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Antagonistic, additive and synergistic interactions in the fumigant toxicity of binary mixes of powders of cloves and citrus fruit peels to adults of *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract

The study evaluated the fumigant toxicity of cloves (*Eugenia aromatica* Baill.) and citrus peels (*Citrus* spp.) powders applied singly and binary combinations (1:1 ratio) to the cowpea storage beetle, *Callosobruchus maculatus* Fabricius under ambient laboratory conditions (28 ± 4°C and 75 ± 10% rh). The powders (singles and mixtures), placed in empty tea bags, were tested at the dosages of 0.0 (control), 0.5, 1.0 1.5 and 2.0 g/20 g of grain in plastic bottles with airtight lids against freshly emerged adult beetles. Adult mortality was observed 24, 48, and 72 h post fumigation. To determine antagonistic, additive and synergistic interactions, corrected mortality data was used to compute co-toxicity factors. Co-toxicity factor for the binary combinations of powders at different dosages at 24 hours indicated antagonistic interaction, ranging from - 40 to -100. Co-toxicity factor at 48 hours ranged from -30 to -90 indicating mainly antagonistic interaction except for Clove + Tangerine, Clove + Lime and Clove + Grape applied at 2.0 g/20 g of grain with -6.7, 0.0, and 6.6 respectively, indicative of additive interaction. Co-toxicity factor at the 0.5 g/20 g of grain dosage at 72 hours ranged from -53.3 to -56.6 indicating antagonistic interaction; at the 1.0 and 1.5 g/20 g ranged from -20 to 6.7 indicating additive interaction; and at the 2.0 g/20 g was 19.7 for Clove and Sweet orange mixture also indicating additive interaction, but 40, 46.7 and 46.7 for Clove and Grape, Clove and Tangerine, and Clove and Lime mixtures, respectively, indicating synergistic interactions.

Keywords: Plant powder mixes; Interaction; *Callosobruchus maculatus*; Adult mortality; Cowpea

1. Introduction

Callosobruchus maculatus Fabricius is considered to be one of the most important storage pest of stored legume seeds in the tropics and subtropics [1, 2]. Its principal legume host in Nigeria is cowpea, *Vigna unguiculata* (L.) Walpers [3], hence the beetle is often referred to as cowpea seed beetle, cowpea storage beetle, cowpea bruchid or cowpea weevil. Damage is initiated by female beetles laying eggs on maturing cowpea pods in the field or on cowpea seeds in store. First instar larvae, upon hatching from eggs, bore into the seeds feeding on the seed resources until pupation. Adult beetles emerge from exit holes demarcated by the last larval instar. As a result of larval feeding, damaged seeds have reduced weight and may suffer embryo damage leading to loss of viability. Damaged seeds are unsuitable for human consumption, and cannot be used for agricultural and commercial purposes [4].

C. maculatus infestation and damage to stored legume seeds is most effectively controlled by application of synthetic chemical insecticides mainly as dusts such as pirimiphos-methyl and permethrin or as fumigants such as aluminium phosphide [5]. However, synthetic insecticides may cause many environmental and health problems such as pollution, diseases and resistance in pests [6]. Besides, the majority of farmers in many developing countries in the tropics and subtropics are resource poor and have neither the means nor the skills to obtain and handle these synthetic chemicals

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appropriately [7]. Imperatively, an environmentally safe and economically feasible pest control practice is needed to replace the hazardous synthetic chemicals. For a few decades, researchers have screened materials from medicinal plants to possibly replace the synthetic pesticides for the control of insect pests of both field and stored crops. Plant-derived insecticidal materials are relatively cheaper and are generally assumed to be more biodegradable, leading to less environmental problems [7]. The efficacy of powder derived from cloves (the dry flower buds of *Eugenia aromatica* Baill.) as well as *Citrus* peels to mitigate damage by *C. maculatus* has been reported by many workers [8, 9]. Inadequate attention appears to have been given to research involving combination of products from different plants for stored products protection, even though in traditional grain storage systems herbal mixes are sometimes used [10]. This paper reports on antagonistic, additive and synergistic interactions in the fumigant toxicity of binary mixes of powders of cloves and citrus fruit peels to adults of *C. maculatus*.

2. Materials and Methods

The study was conducted under ambient laboratory conditions using *Callosobruchus maculatus* maintained in Entomology Unit of Crop, Soil and Pest Management Department of The Federal University of Technology, Akure, Nigeria. The *C. maculatus* cultures are recycled monthly by placing about 100 beetles from old cultures onto 400 g clean cowpea seeds in clear plastic containers with meshed lids for fresh progeny production. Clean Ife Brown cowpea seeds (a well-known bruchid susceptible variety) was used in this study. Dry flower buds of *Eugenia aromatica* Baill. (clove) was purchased from Herbal shop in Akure, and further air dried and pulverized in a laboratory mill and sieved to particle size of 300 µm. Fruit peels of sweet orange (*Citrus sinensis* (L.) Osbeck), grape (*Citrus paradisi* Macfad.) lemon (*Citrus limon* (L.) Osbeck) tangerine (*Citrus tangerine* Tanaka) were collected from fruit traders in Akure, air dried and similarly pulverized in a laboratory mill and sieved to particle size of 300 µm. The powders were kept in separate plastic containers with tightly fitted lids. The powders were used within six months of preparation and were tested separately and as a mixture (ratio 1:1).

