.00 ± 5.77^{bc}	86.67 ± 6.67^{bc}	96.67 ± 3.33^{b}
X. aethiopica	43.33 ± 3.33^{bc}	56.67 ± 3.33^{bc}	76.67 ± 3.33^{b}	90.00 ± 5.77^{b}
E. aromatic	63.33 ± 6.67^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
P. guineense	50.00 ± 5.77^{c}	70.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly ($p > 0.05$) different from each other using Tukey'sTest.

Table 2: Mortality of *C. maculatus* exposed to 0.4 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	33.33 ± 3.33^{b}	56.67 ± 3.33^{b}	80.00 ± 5.77^{b}	$93.3\ 3 \pm 3.33^{b}$
Z. officinale	56.67 ± 3.33^{c}	66.67 ± 3.33^{bc}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
X. aethiopica	53.33 ± 3.33^{c}	60.00 ± 0.00^{b}	80.00 ± 0.00^{b}	100.00 ± 0.00^{c}
E. aromatica	70.00 ± 5.77^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
P. guineense	66.67 ± 3.33^{c}	73.33 ± 3.33^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly ($p > 0.05$) different from each other using Tukey's Multiple Range Test.

Table 3: Mortality of *C. maculatus* exposed to 0.6 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	36.67 ± 3.33^{b}	63.33 ± 3.33^{b}	86.67 ± 3.33^{b}	100.00 ± 0.00^{b}
Z. officinale	63.33 ± 3.33^{c}	73.33 ± 3.33^{b}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
X. aethiopica	60.00 ± 5.77^{c}	73.33 ± 3.33^{b}	93.33 ± 3.33^{bc}	100.00 ± 0.00^{b}
E. aromatica	83.33 ± 3.33^{d}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
P. guineense	73.33 ± 3.33^{cd}	86.67 ± 6.67^{bc}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly ($p > 0.05$) different from each other using Tukey's Test.

Table 4: Mortality of *C. maculatus* exposed to 0.8 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	43.33 ± 3.33^{b}	76.67 ± 3.33^{b}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
Z. officinale	73.33 ± 3.33^{c}	86.67 ± 3.33^{bc}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
X. aethiopica	70.00 ± 5.77^{c}	76.67 ± 3.33^{b}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
E. aromatica	96.67 ± 3.33^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
P. guineense	80.00 ± 0.00^{c}	93.33 ± 3.33^{cd}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly ($p > 0.05$) different from each other using Tukey'sTest.

Table 5: Mortality of *C. maculatus* exposed to 1.0 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	46.67 ± 3.33^{b}	60.00 ± 0.00^{b}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
Z. officinale	76.67 ± 8.82^{c}	86.67 ± 3.33^{bc}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
X. aethiopica	76.67 ± 3.33^{c}	83.33 ± 3.33^{bc}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
E. aromatica	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
P. guineense	96.67 ± 3.33^{cd}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly ($p > 0.05$) different from each other using Tukey'sTest.

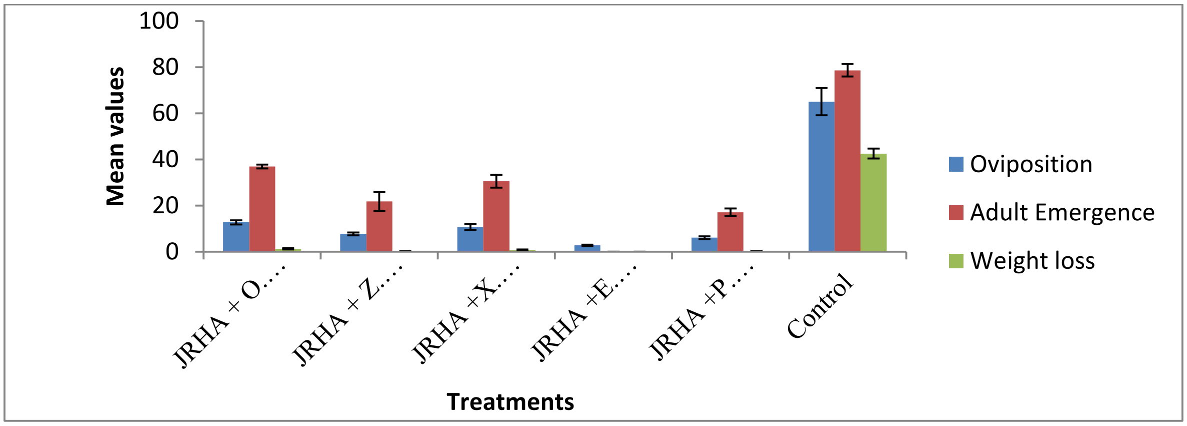

Figure 1: Oviposition, F1 adult emergence of *C. maculatus* and percentage weight loss of the seed exposed to 0.2 g of JRHA in combination with different plant powders

