Synergetic effects of two botanical entomocides as pest-protectants in maize grains

Kayode David Ileke,[1] Jacobs Mobolade Adesina,[2,3] Emmanuel Oluwaseun Obajulaye[1]

[1]Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba, Nigeria; [2]Insect Chemical Ecology Laboratory, Institute of Bioresources and Sustainable Development, Imphal, India; [3]Department of Crop, Soil and Pest Management Technology, Rufus Giwa Polytechnic, Owo, Nigeria

Abstract

Sitophilus zeamais Motschulsky infestation causes severe post-harvest losses of cereal grains in Nigeria leading to major nutritional and economic losses. A laboratory study was conducted to determine the efficacy of *Myrcianthes fragrans* and *Aframomum melegueta* seed powders and methanol extract as well as their synergetic effects on *S. zeamais*. The ability of the plant products to protect maize grain against *S. zeamais* infestation was assessed in terms of mortality 24 to 96 h post-treatment, oviposition, and adult emergence, weight loss and grains damage. *M. fragrans* and *A. melegueta* products significantly cause adult mortality of *S. zeamais. M. fragrans* products were the most toxic as it evoked 100% adult mortality within 24 h of application while *A. melegueta* products evoked 100% mortality of *S. zeamais* at 48 and 72 h post treatment, respectively. The synergetic effects of the plant products caused 100% adult mortality at 48 and 72 h post-treatment, respectively. Oviposition, adult emergence, weight loss and damage of the maize grains treated with the plant products were completely inhibited compared with untreated grains. The results obtained revealed that 2 g and 2% sole application of *M. fragrans* and mixed application of *M. fragrans* and *A. melegueta* products were effective in controlling *Sitophilus zeamais* post-harvest infestation and grain damage and could serve as an alternative to synthetic insecticide in ensuring steady supply of maize grains, thus ensuring food security as the plants are of medicinal and nutritional quality.

Correspondence: Jacobs Mobolade Adesina, Insect Chemical Ecology Laboratory, Institute of Bioresources and Sustainable Development, Takyelpat, 795001 Imphal, Manipur, India.
Tel: +91-8415038748 – Fax: +2348037110905.
Email: moboladesina@yahoo.com

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Introduction

Maize (*Zea mays* L.), is one of the principal food and cash crop cereals grown in the tropical and sub-tropical nations of the world.[1] It constitutes an essential source of cheap, high-quality dietary carbohydrate for resource-poor farmers.[2,3] A major problem in attempting to increase the supply of maize grains in rural and urban households is the highest losses of 45-50% recorded during storage caused by insect infestation.[4,5] *Sitophilus zeamais* is major pest of stored maize causing an average grain loss of 60% within 3-6 months of storage period resulting in weight losses and quality deterioration leading to major economic losses which constitute a threat to food security especially in developing countries.[6]

The use of chemical agents to prevent or control insect infestations has been the main method of grain protection, since it is the simplest and most cost-effective means of dealing with stored product pests.[7] However, current use of synthetic insecticides for the control of storage insect pests is limited because of resistance developed by insect pests, pest resurgence, consumers concern, widespread environmental hazards and increasing costs of application.[8] Thus, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants for the protection of grain products against insect infestations. The use of botanical pesticides to protect plants from pests is very promising because of several distinct advantages such as plant materials are more readily biodegradable, environmental friendly and less toxic to mammals.[9,10] Higher plants are a rich source of novel insecticides.[11] Plant materials with insecticidal properties have been used traditionally for generations throughout the world.[12]

The use of plant products in protecting harvested crops from insect damage is not only gaining prominence,[13] but also with a high degree of success as grain protectants against stored beetle to reduce infestation in storage is also generating positive results.[14-16] Various researchers reported the potential of *Myrcianthes fragrans* (Sw.) McVaugh (syn. *Eugenia aromatica* O. Berg) and *Aframomum melegueta* K. Schum for the management of stored products insect.[17-23] In literature, there appears to be a dearth of empirical information on the utilization of mixture of *M. fragrans* and *A. melegueta* for their insecticidal potential. Therefore, the specific objective of this research is to determine the synergy efficacy of *M. fragrans* and *A. melegueta* powder and methanol extracts in reducing infestation and grains damage by *S. zeamais* on stored maize grains and increase the potency of the plant products constituents.