2.1. Fumigant Activity against Adults of *C. maculatus*

The powders (singles and mixtures) were tested at the dosages of 0.5, 1.0, 1.5 and 2.0 g/20 g of grain in plastic bottles with airtight lids. Each dosage of powder/powder mixture was placed in an empty tea bag that allowed escape of toxic powder fumes but no contact with grain or insects. Fumigant toxicity of each dosage of powder (singles and mixtures) was observed against 10 (five males and five females) of newly emerged *C. maculatus* adults introduced to 20 g cowpea seeds in the airtight plastic bottle. Males and females of *C. maculatus* were determined following the description given by Dobie et al. [11] that females often have strong markings on the elytra consisting of two large marginal dark patches mid-way along the elytra and smaller patches at the rest. Freshly emerged females appear longer than males and the elytra do not quite cover the abdomen. The males are much less distinctly marked [12]. There was a control treatment with an empty tea bag placed in the seeds in the plastic bottle. Adult mortality was taken 24, 48, and 72 hours post treatment. The completely randomized experimental design involving three replications was adopted.

2.2. Data Analysis

Adult mortality data was corrected using Abbott [13] formula. The corrected mortality data was used to assess antagonistic, additive and synergistic interactions, by calculating co-toxicity factors using the following equation from Norris and Bloomquist [14]:

Co-toxicity Factor = Observed Mortality − Expected Mortality/Expected Mortality×100

In this equation, observed mortality was the toxicity observed experimentally in combinations of Clove and *Citrus* powders at the various dosages. Expected mortality was the additive sum of the observed mortality for Clove powder alone and each *Citrus* powder alone. Values > 20 represent synergistic mixtures, −20 ≤ values ≤ 20 represent additive mixtures and values < −20 represent mixtures that are antagonistic [14].

3. Results

Values of co-toxicity factor for the binary combinations of powders for different dosages for 24 hours post fumigation ranged from - 40 to -100 (Table 1) (antagonistic). Values of co-toxicity factor for the binary combinations of powders with fumigation at different dosages for 48 hours ranged from -30 to -90 (Table 2) (antagonistic) except for Clove + Tangerine, Clove + Lime and Clove + Grape applied at 2.0 g/20 g of grain with -6.7, 0.0, and 6.6 respectively (additive).

Values of co-toxicity factor for the binary combinations of powders with fumigation at the 0.5 g/20 g of grain dosage for 72 hours ranged from -53.3 to -56.6 (Table 3) (antagonistic). Values of co-toxicity factor for the binary combinations of powders with fumigation at the 1.0 and 1.5 g/20 g of grain dosage for 72 hours ranged from -20 to 6.7 (additive). Values of co-toxicity factor for the binary combinations of powders with fumigation at the 2.0 g/20 g of grain dosage for 72 hours was 19.7 for Clove and Sweet orange mixture (additive), 40 for Clove and Grape mixture (synergistic) and 46.7 for Clove and Tangerine and Clove and Lime mixtures (synergistic).

Table 1 Computation of Co-toxicity factor for the binary combinations of powders with fumigation at different dosages for 24 hours based on Norris and Bloomquist (2021)

Powder mixture	**Computed Co-toxicity factor with fumigation for 24 hours with:**			
	0.5 g	1.0 g	1.5 g	2.0 g
Clove + Sweet orange	-100	- 76.7	-83.3	-66.7
Clove + Grape	-100	- 76.7	-83.3	-40.0
Clove + Tangerine	-100	- 76.7	-83.3	-70.0
Clove + Lime	-100	- 76.7	-83.3	-70.0

Values > 20 represent synergistic mixtures, −20 ≤ values ≤ 20 represent additive mixtures and values < −20 represent mixtures that are antagonistic.

Table 2 Computation of Co-toxicity factor for the binary combinations of powders with fumigation at different dosages for 48 hours based on Norris and Bloomquist (2021)

Powder mixture	**Computed Co-toxicity factor with fumigation for 48 hours with:**			
	0.5 g	1.0 g	1.5 g	2.0 g
Clove + Sweet orange	-90.0	-36.7	-66.7	-30.0
Clove + Grape	-90.0	-36.7	-66.7	6.6
Clove + Tangerine	-90.0	-36.7	-66.7	-6.7
Clove + Lime	-90.0	-36.7	-56.7	0.0

Values > 20 represent synergistic mixtures, −20 ≤ values ≤ 20 represent additive mixtures and values < −20 represent mixtures that are antagonistic.

