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Lethality of single, binary and ternary combinations of rice husk ash, groundnut pod shell ash and *Eugenia aromatica* Baill powder to adults of *Callosobruchus* species attacking stored grain legumes

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Abstract

The study investigated the lethality of powders made from rice husk ash, dry flower buds of *Eugenia aromatica* (L.) Baill. and groundnut pod shell ash, to adults of C*allosobruchus* maculatus Fabricius and *C. chinensis* Linnaeus at 25 ° C and 65% relative humidity. The powders were tested singly, and in binary, and ternary combinations at the dosages of 0.05, 0.1 and 0.2 g/10 g of grain in glass Petri dishes. The powder combinations were mixed in equal proportions. There was a control involving no powder. Adult mortality of 20 individuals was observed 24, 48 and 72 h post treatment. Data analysis indicated significant differences among treatment means. Adult mortality increased with increase in dosage of powders and period of exposure. When applied singly the three insecticidal powders showed decreasing lethality to the adults beetles in this order *E. aromatica* > rice husk ash > groundnut pod shell ash. In the binary and ternary combinations with equal proportion of materials, the less toxic powders were significantly enhanced in lethality. The use of the binary and ternary combinations may be favoured for use in mitigating *Callosobruchus* damage to stored grain legumes for reasons of affordability, sustainability and durability.

Keywords: Rice Husk Ash; Groundnut Pod Shell Ash; *Eugenia aromatica*; Lethality; *Callosobruchus*

1. Introduction

Seed feeding beetles in the genus *Callosobruchus* (Coleoptera: Chrysomelidae: Bruchinae) are putatively the most damaging insect pests of stored grain legumes in many parts of the tropics and subtropics [1]. Infestation normally begins from the field with females laying eggs on maturing pods and upon eclosion the first instar larvae enter the seeds feeding and developing to metamorphose into adults. Harvested legume seeds often have developing beetle larvae that emerge as adults in the stores. Being multivoltine, many generations of the beetle in stores can completely ravage legume seeds. *C. maculatus* Fabricius and *C. chinensis* Linnaeus are probably the most damaging species. *C. maculatus* is cosmopolitan throughout the tropics and subtropics whereas *C. chinensis* is widespread in Asia.

Bruchid infestations of legumes in commercial quantities is principally controlled by application of synthetic insecticides as fumigants or dusts in Nigeria and many other tropical and subtropical countries of the world and is very effective. However, concern for the environment, and many other drawbacks associated with the use of synthetic pesticides for pest control [2], is restricting the use of these hazardous chemicals worldwide. Rural farmers and other grain handlers in Nigeria traditionally use plant products to protect stored grain against insect depredation [3], but the grains still manifest varying degrees of insect damage as observed in stores and markets [4]. Plants are undoubtedly endowed with myriad of chemical compounds which are used to reduce colonization by phytophagous insects [5] which

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may be usefully exploited for insect pest control. The general opinion is that plant derived insecticides may be biodegradable; less hazardous to man, animals and natural enemies; cheap and can easily be produced by skilled and unskilled farmers and stakeholders [6]. Therefore, many scientists in Nigeria and many other countries have conducted research over many decades aimed at identifying botanicals that may replace the synthetic insecticides [7, 8]. Botanical materials have been investigated as whole plant parts, pulverized material or powder, ash, crude solvent extracts; vegetable oils, essential oils and volatile oils [8]. Single plant species powders have been investigated by many workers as reviewed by Boeke et al. [8] and Ashamo [5]. The major constraints include relatively low efficacy and higher application rates amongst others [9]. In traditional medicine in Nigeria, effective concoctions for mitigating human maladies usually contain many products from different medicinal plants [10]. Farmers in some parts of Nigeria also use mixtures of herbs for stored grain protection [11] putatively to enhance action. Efficacy of binary and ternary mixtures of botanical powders for stored grain protection has not been adequately addressed by researchers in Nigeria. In this paper, the results of a study of lethality of single, binary and ternary combinations of rice husk ash, groundnut pod shell ash and *Eugenia aromatica* Baill dry flower bud powder to adults of the two *Callosobruchus* species attacking stored grain legumes are reported.

2. Material and methods

The study was carried out at the Federal Research Centre for Cultivated Plants (Julius Kuehn Institute) (JKI), Institute of Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany under controlled conditions of 25° C and 65% relative humidity in chambers.