Materials and Methods

Experimental condition and *Sitophilus zeamais* culture

The study was conducted between February-November, 2015 in the Animal and Environmental Biology Research Laboratory, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria (5° 44' E and 7° 28' N) under ambient condition of 28±2°C temperature, 75±5% relative humidity and 12L:12D photoperiod. The insects used to establish a laboratory colony of *S. zeamais* were derived from a colony originating from infested maize grains collected from Erekesan market, Akure, Ondo State, Nigeria (5° 12' E and 7° 15' N). The emerged adults were sub-cultured on a susceptible local maize cultivar and maintained in kilner jars under ambient conditions in the laboratory to allow for oviposition to produce a steady and sufficient supply of weevils of known age for experimental purpose.[6]

Collection and preparation of plant materials

M. fragrans (seeds) and *A. melegueta* (pods) were purchased from Oja-Oba market, Akure, Ondo State, Nigeria (5° 12' E and 7° 15' N). These plant materials were rinsed with tap water to remove dirt and air dried in the laboratory under ambient condition to avoid volatilization of the active ingredients[17] before being milled into very fine powder using an electric blender (Philips mix grinder HL 1606/03 model). The powders were 1 mm sieved and were packed in plastic containers with tight lids and stored in a cool dry place prior to use. Methanol extracts of *M. fragrans* and *A. melegueta* were carried out using cold extraction method.[24] About 150 g of the powders were soaked separately in an extraction bottle containing 250 mL methanol for 72 h and the mixture was stirred occasionally with a glass rod. The resulting mixture was filtered using a double layer of Whatman No. 1 filter paper and the solvent was evaporated using a rotary evaporator at 30 to 40°C with rotary speed of 3 to 6 rpm for 8 h.[25] The resulting materials were air dried in order to remove traces of solvents.

Collection of maize seeds

Maize seeds used for the study was purchased from Erekesan market Oja Oba Akure, Ondo State, Nigeria. The seeds were thoroughly cleaned, sun dried, cooled, and stored with 10 ± 2% moisture content. Prior to use, the maize seeds were then placed inside an oven at 50°C for 4 hours to kill all hidden infestations (if any) and left to equilibrate at room temperature for 1-2 hrs before 20 g of maize seeds were weighed using digital weighing balance into small plastic containers for bioassay.[26,27]

Toxicity of single and mixed plants powders on adult mortality and progeny development of *Sitophilus zeamais*

2.0 g of *M. fragrans* and *A. melegueta* powder were measured and thoroughly stirred with glass rod to ensure proper mixing from which 2.0 g was admixed separately in single and mixed formulation with 20 g of maize grain in 250 mL plastic containers properly agitated to ensure uniform coating of the grains with the plant powders. Thereafter, adult *S. zeamais* (10 males and 10 females) were introduced into each of the containers and covered. The morphology of the insect proboscis was used for sexual differentiation.[28-30] Four replicates of the treated and untreated controls were laid out in Complete Randomized Block Design (CRD) and arranged on the laboratory work bench for observation. Insect mortality was assessed every 24 hours for four days. Adults were considered dead when probed with sharp objects and made no responses; to avoid the possibility of death mimicry, the insects were watched for 2 min and again subjected to gentle probe.[23] Percentage adult mortality was corrected using Abbott[31] formula. At the end of 96h after exposition, all insects, both dead and alive were removed from each container and number of eggs laid was recorded before returning the grains to their respective containers. This was achieved by identifying the egg plugs of *S. zeamais* after staining with acid fuchsin dye solution.[32]

The experimental set up was kept undisturbed on the laboratory work bench for further 30 days till the emergence of the first filial (F_1) generation. The containers were sieved out and newly emerged adult maize weevil were counted and recorded. The percentage adult emergence was calculated using the method of Odeyemi and Daramola.[30]

Percentage weight loss of the cowpea seeds was determined by re-weighing after 35 days and the % loss in weight was determined following the formula adopted by Mansoor.[33] After re-weighing, the numbers of damaged maize seeds were evaluated by counting wholesome grains and grains with adult weevil emergent holes and this was used to determine percentage seed damaged and Weevil Perforation Index (WPI) following the formula adopted by Enbakhare and Law-Ogbomo[34] and Fatope *et al.*[35] respectively, where WPI value exceeding 50 was regarded as enhancement of infestation by the beetle or negative protectability of the extract tested.