Table 3 Computation of Co-toxicity factor for the binary combinations of powders with fumigation at different dosages for 72 hours based on Norris and Bloomquist (2021)

Powder mixture	**Computed Co-toxicity factor with fumigation for 72 hours with:**			
	0.5 g	1.0 g	1.5 g	2.0 g
Clove + Sweet orange	-53.3	-3.3	-13.3	19.7
Clove + Grape	-56.6	-13.3	-20	40.0
Clove + Tangerine	-53.3	-6.6	-6.6	46.7
Clove + Lime	-53.3	6.7	6.7	46.7

Values > 20 represent synergistic mixtures, −20 ≤ values ≤ 20 represent additive mixtures and values < −20 represent mixtures that are antagonistic.

4. Discussion

The different binary combinations of cloves and citrus peels powders produced fumigant effects which may be classified as antagonistic, additive and synergistic interactions [14]. Specifically, values of co-toxicity factor for the binary combinations of clove and citrus peels powders with fumigation at different dosages for 24 hours ranged from - 40 to -100 indicating antagonistic interaction. Values of co-toxicity factor for the binary combinations of powders with fumigation at different dosages for 48 hours ranged from -30 to -90, similarly indicating antagonistic interaction except for Clove + Tangerine, Clove + Lime and Clove + Grape applied at 2.0 g/20 g of grain with -6.7, 0.0, and 6.6 respectively, which indicated additive interaction. Values of co-toxicity factor for the binary combinations of powders with fumigation at the 0.5 g/20 g of grain dosage for 72 hours ranged from -53.3 to -56.6 also indicating antagonistic interaction. Co-toxicity factor values for the binary combinations of powders with fumigation at the 1.0 and 1.5 g/20 g of grain dosage for 72 hours ranged from -20 to 6.7, indicative of additive interaction. Co-toxicity factor values for the binary combinations of powders with fumigation at the 2.0 g/20 g of grain dosage for 72 hours was 19.7 for Clove and Sweet orange mixture (additive interaction), 40 for Clove and Grape mixture (synergistic interaction) and 46.7 for Clove and Tangerine and Clove and Lime mixtures (synergistic interaction). Clearly, majority of the binary combinations of cloves and citrus peel powders acted antagonistically as regards fumigant toxicity to adults of *C. maculatus*. Khorrami et al. [15] similarly reported that combination of plant powders elicited antagonistic effects to *Tribolium castaneum* Herbst. In a review quantifying synergy by considering toxicity studies of binary combinations of pesticides within environmental toxicology, Cedergreen [16] observed that synergy occurred in 70%, 3% and 26% of the 194, 21 and 136 binary pesticides, metal and antifoulants mixtures included in the data compilation on frequency. Cedergreen [16] concluded that true synergistic interaction between chemicals are rare and often occur at high concentrations. This has been corroborated by the results of this study. Of the forty eight (48) binary combinations of cloves and citrus peels powders tested in this study only three (3): Clove and Grape, Clove and Tangerine, and Clove and Lime applied at the highest dosage of 2.0 g/20 g of grain was synergistic and it occurred after 72 hours of fumigation. Presumably, exposure time may also have influence on the expression of synergistic interaction in the efficacy of binary combinations of pesticides against insects. In their study on effectiveness of binary combinations of *Plectranthus glandulosus* leaf powder and *Hymenocardi acida* wood ash against *Sitophilus zeamais*, the mixture of 75% *P. glandulosus* and 25% *H. acida* produced synergistic effect, whereas the mixture of 50:50 had antagonistic effect in weevil mortality [7]. Putatively, varying the proportional composition of materials in a binary combination may further produce different interactions with regards to toxicity. This may be subject for further investigation with cloves and citrus peels powders mixes.

The combination of insecticidal materials has the advantages of increased efficacy by complementing the bio-efficacy of the individual products and simultaneously lowering their doses [7]. Miresmailli and Isman [17] also opined that the use of mixtures of plant derived insecticidal materials rather than single material interferes with the development of resistance by a pest, since it becomes entangled with detoxifying a more complex agglomeration of chemical compounds. Additionally, a mixture may target more than a single site of action, acting on both physiological and behavioral parameters thereby broadening the spectum of activity and similarly reducing the chance of resistance development [7, 18]. *C. maculatus* has been demonstrated as capable of developing resistance to Clove powder over many generations [19]. Deploying a formulation combining clove and citrus peels powders may presumably delay the development of resistance or tolerance by *C. maculatus*. Therefore, the contemporary idea of utilizing synergistic mixtures of different botanical formulations should putatively enhance effectiveness of the botanical pesticides in order to mitigate insect damage to crops and needs further exploitation.

5. Conclusion

The binary combinations of cloves and citrus peels powders provoked adult mortality of *C. maculatus* through fumigant action. The fumigant effects may be classified as mostly antagonistic, less of additive and much less of synergistic interactions. The contemporary idea of utilizing synergistic mixtures of different botanical formulations should be further explored and exploited to enhance effectiveness of the botanical pesticides in mitigating insect damage to stored grain.

Compliance with ethical standards

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This work forms part of a dissertation submitted by the first author to the School of Postgraduate Studies, The Federal University of Technology, Akure, Nigeria, for the award of degree of Master in Pest Management.

Disclosure of conflict of interests

No conflict of interests whatsoever.

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