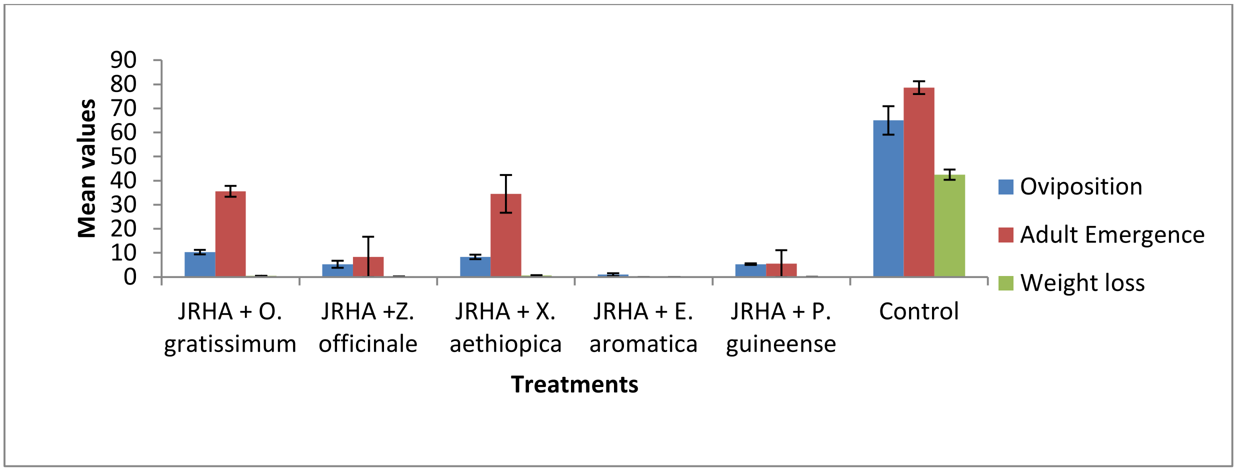

Figure 2: Oviposition, F1 adult emergence of *C. maculatus* and percentage weight loss of grain exposed to 0.4 g of JRHA in combination with different plant powders

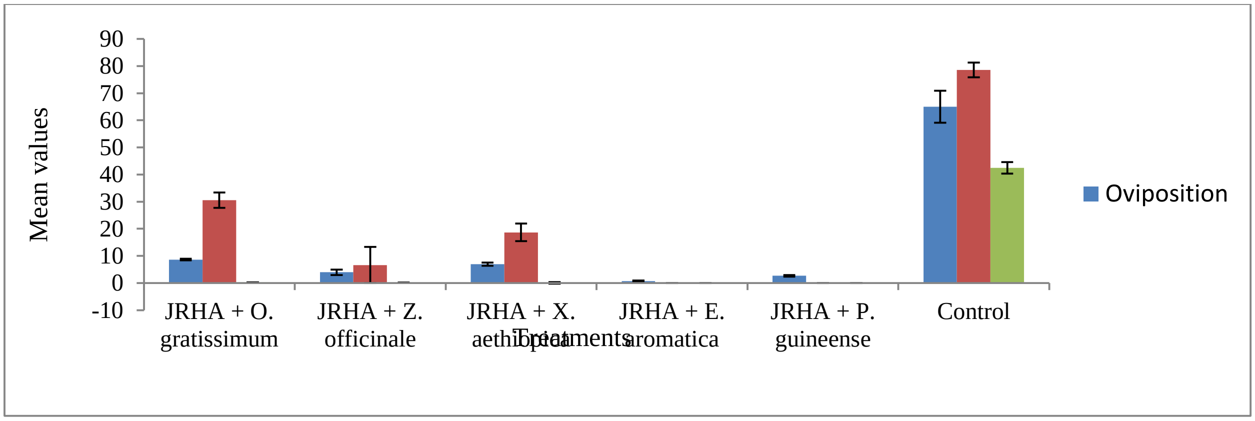

Figure 3: Oviposition, F1 adult emergence of *C. maculatus* and percentage weight loss of grain exposed to 0.6 g of JRHA in combination with different plant powders