Toxicity of single and mixed plants extracts on adult mortality and progeny development of *Sitophilus zeamais*

From each plant extract 5ml were measured into a 15 mL culture tube and thoroughly mixed and applied at 2.0% concentration mixed formulation, while 2% concentration each of *M. fragrans* and *A. melegueta* extracts were mixed separately with 20g of sterilized maize seeds in 250 mL plastic containers. The extracts and maize seeds were thoroughly mixed using a glass rod and then agitated for 5 min to ensure uniform coating. The containers were left open for 30 min to allow solvent traces to evaporate off. The experiment was set up following the procedures described above and data were collected accordingly.

Statistical analysis

All the data obtained were subjected to one way analysis of variance using Statistical Analysis Software (SAS) package[36] and significant treatment means were separated using Tukey's Test at 5% significant level. Percentages and egg counts data were subjected to square root transformation and arcsine transformed respectively before analysis.[37]

Results

Effect of *Myrcianthes fragrans* and *Aframomum melegueta* products on adult mortality of *Sitophilus zeamais*

Results presented in Figure 1 show that the plant powders significantly (P<0.05) reduce the longevity of adults *S. zeamais* on treated maize grain compared to untreated maize grains. *M. fragrans* powder was effective against *S. zeamais* evoking 100% mortality within 24 hour of exposure; same trend was equally observed in maize grains protected with sole application of *M. fragrans* extract (Figure 1) and *A. melegueta* powder recorded 100% weevil mortality 72 h after exposure (Figure 1) while, the extract of *A. melegueta* was able to exert 100% mortality 48 h after exposure. Same scenario was recorded in grains protected with the mixture of *M. fragrans* and *A. melegueta* extracts (Figure 1). In the control dishes, no mortality was recorded during the exposure time. Adult insect mortality was observed to be directly proportional to increase with duration of exposure to plant products in sole application of *A. melegueta* and in insects exposed to the mixture of both plant products.

Effect of *Myrcianthes fragrans* and *Aframomum melegueta* products on oviposition and adult emergence of *Sitophilus zeamais*

The different plant products significantly (P<0.05) reduced the oviposition, F_1 progeny development and emergence of adult *S. zeamais* (Figure 2). Sole application of *M. fragrans* powder and mixture of both *M. fragrans* and *A. melegueta* products completely inhibit *S. zeamais* from laying eggs on treated maize grains, while sole application *A. melegueta* powder significantly (P<0.05) reduced mean number of eggs laid (2.75) compared to untreated maize seeds (36.75). Percentage adult emergence in the untreated maize seeds was significantly different (P<0.05) from progeny emergence observed in the treated seeds (Figure 2). *M. fragrans* and *A. melegueta* products completely inhibited the progeny development or emergence of *S. zeamais* from treated maize grains compared to untreated maize grains that recorded 57.15% adult emergence.

Effect of *Myrcianthes fragrans* and *Aframomum melegueta* products on maize grain damage

Application of *M. fragrans* and *A. melegueta* products singly or in mixture completely prevented the infestation and damage of the maize grains compared to control treatment that suffered significant (P<0.05) seeds damage and percentage weight loss as a results of infestation and feeding activity of *S. zeamais* (Figure 2). WPI was zero (0) for maize seeds treated with the plants products.