2.1. Insects

Cultures of *C. maculatus* and *C. chinensis* are maintained at Institute of Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany using standard procedures [12]. These cultures served as source of adults used for this study on blackeye cowpea.

2.2. Rice Husk Ash (RHA) and Groundnut Pod Shell Ash (GPSA)

RHA was produced from the Jemila variety of rice grown in Kaduna State, Nigeria (10.3333° N, 7.7500° E), and has been reported to be insecticidal to *C. maculatus* [13]. GPSA was produced from groundnut pod shell obtained from traders in Oba Market in Akure, Nigeria. The rice husk and groundnut pod shell were first pulverized in an electric blender into coarse powder which was thereafter converted to ash material in electric oven at 550° C for three hours [14]. The RHA and GPSA was separately pulverized in a laboratory mill into a fine powder with particle size of ≤ 150 µm using a British standard sieve [15]. The ash powders (approximately 100 g for each material) was then put in separate plastic containers with tight fitted lids.

2.3. Eugenia aromatica Powder (EAP)

Dry flower buds of *E. aromatica* were purchased from the Central Spices Market in Kaduna, Nigeria (10.3333° N, 7.7500° E). Identity of the plant material was confirmed at Obafemi Awolowo University Herbarium, Ile-Ife, Nigeria. The dry flower buds were further oven dried at 80 °C for a period of 24 h and thereafter pulverized in a laboratory mill and sieved to powder of particle size of ≤ 150 µm using British standard sieve [15]. EAP was stored in a plastic container with tight fitted lid and used within six months of preparation.

2.4. Effect of Single, Binary and Ternary Application of Botanicals on Mortality of Adult Beetles

The lethality of single, binary and ternary combinations of RHA, GPSA and EAP was tested at 0.05, 0.1 and 0.2 g per 10.g of cowpea seeds in glass Petri dishes (5.0 cm diameter). Twenty unsexed adults of *C. maculatus* and *C. chinensis* (< 2 days old) were introduced into a dish containing a dose of material and shaken to ensure contact with the insects. The binary and ternary combinations had equal proportions of the materials. There was a control treatment with no plant material. The experimental design was the completely randomized design. Adult mortality was observed 24, 48 and 72 h post-treatment. The experiment was replicated thrice.

2.5. Data Analysis

Mortality data was corrected as recommended by Abbott [16] and the calculated percentage mortality data subjected to one-way analysis of variance (ANOVA). Where the ANOVA indicated significant difference between treatments, Fisher's least significant difference method was used to separate means at 5% level of probability.

3. Results

Mortality of *C. maculatus* adults exposed to single, binary and ternary combinations of Rice husk ash (RHA), Groundnut pod shell ash (GPSA) and *E. aromatica* dry flowers buds powder (EAP) varied significantly with rate of application and period of exposure (Table 1). At 24 h post treatment, mortality increased significantly with increase in dosage of plant powders; EAP causing the highest mortality of 85%, followed by the ternary combination (RHA + EAP + GPSA) of 80% at the dosage of 0.05g/10 g of grain. GPSA produced the lowest mortality of 6.7%. At the dosage rate of 0.1 g/10 g of grain, EAP and RHA + EAP + GPSA treatments caused 100% mortality of the exposed adults. At the dosage rate of 0.2 g/10 g of grain, EAP, RHA + EAP and RHA + EAP + GPSA treatments caused 100% mortality of the exposed adults. At 48 h post treatment all treatments produced 100% mortality of *C. maculatus* adults except RHA, GPSA, RHA +EAP, RHA + GPSA, EAP + GPSA applied at 0.05 g, and GPSA and RHA + GPSA applied at 0.1 g and GPSA applied at 0.2 g. At 72 h post treatment all treatments produced 100% mortality of *C. maculatus* adults except GPSA, RHA + GPSA and EAP + GPSA applied at 0.05 g, and GPSA applied at 0.1 g and 0.2 g.