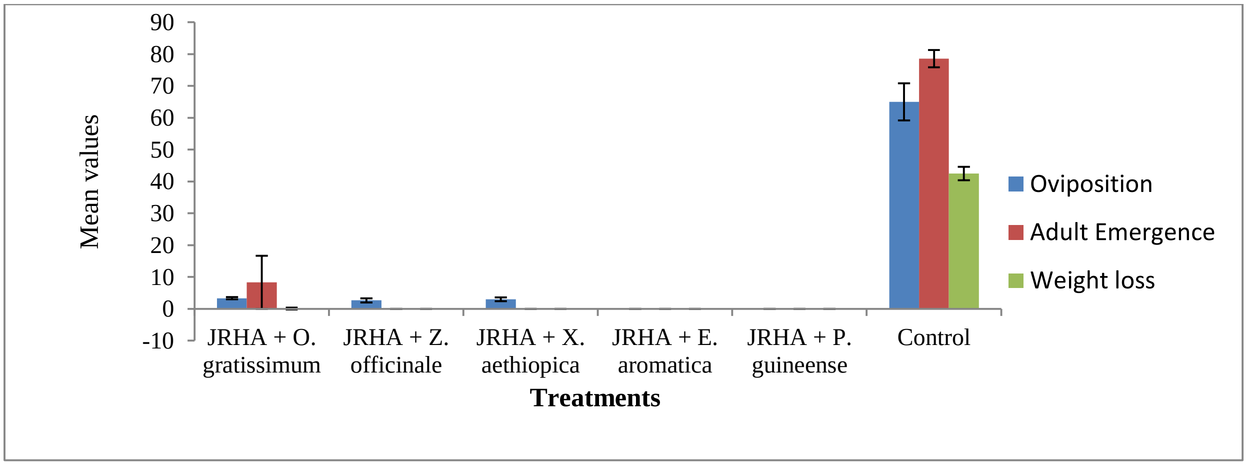

Figure 4: Oviposition, F1 adult emergence of *C. maculatus* and percentage weight loss of grain exposed to 0.8 g of JRHA in combination with different plant powders

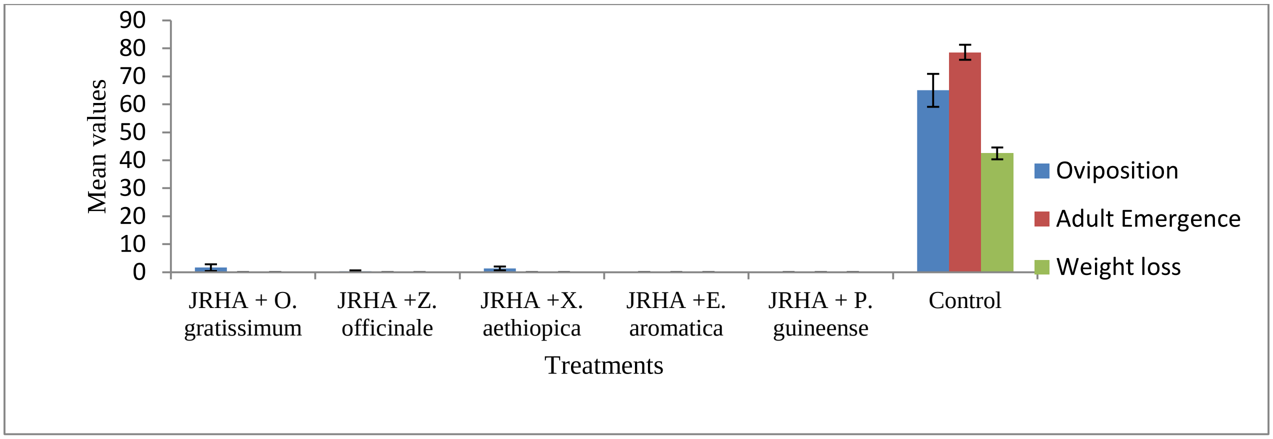

Figure 5: Oviposition, F1 adult emergence of *C. maculatus* and percentage weight loss of grain exposed to 1.0 g of JRHA in combination with different plant powders