Discussion

The various shortcomings associated with the use of synthetic insecticides for the management of insect pests has stimulated renewed interest in the utilization and development of alternative control strategies such as botanicals to combat the qualitative and quantitative loss resulting from the feeding activities and damage of the stored grains owing to insect infestation. The act of admixing *M. fragrans* and *A. melegueta* products in this study was aimed at producing a botanical insecticide that is more potent in controlling the stored-product insect pests. The results obtained from the study show that both plant products have insecticidal effects against *S. zeamais* causing high adult mortality between 24 to 72 h post treatments.

The rapid action of the plant products or its constituents against *S. zeamais* as observed in this study is indicative (or an indication) of neurotoxic actions.[38-41] It appears that death of the adults, larvae, oviposition inhibition and development inhibition may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism.[42] The mode of action of botanicals is partially attributed to interference in normal respiration that results in suffocation.

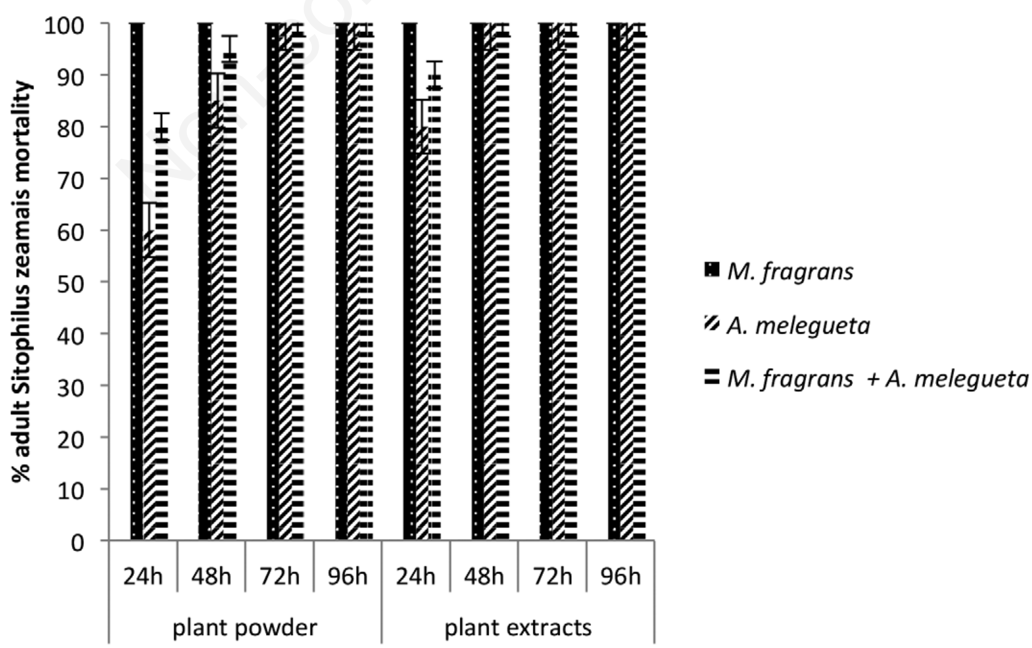

Figure 1. Mortality rate of adult *Sitophilus zeamais* exposed to *Myrcianthes fragrans* and *Aframomum melegueta* products. Bars represent mean values of three replicates.

However, factors other than oxygen starvation probably play an important role in their mode of action.[43,44] It is also thought that botanicals exert some lethal action on developing embryos and first instar larvae e.g. by the reduction in gaseous exchange due to a *barrier* effect and direct toxicity by penetrated botanicals fractions.[42] Another hypothesis is that botanical infiltration under the operculum may block respiration and disrupt the water balance of eggs and developing embryos.[45]

