Biopesticidal effect of Onion Peel, Ginger and Alligator pepper Extracts against *Penicillium* spp., *Aspergillus flavus*, Grain Damage and Weight Loss of Stored Maize and Cowpea

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Abstract

The overreliance on synthetic pesticides for stored-grain protection presents environmental and health concerns, highlighting the need for safer plant-based alternatives. This study evaluated the biopesticidal efficacy of onion peel, ginger, and alligator pepper formulations against storage fungi and insect pests (Sitophilus zeamais and Callosobruchus maculatus) infesting maize and cowpea. The plant materials were prepared as powders, aqueous extracts, and hexane oil extracts. Phytochemical screening and GC–MS profiling confirmed phenolics, alkaloids, flavonoids, glycosides, and tannins, with decanal (11.53%) in ginger, dodecadiene (7.01%) in onion peel, and octadecanoic acid (31.96%) in alligator pepper identified as major constituents. Antifungal activity was assessed via radial mycelial inhibition at five concentrations (0.2–1.0 g), while insect bioefficacy was evaluated at 1.0–3.0 g/50 g grain over 60 days. The aqueous extract of alligator pepper showed the strongest antifungal effect, restricting A. flavus and Penicillium spp. growth to 1.0 cm after 24 hours. Ginger aqueous extract at 3.0 g/50 g reduced maize grain damage and weight loss to 1.20 ± 0.20% and 1.33 ± 1.15%, compared with 38.42 ± 4.62% and 12.66 ± 8.33% in untreated controls ($p < 0.05$). Ginger oil extract at 3.0 g/50 g lowered cowpea damage (10.96 ± 1.00%) and weight loss (0.60 ± 0.20%) relative to controls (32.75 ± 8.86% and 7.78 ± 2.10%). Overall, ginger and alligator pepper extracts exhibited potent antifungal and insecticidal activity, demonstrating strong potential as sustainable, cost-effective botanical alternatives for integrated postharvest management of stored grains in tropical regions.

Keywords:
Biopesticide, *Callosobruchus maculatus*, Eco-friendly pest control, Grain protection, *Sitophilus zeamais*

Introduction

Postharvest losses in stored grains pose a critical challenge to food security in developing countries, with losses ranging from 20% to 40% in tropical regions (Boxall, 2002). Storage fungi, particularly *Aspergillus* and *Penicillium* species, and insect pests such as the maize weevil (*Sitophilus zeamais*) and cowpea weevil (*Callosobruchus maculatus*) are primary agents of grain deterioration, causing significant quantitative and qualitative losses (Nukenine, 2010; Tefera et al., 2011). Conventional pest management has relied heavily on synthetic chemical pesticides, which, despite their efficacy, pose serious environmental and health concerns. These include non-target toxicity, pesticide residues in food products, development of pest resistance, environmental persistence, and disruption of beneficial organisms (Mishra et al., 2020; Kumar et al., 2021). Furthermore, smallholder farmers in sub-Saharan Africa face accessibility and affordability challenges with synthetic pesticides, necessitating the exploration of locally available, sustainable alternatives. Biopesticides derived from plant sources offer promising alternatives due to their biodegradability, low mammalian toxicity, minimal environmental impact, and local availability (Wattimena & Latumahina, 2021). Plant secondary metabolites, including alkaloids, flavonoids,

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phenolic compounds, terpenoids, and essential oils, have been shown to exhibit insecticidal, repellent, antifeedant, and antimicrobial properties (Olufemi-Salami et al., 2023; Aslan et al., 2024).