Table 6: Summary of regression analysis of mortality data on *C. maculatus* exposed to JRHA in combination with different plant powders within 24h of observation

JRHA +	Slope ± S.E	Intercept ± S.E	X^{2}	LD$_{50}$	95%FL	Sign
O.gratissimum	0.77 ± 0.14	-0.65 ± 0.07	15.66	3.99	2.17-4.35	0.268
Z. officinale	1.20 ± 0.14	-0.20 ± 0.06	51.98	1.47	0.78-1.79	0.000
X. aethiopica	1.31 ± 0.14	-0.23 ± 0.06	18.76	1.49	0.95-1.99	0.131
E.aromatica	2.28 ± 0.17	0.14 ± 0.07	93.44	0.02	0.01-0.04	0.0001
P.guineense	1.86 ± 0.15	-0.09 ± 0.07	53.42	0.05	0.04-0.34	0.0001

LD$_{50}$ = lethal dosage; SE = standard error; X^{2}= Chi-square; FL=Fiducial limits.

Table 7: Mortality of *S. zeamais* exposed to 0.2 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	3.33 ± 3.33^{a}	20.00 ± 0.00^{b}	40.00 ± 0.00^{b}	53.33 ± 3.33^{b}
Z. officinale	13.33 ± 3.33^{b}	33.33 ± 3.33^{b}	50.00 ± 5.77^{bc}	60.00 ± 5.77^{b}
X. aethiopica	10.00 ± 0.00^{ab}	26.67 ± 3.33^{b}	43.33 ± 6.67^{b}	56.67 ± 3.33^{b}
E. aromatica	46.67 ± 3.33^{c}	76.67 ± 6.67^{c}	90.00 ± 5.77^{d}	100.00 ± 0.00^{c}
P. guineense	36.67 ± 3.33^{c}	60.00 ± 5.77^{c}	73.33 ± 6.67^{cd}	90.00 ± 0.00^{c}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly ($p > 0.05$) different from each other using Tukey's Test.

Table 8: Mortality of *S. zeamais* exposed to 0.4 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	13.33 ± 3.33^{b}	36.67 ± 3.33^{b}	53.33 ± 3.33^{b}	73.33 ± 3.33^{b}
Z. officinale	20.00 ± 0.00^{b}	43.33 ± 3.33^{b}	60.00 ± 0.00^{b}	80.00 ± 5.77^{bc}
X. aethiopica	16.67 ± 3.33^{b}	40.00 ± 0.00^{b}	56.67 ± 3.33^{b}	80.00 ± 0.00^{bc}
E. aromatica	66.67 ± 3.33^{d}	90.00 ± 0.00^{d}	93.33 ± 3.33^{c}	100.00 ± 0.00^{d}
P. guineense	46.67 ± 3.33^{c}	76.67 ± 3.33^{c}	86.67 ± 3.33^{c}	93.33 ± 3.33^{cd}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly (p > 0.05) different from each other using Tukey's Test.

Table 9: Mortality of *S. zeamais* exposed to 0.6 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	23.33 ± 3.33^{b}	43.33 ± 3.33^{b}	63.33 ± 3.33^{b}	76.67 ± 6.67^{b}
Z. officinale	43.33 ± 3.33^{c}	60.00 ± 0.00^{c}	70.00 ± 0.00^{b}	83.33 ± 3.33^{bcd}
X. aethiopica	43.33 ± 3.33^{c}	53.33 ± 3.33^{bc}	66.67 ± 3.33^{b}	80.00 ± 0.00^{bc}
E. aromatica	76.67 ± 3.33^{d}	93.33 ± 3.33^{e}	96.67 ± 3.33^{c}	100.00 ± 0.00^{d}
P. guineense	63.33 ± 3.33^{d}	76.67 ± 3.33^{d}	90.00 ± 0.00^{c}	96.67 ± 3.33^{cd}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly (p > 0.05) different from each other using Tukey's Test.

Table 10: Mortality of *S. zeamais* exposed to 0.8 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	26.67 ± 3.33^{b}	46.67 ± 3.33^{b}	66.67 ± 6.67^{b}	80.00 ± 0.00^{b}
Z. officinale	53.33 ± 3.33^{c}	66.67 ± 3.33^{c}	83.33 ± 3.33^{bcd}	93.33 ± 3.33^{cd}
X. aethiopica	50.00 ± 0.00^{c}	63.33 ± 3.33^{c}	76.67 ± 3.33^{bc}	90.00 ± 0.00^{c}
E. aromatica	86.67 ± 3.33^{d}	96.67 ± 3.33^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
P. guineense	76.67 ± 3.33^{d}	90.00 ± 0.00^{d}	93.33 ± 3.33^{cd}	100.00 ± 0.00^{d}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly (p > 0.05) different from each other using Tukey's Test.