Table 1 Mean percentage mortality of *C. maculatus* adults exposed to single, binary and ternary combinations of Rice husk ash (RHA), Groundnut pod shell ash (GPSA) and *E. aromatica* (EAP) powders applied at different rates overtime

Rate: g/10 g of grain	Plant powder (s)	Computed % Abbott mortality in:		
		24 h	48 h	72 h
0.05	RHA	20.0	73.3	100.0
	EAP	85.0	100.0	100.0
	GPSA	6.70	18.3	31.7
	RHA + EAP	55.0	86.7	100.0
	RHA + GPSA	31.7	60.0	80.0
	EAP + GPSA	25.0	71.7	85.0
	RHA + EAP + GPSA	80.0	100.0	100.0
0.1	RHA	51.7	100.0	100.0
	EAP	100.0	100.0	100.0
	GPSA	16.7	35.0	45.0
	RHA + EAP	91.7	100.0	100.0
	RHA + GPSA	55.0	81.7	100.0
	EAP + GPSA	78.3	100.0	100.0
	RHA + EAP + GPSA	100.0	100.0	100.0
0.2	RHA	68.3	100.0	100.0
	EAP	100.0	100.0	100.0
	GPSA	31.7	55.0	75.0
	RHA + EAP	100.0	100.0	100.0
	RHA + GPSA	71.7	100.0	100.0
	EAP + GPSA	71.7	100.0	100.0
	RHA + EAP + GPSA	100.0	100.0	100.0
LSD (0.05)		0.00	0.00	0.00

RHA = Rice husk ash; EAP = *Eugenia aromatica* powder; GPSA = Groundnut pod shell ash

Mortality of *C. chinensis* adults exposed to single, binary and ternary combinations of Rice husk as (RHA), Groundnut pod shell ash (GPSA) and *E. aromatica* dry flowers buds powder (EAP) varied significantly with rate of application and period of exposure (Table 2), following the pattern observed for *C. maculatus*. At 24 h post treatment, mortality

increased significantly with increase in dosage of plant powders; EAP and the ternary combination (RHA + EAP + GPSA) causing mortality of 100% at the dosage of 0.05g/10 g of grain. GPSA produced the lowest mortality of 20.0%. At the dosage rate of 0.1 and 0.2 g/10 g of grain, EAP, RHA + EAP, EAP + GPSA and RHA + EAP + GPSA treatments caused 100% mortality of the exposed adults. At 48 h post treatment all treatments produced 100% mortality of *C. chinensis* adults except RHA, GPSA, RHA + GPSA, EAP + GPSA applied at 0.05 g, and GPSA and RHA + GPSA applied at 0.1 g and GPSA applied at 0.2 g. At 72 h post treatment all treatments produced 100% mortality of *C. chinensis* adults except GPSA, RHA + GPSA and EAP + GPSA applied at 0.05 g, and GPSA and RHA + GPSA applied at 0.1 g; and GPSA applied at 0.2 g. At 72 h post treatment all treatments produced 100% mortality of *C. chinensis* adults except GPSA, RHA + GPSA and EAP + GPSA applied at 0.05 g, and GPSA applied at 0.1 g and 0.2 g.

Table 2 Mean percentage mortality of C. chinensis adults exposed to single, binary and ternary combinations of Rice husk ash (RHA, Groundnut pod shell ash (GPSA) and E. aromatica (EAP) powders applied at different rates overtime

Rate: g/10 g of grain	Plant powder (s)	Computed % Abbott mortality in:		
		24 h	48 h	72 h
0.05	RHA	26.7	70.0	100.0
	EAP	100.0	100.0	100.0
	GPSA	20.0	30.0	51.7
	RHA + EAP	86.7	100.0	100.0
	RHA + GPSA	33.3	66.7	86.7
	EAP + GPSA	43.3	75.0	90.0
	RHA + EAP + GPSA	100.0	100.0	100.0
0.1	RHA	73.3	100.0	100.0
	EAP	100.0	100.0	100.0
	GPSA	23.3	48.3	65.0
	RHA + EAP	100.0	100.0	100.0
	RHA + GPSA	63.3	88.3	100.0
	EAP + GPSA	100.0	100.0	100.0
	RHA + EAP + GPSA	100.0	100.0	100.0
0.2	RHA	83.3	100.0	100.0
	EAP	100.0	100.0	100.0
	GPSA	50.0	68.3	81.7
	RHA + EAP	100.0	100.0	100.0
	RHA + GPSA	76.7	100.0	100.0
	EAP + GPSA	100.0	100.0	100.0
	RHA + EAP + GPSA	100.0	100.0	100.0
LSD (0.05)		0.00	0.00	0.00

