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Efficacy of binary combinations of powders from selected botanicals against *Callosobruchus maculatus* Fabricius damage in stored cowpea seeds

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Abstract

The efficacy of powder mixes of two different insecticidal plants against *Callosobruchus maculatus* Fabricius was determined at the Entomology Research Laboratory, Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Ondo State, Nigeria, under ambient laboratory conditions of 26 ± 4° C and 65 ± 10% RH. The powder combinations were made from *Azadirachta indica* leaf *Thaumatococcus daniellii* rhizome *Eugenia aromatica* dry flower buds, seeds of *Piper guineense* and rhizome of Zingiber officinale in a 1:1 ratio. The binary combinations were assessed at the dosages of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 g per 20 g of cowpea seeds in separate 250 ml plastic containers with lids, against five pairs of newly emerged *C. maculatus* adults. Insect mortality was recorded 24, 48 and 72 hours post treatment and the number of F_1 adult emergence was recorded after up to 35 days post treatment. The combination of *E. aromatica* and *T. daniellii*, and *E. aromatica* and *Z. officinale* produced 60% and 53% mortality respectively, of adults of *C. maculatus* at the lowest tested dosage (0.2 g/20 g of grain), and 100% and 83% mortality respectively, at the highest tested dosage (2.0 g/20 g of grain). Oviposition and progeny production of *C. maculatus* were also significantly lower in treatments involving the use of these two combinations; significantly fewer seeds bore beetle eggs and adult exit holes. The combination of *E. aromatica* and *T. daniellii*, and *E. aromatica* and *Z. officinale* may be included in pest management strategies for mitigating *C. maculatus* damage to stored cowpea.

Keywords: Binary; Plant powder; *Callosobruchus maculatus*; Cowpea damage

1. Introduction

The cowpea storage beetle, *Callosobruchus maculatus* Fabricius is generally considered as the most damaging insect pest of stored cowpea (*Vigna unguiculata* (L.) Walp.), an important source of dietary protein in Nigeria and many other tropical and subtropical parts of the world [1]. Damaged seeds are defaced with beetle egg covers, riddled with adult exit holes leading to reduced weight and poor seed germinating capacity. Severely damaged seeds are unfit for human consumption being reduced to powdery mass which often becomes mouldy. The control of *C. maculatus* has been based primarily on the use of synthetic insecticides with either contact or fumigant action, and is quite effective [2]. Over the years, however, use of synthetic insecticides for pest control has been accompanied by adverse effects such as toxicity to non-target organisms including man and development of pest tolerance or resistance [3]. The increasing concern over the negative effects of synthetic chemicals on the environment and human health has forced researchers to look for alternatives to them. Plants are endowed with myriad of chemical compounds which can be usefully exploited for plant protection purposes [4]. Therefore, in the past few decades, scientists have focused on botanicals as likely source of materials that could replace the hazardous synthetic chemicals. Plant derived insecticidal materials are thought to be safer and more environment friendly than synthetic chemicals [2, 5]. Traditionally, grain producers also use plant materials to mitigate insect damage to stored grain [6]. Efficacy of the use of plant materials in the mitigation of insect damage to stored grain has been subjected to empirical verification by many workers, but with varying degrees of

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success [7, 8]. Products from single plant species have been involved in the efficacy studies against storage insect pests, but in traditional human medicine, and sometimes in grain storage, a mixture of herbs is deployed against pest organisms [9, 10]. There appears to be inadequate research reports verifying the efficacy of herbal mixtures in the protection of stored grain against insect depredation. The efficacy of ten binary combinations of powders from selected insecticidal plants against *C. maculatus* damage in stored cowpea seeds is therefore, reported in this paper.

2. Material and methods

The experiment was carried out at the Entomology Research Laboratory, Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure, Ondo State. Nigeria, under ambient laboratory conditions, 26 ± 4°C and 65 ±10% relative humidity.