Table 11: Mortality of *S. zeamais* exposed to 1.0 g JRHA in combination with different plant powders

JRHA +	% adult mortality in hours post treatment			
	24	48	72	96
O. gratissimum	33.33 ± 3.33^{b}	60.00 ± 0.00^{b}	83.33 ± 3.33^{b}	96.67 ± 3.33^{b}
Z. offininale	66.67 ± 3.33^{c}	80.00 ± 5.77^{cd}	93.33 ± 3.33^{bc}	100.00 ± 0.00^{b}
X. aethiopica	60.00 ± 0.00^{c}	73.33 ± 3.33^{bc}	83.33 ± 3.33^{b}	100.00 ± 0.00^{b}
E. aromatica	90.00 ± 5.77^{d}	100.00 ± 0.00^{e}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
P. guineense	83.33 ± 3.33^{d}	93.33 ± 3.33^{de}	100.00 ± 0.00^{c}	100.00 ± 0.00^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is mean ± standard error of three replicates. Values followed by the same alphabet within a column are not significantly (p > 0.05) different from each other using Tukey's Test.

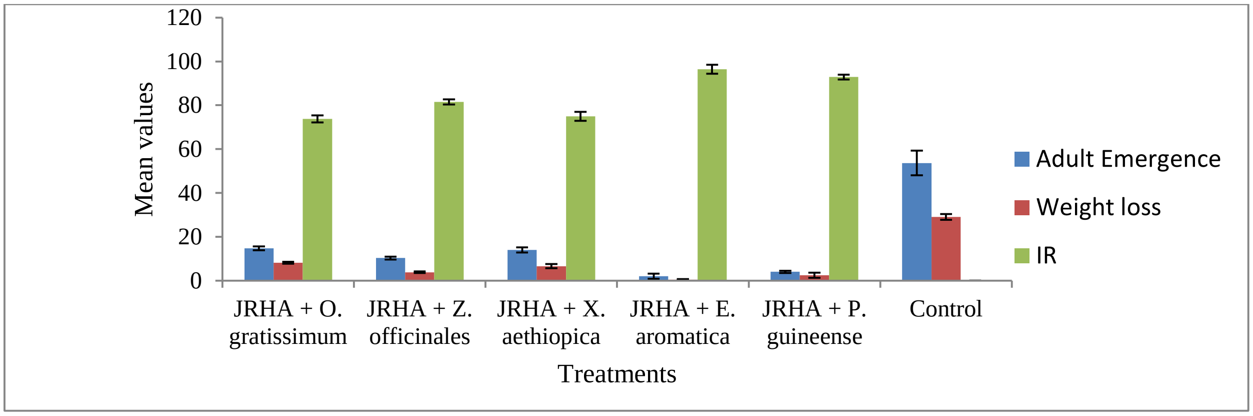

Figure 6: F1 adult emergence, percentage weight loss of the protected maize grain and IR of *S. zeamais* at 0.2 g KRHA in combination with different plant powders