RHA = Rice husk ash; EAP = *Eugenia aromatica* powder; GPSA = Groundnut pod shell ash

4. Discussion

Mortality of *C. maculatus* and *C. chinensis* adults exposed to single, binary and ternary combinations of Rice husk ash (RHA), Groundnut pod shell ash (GPSA) and *E. aromatica* dry flowers buds powder (EAP) was observed to vary significantly with rate of application and period of exposure. Adult mortality generally increased with increase in dosage of powders and period of exposure. When applied singly the three insecticidal powders showed decreasing lethality to adults of the two beetles in this order *E. aromatica* > rice husk ash > groundnut pod shell ash. In the binary and ternary

combinations of equal proportion of materials, the less toxic powders were significantly enhanced in lethality. For instance, the lethality of RHA and GPSA to the adult beetles was significantly enhanced in binary combination with EAP in comparison with when applied singly, especially within the 24 h exposure period. For *C. maculatus*, at the 0.05 g dosage RHA and GPSA only produced 20.0% and 6.7% mortality, respectively, whereas RHA + EAP and EAP + GPSA produced 55.0% and 25.0% mortality, respectively. Similarly, the lethality of RHA and GPSA to the adult beetles was significantly enhanced in ternary combination with EAP in comparison with when applied singly and in binary combination, especially within the 24 h exposure period and the lower dosages. For instance, with *C. chinensis* at the 0.05 g dosage RHA + EAP and EAP + GPSA caused 86.7% and 43.3% mortality, respectively within 24 h of exposure, whereas RHA + EAP + GPSA produced 100% mortality.

The insecticidal activity of EAP has been attributed mainly to the chemicals eugenol and caryophylene by many workers [17, 5] whilst that of RHA is said to be due to the high silica content [18]. Since GPSA also has an appreciable amount of silica [19], its toxicity to insects may presumably be partly due to the chemical. These chemicals may have interacted favourably in the binary and ternary combinations of RHA, EAP and GPSA, to produce the lethality observed against the adult beetles.

Efficacy of some binary mixtures of botanical powders for protection of stored cowpea against damage by *C. maculatus* has been reported by some other workers [20; 21; 22; 23; 24; 25; 26]. In most of these binary combinations, insecticidal activity of each material was not mitigated by mixing the two against the beetle, but the interaction was not clearly additive or synergistic. Data obtained in this study are generally along this line. However, Tofel et al. [24] did not observe any advantage in binary combinations of NeemAzal (neem seed based powder) and *Plectranthus glandulosus* leaf powder against cowpea and maize weevils since the binary combinations gave similar results as when they were applied singly. Aweke et al. [25] reached a similar conclusion. Nevertheless, cocktail effect and synergistic interaction was observed by Azeez and Pitan [26] in the ternary combination of powders from *Hyptis suaveolens*, *Cymbopogon citratus*, and *Alstonia boonei* against *C. maculatus*. The lethal effect of the ternary combination of RHA, EAP and GPSA on the adult beetles in this study may similarly be suggestive of additive or synergistic interaction especially with the lowest dosage. It is plausible that application at lower dosages may reveal a clearer and more definite pattern of action.

It is unequivocal from the results of this study that there are good prospects in combining insecticidal botanical powders to reduce the amounts needed for the mitigation of insect damage. The relatively large quantity of insecticidal plant material, especially as powders or dusts, required for appreciably reducing depredation by storage insects, has been a major drawback in their use for pest control in large scale grain storage situations [7, 8]. Combining insecticidal plant materials that are cheaply and easily obtainable by reason of relative abundance like rice husk ash or groundnut pod shell ash, with *Eugenia* powder which may be more difficult to obtain in sufficient quantity, will be more affordable and sustainable. Besides, there is the presumption that it may be more difficult for insects to develop resistance or tolerance to botanical mixtures [27], thereby ensuring durability in efficacy.

5. Conclusion

Single, binary and ternary combinations of rice husk ash, groundnut pod shell ash and *Eugenia aromatica* powder have been proven to be sufficiently lethal to adults of *Callosobruchus* species that may warrant their use for mitigating the damage of the beetles to stored grain legumes. The use of the binary and ternary combinations may however be favoured for use for reasons of enhanced efficacy, affordability, sustainability and durability.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interests whatsoever.

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