2.1. Collection and Preparation of plant powders

The plant parts, *Azadirachta indica* A, Juss. leaves and *Thaumatococcus daniellii* (Benn.) Benth. rhizomes used for the study were collected from the Teaching and Research Farm and Forestry Unit of the Federal University of Technology, Akure, Ondo State, Nigeria while *Eugenia aromatica* (L.) Baill. dry flower buds, seeds of *Piper guineense* Schum. & Thonn. and rhizomes of *Zingiber officinale* Roscoe, were purchased from local herbal sellers in Oba market, Akure, Ondo state, Nigeria. The collected plant parts were air dried in the laboratory for four (4) weeks until a moisture content of 10-12% was achieved and thereafter, milled into powder using grinding machine with GX200 petrol engine - 6.5 HP and later into finer particles using electric blender (Marlex Excella). The powders were further sieved to 300 μm particle size and stored in separate airtight 250 ml plastic containers with lids. The powders were used within six months of preparation.

2.2. Testing powder mixtures against *C. maculatus* infestation of cowpea seeds

The prepared plant powders were permutated to form ten (10) mixtures at ratio 1:1, comprising *E. aromatica* + *Z. officinale* (EAP + ZOP); *E. aromatica* + *A. indica* (EAP + AIP); *E. aromatica* + *P. guineense* (EAP + PGP); *E. aromatica* + *T. daniellii* (EAP + TDP); *Z. officinale* + *A. indica* (ZOP + AIP); *Z. officinale* + *P. guineense* (ZOP + PGP); *Z. officinale* + *T. daniellii* (ZOP + TDP); *A. indica* + *P. guineense* (AIP + PGP); *A. indica* + *T. daniellii* (AIP + TDP); and *P. guineense* + *T. daniellii* (PGP + TDP).

The mixtures were tested at various doses of 0.2, 0.4, 0.6, 0.8, 1.0 and 2 g per 20 g of cowpea seeds against 10 newly emerged *C. maculatus* adults (5 males and five females) in 250 ml plastic containers. There was a control treatment having no powder. All treatments were replicated three times, including the control. The experimental design was the completely randomized design. Mortality of introduced insects was observed 72 hours post treatment. Total number of eggs laid on the seeds was counted 14 days post-introduction of the adult insects at which time they had died and sieved out. Number of seeds with and without eggs was noted. Progeny production (F_1 adults) from infested cowpea seeds subjected to the various treatments was recorded up to 38 days post-treatment. Number of seeds with and without adult exit holes was noted.

2.3. Data analysis

Percentage mortality data was corrected using Abbott's formula [11]. Data based on counts were reduced to their square-roots and percentages were subjected to arcsine transformation. The transformed data were subjected to analysis of variance and Fishers Least Significant Difference value was used to separate means at the 5% level of significance.

3. Results

There were significant differences ($P < 0.05$) amongst the binary combinations of the different plant powders with respect to mortality of *C. maculatus* adults, with each dosage 72 hours post treatment (Table 1). Beetle mortality generally increased with increase in dosage. The binary combination of *E. aromatica* and *T. daniellii* rhizome powders (EAP + TDP) caused significantly higher beetle mortality at each rate of application in comparison to others and produced 100% beetle mortality at the 2.0 g application rate within 72 hours post treatment. This was followed by the binary combinations of *E. aromatica* and *Z. offficinale* (EAP + ZOP) (83.3%) and *E. aromatica* and *A. indica* (EAP + AIP) (80.0%). There were no significant differences in the mortality caused by the binary combinations of *Z. offficinale* and *T. daniellii* rhizome (ZOP + TDP), *Z. offficinale* and *A. indica* leaf (ZOP + AIP), *P. guineense* and *A. indica* leaf (PGP + AIP), *A.indica* leaf and *T. daniellii* rhizome (AIP + TDP) and *P. guineense* and *T. daniellii* rhizome (PGP + TDP).

The number of eggs laid on the seeds treated with binary combination of *E. aromatica* and *T. daniellii* rhizome (EAP + TDP) (9.6) was significantly lower than in other treatments (Table 2). The number of eggs laid on treatments involving combination of *E. aromatica* and *Z. offficinale* (EAP + ZOP) (13.6) and *E. aromatica* and *A. indica leaf* (EAP + AIP) (13.7) was significantly lower than the remaining treatments. The highest number of eggs was laid in the control. Progeny production followed the same trend as oviposition. The lowest number of progeny was recorded in seeds treated with EAP + TDP, while the highest value was recorded in the control.