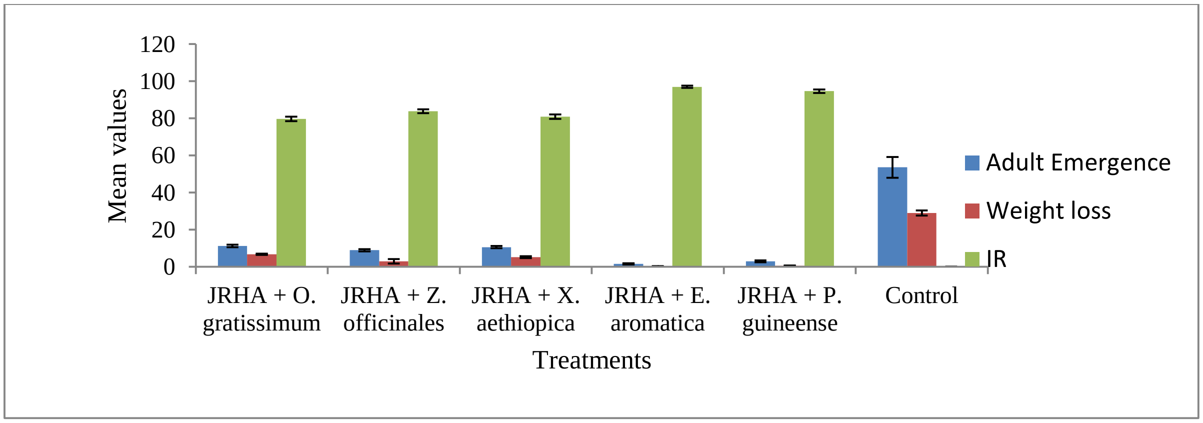

Figure 7: F1 adult emergence, percentage weight loss of the protected maize grain and IR of *S. zeamais* at 0.4 g JRHA in combination with different plant powders

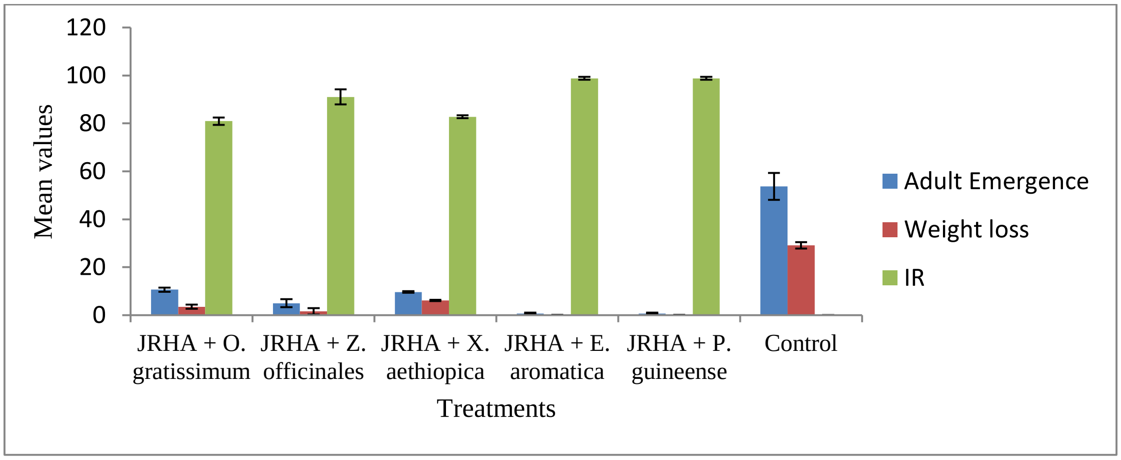

Figure 8: F1 adult emergence, percentage weight loss of the protected maize grain and IR of *S. zeamais* at 0.6 g JRHA in combination with different plant powders

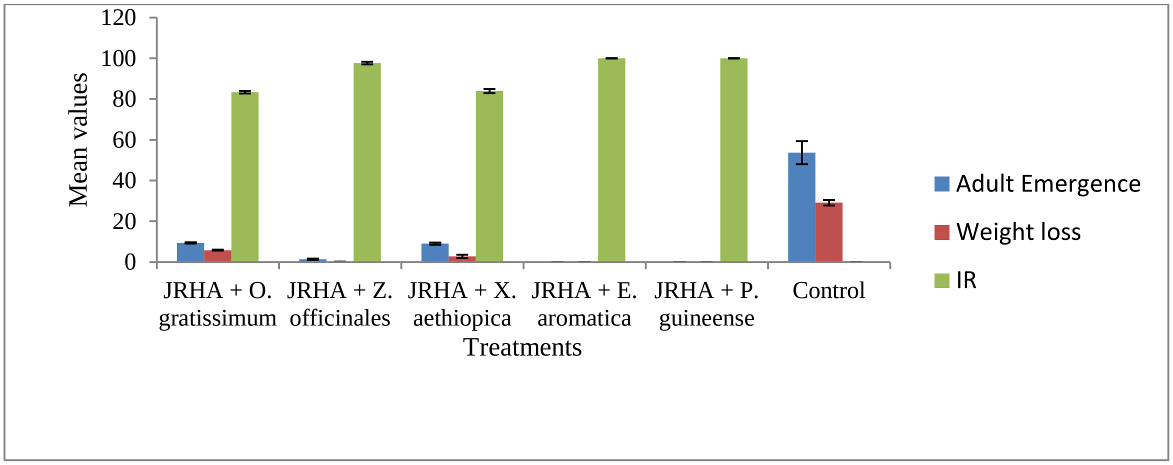

Figure 9: F1 adult emergence, percentage weight loss of the protected maize grain and IR of *S. zeamais* at 0.8 g JRHA in combination with different plant powders