The powder and extract of *M. fragrans* and *A. melegueta* and the mixture of both plants significantly reduce or prevent oviposition and adult emergence at 35days of exposure. The reduced number of eggs was a consequence of reduced longevity/survival time since most female bruchids deposit most of their eggs within 3 days of adult life[46,47] and any reduction in adult life span is expected to contribute to reduced oviposition.[23] The low adult emergence could result from mortality of eggs and death of immature stages or reduction of egg hatching as a result of treatment. The active components of the two plants might be responsible for the reduced fecundity. Bamaiyai *et al* also opined that the ovicidal properties of botanical powders suppressed the emergence of pulse beetle.[48] The semiochemical nature, pungent and peppery odour of the plant products might alter the behaviour and physiology of beetles adversely and thus preventing them from oviposition. Javid and Poswal[49] reported that admixing 2% (w/w) powdered flower buds of *Syzigium aromaticum* with cowpeas prevented oviposition and emergence of *Callosobruchus maculatus*. This is also in agreement with the findings of Adedire and Akinneye,[50] and Adesina *et al.*[23] who found *E. aromatica* and *A. melegueta* significantly reduce or prevent the oviposition and adult emergence of *C. maculatus* on cowpea. The ability of the evaluated plants to significantly ($P<0.05$) suppress adult emergence suggested that the plants might possess ovicidal and larvicidal properties.

The heavy grains damage recorded from untreated maize grains is that an increase in progeny emergence apparently resulted in increase in percentage grain damaged as rightly observed in the untreated control due to insect developmental and feeding activity within the grains. WPI value lower than 50 is an index of positive protectant effect of the evaluated plant products while WPI greater than 50 is an index of negative grains protectant. The reduction in seed damage as reflected by the mean percentage seed damage, weight loss and WPI is in tandem with the mean number of adult emergence from the untreated grains.[51] This confirms the assertion that weight loss recorded could be attributed to the reduction in F1 progeny as a result of low egg hatchability and adult emergence in the treated grains[52,53] and to certain anti feeding nature exhibited by the pungent and irritating odour of the plant product.[23,54]

Synergetic effects of both *M. fragrans* and *A. melegueta* evoked significant adult mortality, prevented oviposition and adult emergence, maize grain damage, WPI, and weight loss of treated maize grain. The mechanism of synergism in this study is not known; but[55] stated that synergism might be due to phytochemicals inhibiting the ability of the insect to employ detoxifying enzymes against synthetic chemicals. Agona and Muyinza[56] opined that when some botanicals are combined as binary formulations, their biological activities against *Acanthoscelides obtectus* on stored beans is enhanced. Use of synergistic combinations of pesticides is already being applied to increase the potency of pesticides in many formulations of agricultural pesticides.[57]

Results of the qualitative phytochemical analysis of *M. fragrans and A. melegueta* as conducted by different researchers[23,24,38] indicate the presence of flavonoids, alkaloids, saponins, tannins and cardiac glycosides (Table 1) that may be responsible for its high insecticidal activity.

Insecticidal, ovicidal and larvicidal activity caused by the different plant products on larval and adult insects might be due to the differential toxicity of the active ingredients. The varying results were probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant.[58]