Significantly fewer seeds were found with eggs or exit holes in the EAP + TDP treatment compared to other treatments and the control while the highest number of seeds without eggs or exit holes was recorded in the same treatment combination (Table 3).

Table 1 Mortality of adults of *C. maculatus* infesting cowpea seeds treated with different rates of mixtures of some plant powders at 72 h post treatment

Powder combination*	**Mean % mortality of *C. maculatus* adults at application rate in g/20 g of seed:**					
	0.2 g	**0.4 g**	**0.6 g**	**0.8 g**	**1.0 g**	**2.0 g**
EAP + ZOP	53.3c	56.7c	60.0cd	73.3cd	76.7c	83.3d
EAP + AIP	50.0c	53.3c	56.7c	66.7c	76.7c	80.0d
EAP + PGP	33.3a	36.7a	36.7a	50.0b	53.3b	73.3c
EAP + TDP	60.0c	66.7d	66.7d	76.7d	93.3d	100.0e
ZOP + AIP	43.3b	43.3a	43.3b	46.7a	46.7a	56.7ab
ZOP + PGP	40.0a	46.7b	46.7b	53.3b	53.3b	60.0b
ZOP + TDP	43.3b	46.7b	46.7b	46.7a	53.3b	53.3ab
PGP + AIP	43.3b	46.7b	46.7b	46.7a	53.3b	56.7ab
AIP + TDP	36.7a	40.0a	40.0ab	46.7a	46.7a	56.7ab
PGP + TDP	33.3a	36.7a	40.0ab	40.0a	43.3a	50.0a

Means followed by different letters are significantly different at the 5% level of probability; *EAP = *Eugenia aromatica* powder; ZOP = *Zingiber officinale* powder; AIP = *Azadirachta indica* powder; PGP = *Piper guineense* powder; TDP = *Thaumatococcus daniellii* powder.

Table 2 Oviposition and progeny production of *C. maculatus* infesting cowpea seeds protected with a mixture of different plant powders

Powder combination*	**Mean number of eggs laid**	**Mean number of progeny produced**
EAP + ZOP	13.6b	8.6b
EAP + AIP	13.7b	8.8b
EAP + PGP	14.4c	8.7b
EAP + TDP	9.6a	8.3a
ZOP + AIP	32.0f	29.9e
ZOP + PGP	32.2f	29.5d
ZOP + TDP	32.0f	28.9c
PGP + AIP	29.6d	29.6d
AIP + TDP	31.6e	29.7e
PGP + TDP	32.0f	29.8e
CONTROL	37.1g	31.5f

Means are square-root transformed values; Means followed by different letters are significantly different at the 5% level of probability; *EAP = *Eugenia aromatica* powder; ZOP = *Zingiber officinale* powder; AIP = *Azadirachta indica* powder; PGP = *Piper guineense* powder; TDP = *Thaumatococcus daniellii* powder.

Table 3 Some seed damage parameters in cowpea treated with mixtures of plant powders applied against *C. maculatus*

Powder combination*	Mean number of seeds with eggs	Mean number of seeds without eggs	Mean number of seeds with holes	Mean number of seeds without holes
EAP + ZOP	4.6a	9.9d	4.7ab	9.9b
EAP + AIP	5.7ab	9.8d	5.9ab	10.0b
EAP + PGP	7.6b	8.2c	7.2b	8.8b
EAP + TDP	2.7a	12.8e	3.2a	12.5c
ZOP + AIP	12.4c	0.9b	12.6c	1.0a
ZOP + PGP	12.3c	0.9b	12.5c	1.0a
ZOP + TDP	12.4c	0.9b	12.7c	1.0a
PGP + AIP	12.3c	0.9b	12.5c	1.0a
AIP + TDP	12.5c	0.9b	12.7c	1.0a
PGP + TDP	12.4c	0.9b	12.5c	1.0a
CONTROL	13.0c	0.7a	12.5c	0.7a

Means are square-root transformed values; Means followed by different letters are significantly different at the 5% level of probability; *EAP = *Eugenia aromatica* powder; ZOP = *Zingiber officinale* powder; AIP = *Azadirachta indica* powder; PGP = *Piper guineense* powder; TDP = *Thaumatococcus daniellii* powder.