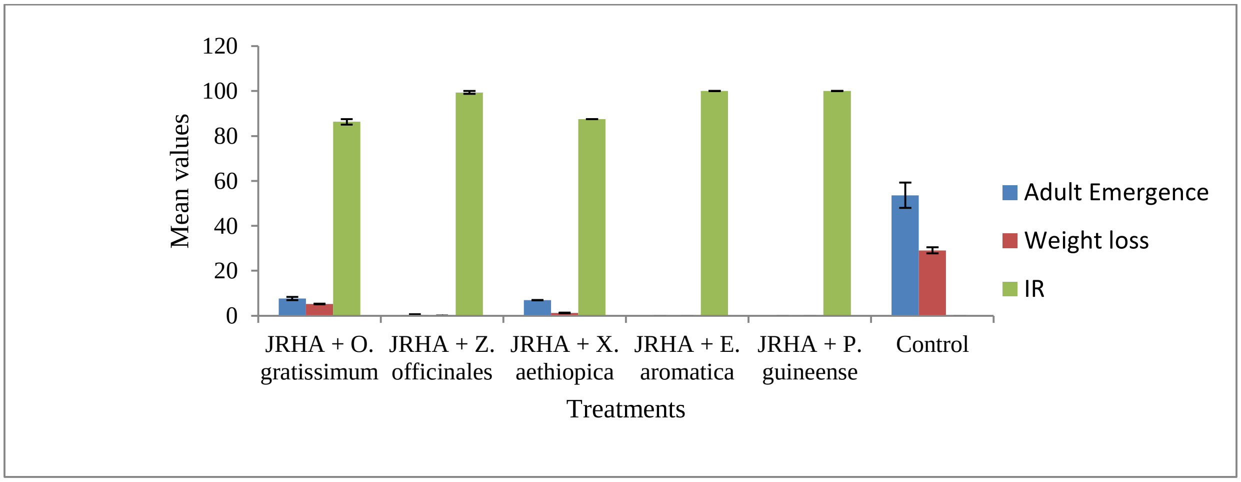

Figure 10: F1 adult emergence, percentage weight loss of the protected maize grain and IR of *S. zeamais* at 1.0 g JRHA in combinatio**n with different plant powders**

Table 12: Summary of regression analysis of mortality data on *S. zeamais* exposed to JRHA in combination with different plant powders within 24 h of observation

Treatment: JRHA +	**Slope ± S.E**	**Intercept ± S.E**	**X^2**	**LD_{50}**	**95%FL**	**Sign**
O. gratissimum	1.11 ± 0.15	-1.47 ± 0.09	295.88	5.24	4.32-10.03	0.0432
Z. officinale	2.02 ± 0.16	-1.35 ± 0.08	123.82	2.63	2.12 - 3.26	0.321
X. aethiopica	1.77 ± 0.14	-0.74 ± 0.07	58.95	2.67	2.51 - 3.75	0.006
E. aromatica	2.17 ± 0.14	-0.67 ± 0.07	58.33	0.33	0.30 - 0.40	0.0001
P. guineense	2.22 ± 0.15	-0.44 ± 0.07	49.38	0.56	0.45 - 1.37	0.0001

LD_{50} = lethal dosage; SE = standard error; X^2= Chi-square; FL=Fiducial limits.