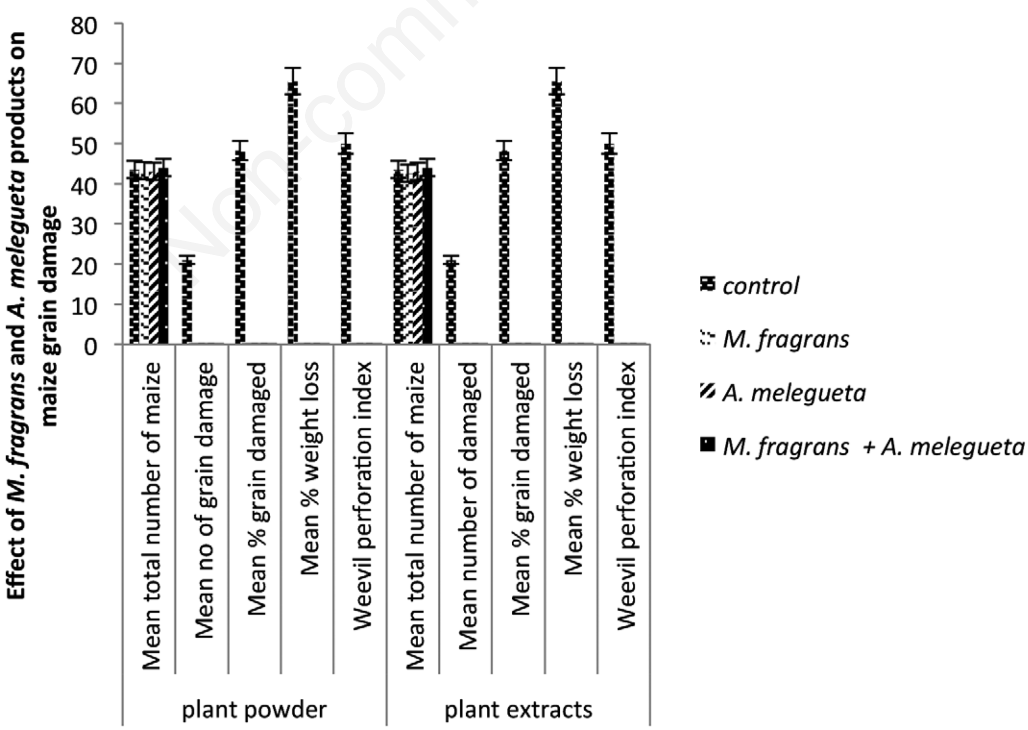

Figure 2. Effect of *Myrcianthes fragrans* and *Aframomum melegueta* products on maize grain damage. Bars represent mean values of three replicates.

Table 1. Phytochemical constituents of *Myrcianthes fragrans* and *Aframomum melegueta*.

Phytochemicals	Methanol extract of *M. fragrans*	Powder of *M. fragrans*	Methanol extract of *A. melegueta*	Powder of *A. melegueta*
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phlobatannin	-	-	-	-
Anthraquinones	-	-	-	-
Flavanoids	+	+	+	+
Cardiac glycosides	+	+	+	+

M. fragrans, Myrcianthes fragrans; *A. melegueta*, *Aframomum melegueta*; -, absent; +, present.

Feng and Isman[59] reported that complex mixtures of secondary compounds in plant extracts contribute to a great deal for synergism, which enhances the joint action of active compounds against insect and reduces the rate of resistance development. *A. melegueta* was reported to contain the following bioactive molecules: α-caryophyllene, β-caryophyllene, E-nerolidol, linalool, gingerdione, gingerol, 2-heptanol, 2-heptyl acetate, paradol, shagaol and humulene.[60-63] While, *M. fragrans* (*E. aromatica*) contains: eugenol, eugenyl acetate, cariofilen β-caryophyllene, 1,8-Cineole and -Cadinene which produce characteristic smell and insecticidal activity[64-67] which is responsible for the sharp, peppery taste, characteristic smell, insecticidal and biological activity of *A. melegueta* and *M. fragrans* (*E. aromatica*).[17-23,60-68]

Conclusions

The insecticidal potential of the two plants have been established and results obtained from this study show that the plant products were effective in check mating *S. zeamais* infestation and the mechanisms of its protective action can be deduced from the great and rapid mortality of adult insects, strong inhibition of oviposition, and the remarkably high reduction in survival to adulthood of immature stages of the insects in treated seeds in comparison with the control which ultimately leads to grains damage suppression.

Various authors reported that the plants efficacy increased with an increase in concentration and confirmed the plants potency at a range varying between 2-2.5g and 2-5% concentration.[18-23,27,60-61,69] The use of one concentration is veritable means of comparison between the two protectants in this study because the rate adopted has been reported to confer appreciable level of protection against insect infestation and damages on stored food grains and since the objective of the study was to test for the synergetic effect of the plant products. Research findings indicate that combination or mixture of plant products offer greater grains protection ability due to the different bioactive molecules present in the plants.[55-59] To minimize the severe damage caused by *S. zeamais*, the synergetic utilization of *A. melegueta* and *M. fragrans* products, proved to be highly effective against the insects infestation on stored maize grains. Its application as botanical insecticides will benefit agricultural sector of developing countries, as these substance are not only of low cost, but they also have no health or environmental hazard as the plant is of high medicinal and nutritional importance in tropical and sub-tropical countries.

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