4. Discussion

The results of this study has shown unequivocally that binary combinations of some insecticidal plant powders can be used to mitigate infestation and damage to cowpea by *C. maculatus*. The combination of *E. aromatica* and *T. daniellii*, and *E. aromatica* and *Z. officinale* produced 60% and 53% mortality respectively, of adults of *C. maculatus* at the lowest tested dosage (0.2 g/20 g of grain), and 100% and 83% mortality respectively, at the highest tested dosage (2.0 g/20 g of grain). Clearly, beetle mortality increased with increase in dosage of the mixtures. Oviposition and progeny production of *C. maculatus* were also significantly lower in treatments involving the use of these two combinations. This may be explained by the higher adult mortality such that fewer adults could lay eggs to produce progeny. Correspondingly, significantly fewer seeds bore beetle eggs and adult exit holes in treatments involving use of these two combination of plant powders, indicating lower damage and infestation by the beetle.

Efficacy of some binary mixtures of botanical powders for protection of stored grain against damage by storage beetles has been reported by some other workers [12, 13, 14, 15, 16, 17, 18]. In most of these reported binary combinations, insecticidal activity of the materials appeared to be complementary, against the beetles. This may have been corroborated by the recorded activity of the combination of *E. aromatica* and *T. daniellii*, and *E. aromatica* and *Z. officinale* against *C. maculatus* in this study. However, Tofel et al. [17] did not observe any advantage in binary combinations of NeemAzal (neem seed based powder) and *Plectranthus glandulosus* leaf powder against cowpea and maize weevils. Similarly, many of the mixtures used in this study were not efficacious against *C. maculatus* when compared with the untreated control in terms of seeds infestation with beetle eggs and perforations. Aweke et al. [18] did not also observe any difference between single and binary combinations of neem seed and garlic bulb powders as biopesticides against adults of *Sitophilus zeamais* Motschulsky. Single and binary combinations were not compared in this study.

The insecticidal activity of many plants have been attributed to a vast array of their constituent chemical compounds which adversely affect pest insects [4]. For instance, the insecticidal activity of EAP has been attributed mainly to the chemicals eugenol and caryophylene by many workers [19, 8]; TDP, alkaloids, saponins, etc (; AIP, azadirachtin, etc. [8] and ZOP, sesquiterpenes [20]. These chemicals may have interacted favourably in the binary combinations of EAP + TDP, EAP + ZOP and EAP + AIP to produce the mitigation effects observed against *C. maculatus*.

The binary combination of EAP + TDP proved most superior amongst others in protecting cowpea seeds against *C. maculatus* infestation and damage and may be included in pest management strategies for mitigating damage by this pest. Nevertheless, in African traditional medicine, more than two plant products are frequently concocted to resolve human and animal maladies [9]. It may be rewarding to include EAP and TDP in ternary and quaternary mixtures for further investigations aimed at the mitigation of stored products damage by insects. The combination of insecticidal materials has the advantages to increase efficacy by complementing the bio-efficacy of the individual products and simultaneously lowering their doses on the one hand, and broadening the spectrum of activity and reducing the chance of resistance development on the other hand [5]. For instance, cocktail effect and synergistic interaction was observed by Azeez and Pitan [21] in the ternary combination of powders from *Hyptis suaveolens* (L.) Kuntze, *Cymbopogon citratus* (DC.) Stapf and *Alstonia boonei* De Wild. against *C. maculatus*.

5. Conclusion

It can be concluded that the combination of *E. aromatica* and *T. danielli*, and *E. aromatica* and *Z. officinale* powders may be included in pest management strategies for mitigating *C. maculatus* damage to stored cowpea. It is suggested that more studies are carried out on using botanical cocktails against stored products insect pests because use of confirmed efficacious cocktails may be the solution to finding suitable replacements for synthetic chemicals for stored products insect pest management.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest at all.

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