Discussion

The results of this study showed that all the binary combinations of RHA with some other insecticidal plant powders significantly protected the infested grain against damage by C. *maculatus* and *S. zeamais* when compared with the unprotected grain. The protection of the grain by these mixtures from damage by *C. maculatus* may be explained by the significant lethality to the adult beetles, reduced egg-laying by female beetles and reduced adult emergence in the first filial generation. With *S. zeamais*, toxicity to adults and the reduced adult emergence in the first filial generation may explain the protection of the grain by these mixtures. The overall effect was that protected grain had significantly lower loss in weight caused by the beetles. Efficacy of binary mixtures of plant materials for stored grain protection has been subjected to empirical verification by some other workers (Ogunwolu and Idowu, 1994; Dawodu and Ofuya, 2000; Emeasor *et al*., 2007).The protection of stored grain against F1 adult emergency production was likely caused by toxicity of the treatments to eggs. This is in agreement with Ofuya *et al*., (2015) who found that binary mixture of *Piper guineense* seed powder and Diatomaceous Earth significantly reduced oviposition and F1 adult emergency caused by *C. maculatus* on cowpea in this study, it appears that the insecticidal activity of each material against the test insects, was not mitigated by mixing them together. In other words, the materials did not appear to be antagonistic in insecticidal action against each other. A similar conclusion was reached by Ogunwolu and Idowu (1994) in a binary mixture comprising *Azadirachta indica* seed powder and *Zanthoxyles zanthoxyloides* root bark powder against *C. maculatus*. However, synergistic or additive effects would be desirable to greatly enhance efficacy of binary botanical mixtures. Agona and Muyinza (2003) indicated that when some botanicals are combined as binary formulations, the biological activities against *Acanthoscelides obtectus* (Say) on stored beans is synergistically enhanced. Synergistic bioefficacy of binary formulation of *Chenopodium ambrosioides* (L.) with *Datura stramonium* (L.) and *Jatropha curcas* (L.) with *Schinusmolle* (L.) against the bruchid, *Zabrotes subfasciatus* (Say) has also been reported (Tamiru *et al*., 2016).Ileke *et al.*, (2016) similarly reported synergetic effect of a mixture of *Myrcianthes fragrans* and *Aframomum melegueta* powders in inflicting high mortality to adults of *S. zeamais*. Furthermore, a mixture of 75% *Plectranthus glandulosus* leaf powder and 25% *Hymenocardia acida* wood ash produced synergistic effect against *S. zeamais* (Goudoungou *et al*., 2018). The data and experimental design in this study does not give clear indication of either synergism or additivity. However, the results are somewhat consistent with findings of Agona and Muyinza (2016) and Ileke *et al*., (2016) but conflict with Goudoungou *et al*., (2018) in which the binary mixture of 50:50 had antagonistic effect on weevil mortality.

The insecticidal activities of plant materials are linked to the gamut of debilitating bioactive chemical compounds they contain (Dales, 1996; Boulogue *et al*., 2012). The main insecticidal active material in RHA is silica (Naito, 1999), whilst that of EAP are eugenol and caryophyllene (Akinneye *et al*., 2019). The favourable interactions of these bioactive compounds against the storage beetles may have produced the lethality as well as other adverse effects observed. Ileke *et al.* (2016) reached a similar conclusion in the synergetic effects of the formulation containing *M. fragrans* and *A. melegueta* against *S. zeamais*. A major advantage in using a combination of insecticidal plants in a formulation is the presumption that it may be more difficult for insects to develop tolerance or resistance to them (Lale, 2002).

The effective rates of insecticidal plant powders that has been reported by many workers range generally from less than 1 g/kg of grain to 20 g/kg of grain; the amount of powder not constituting more than 2% of the weight of grain (Lale, 1995). The 0.2 g and 0.4 g doses included in this study fall within the range. At these two dosages, the combination of Jemila RHA with *E. Aromatic* powder in equal proportion proved superior to other similar combinations of the RHA with other insecticidal plant powders. The superiority was in terms of higher mortality of adult beetles and reduced adult emergence in the first filial generation, which translated into reduced weevil perforation and weight loss in protected grain. In the specific case of *C. maculatus*, egg-laying by the female beetles was more significantly reduced with Jemila RHA. Correspondingly, the computed lethal dose (LD_{50}) of the mixture containing JRHA and EAP was lowest for both beetles. Many African farmers undoubtedly use mixtures of herbs for storage protection against insects (Obeng-Ofori, 2010) putatively to enhance action. The results of this study has provided substantive additional evidence to justify the use of admixture of herbs for stored grain protection against storage beetle pests. The JRHA and *E. aromatica* mixture is unequivocally a candidate for consideration for recommendation for use in grain protection against insect pest depredation. Further studies are however required to determine its potency against other storage insect pests, its efficacy in comparison with synthetic insecticidal dusts; its shelf-life and effect on germination potential of treated grain; and its effect on the organoleptic properties of treated grain. It may also be curious to investigate the biochemical changes in the insects exposed to the formulation.

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