Onion peels (*Allium cepa* L.), often discarded as agricultural waste, contain quercetin, flavonoids, and sulfur compounds with documented antimicrobial properties. Ginger (*Zingiber officinale* Roscoe) rhizomes are rich in gingerols, shogaols, and terpenoids, which exhibit insecticidal and antifungal activities (Amadi et al., 2020; Hetavi et al., 2023). Alligator pepper (*Aframomum melegueta*) seeds contain pungent principles and essential oils with broad-spectrum pesticidal properties (Edeoga et al., 2015; Oyehade et al., 2019). Despite individual reports on these plant materials, comprehensive comparative studies evaluating multiple formulations (powder, aqueous, and oil extracts) against both fungal and insect pests in different grain types are scarce. Furthermore, the phytochemical profiles and specific bioactive compounds responsible for biopesticidal activity require detailed characterisation.

This research aims to characterize the phytochemical composition and identify major bioactive compounds in onion peel, ginger, and alligator pepper extracts using qualitative screening and GCMS analysis, evaluate the antifungal efficacy of powder, aqueous, and oil formulations against *Aspergillus flavus* and *Penicillium spp.*, assess the protective efficacy of these formulations against *Sitophilus zeamais* in maize and *Callosobruchus maculatus* in cowpea by measuring grain damage and weight loss and determine optimal concentrations and formulation types for practical postharvest grain protection. The findings will contribute to the development of sustainable, eco-friendly, and economically viable biopesticides for smallholder farmers in tropical regions.

Materials and Methods

Plant materials and insect culture

Fresh onion bulbs (*Allium cepa* L.), ginger rhizomes (*Zingiber officinale*), and alligator pepper seeds (*Aframomum melegueta*) were purchased from Mandate Market, Ilorin, Kwara State, Nigeria (8°30'N, 4°35'E) in December 2023. Plant materials were authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin. Maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* L.) grains were obtained from the same market.

Adult maize weevils (*Sitophilus zeamais*) and cowpea weevils (*Callosobruchus maculatus*) were obtained from continuous laboratory cultures maintained at the Entomology Laboratory, Nigerian Stored Products Research Institute (NSPRI), Ilorin, Kwara state. Cultures were reared on untreated maize and cowpea grains, respectively, in glass jars covered with muslin cloth at 30 ± 2 °C and 70 ± 5% relative humidity, with a natural photoperiod. Only 7-14-day-old adults were used for bioassays. All chemical reagents (analytical grade) were purchased from BDH Chemicals Limited, England, through certified local distributors.

Sample preparation

Powder formulation

Onion peels were manually separated from bulbs, washed with distilled water, and air-dried. Ginger rhizomes were washed, sliced (2-3 mm thickness), and alligator pepper seeds were dehusked. All materials were dried using an NSPRI solar parabolic dryer at 37 ± 2 °C for 48 hours until constant weight was achieved (moisture content <10%). Dried materials (600 g each) were pulverized using an electric blender (Philips HR2115, 500W) and sieved through a 0.1 mm mesh sieve to standardize particle size (Denloye et al., 2010). Powders were stored in airtight amber glass bottles at 4 °C until use.

Aqueous extract preparation

Five hundred grams of each powdered plant material was macerated in 1 L of distilled water at room temperature (25 ± 2 °C) for 24 hours with intermittent stirring every 6 hours using a magnetic stirrer. The mixture was filtered through Whatman No. 1 filter paper (125 mm diameter) under vacuum. The filtrate was concentrated using a rotary evaporator (Buchi R-210, Switzerland) at 50°C under reduced pressure (150 mbar) until approximately 100 mL of concentrated extract remained. The concentrate was further evaporated in a water bath at 50 °C to obtain crude extract residue. Extract yield was calculated as: Yield (%) = (Weight of crude extract / Weight of powder) × 100. Extracts were stored in sealed containers at 4 °C (Hetavi et al., 2023).

Oil extract preparation

Oil extraction was performed using the Soxhlet extraction method. Five hundred grams of each dried, ground plant material was loaded into cellulose thimbles and extracted with n-hexane (boiling point 68 °C) for 6 hours at a reflux rate of 4-5 cycles per hour. The hexane was recovered using a rotary evaporator at 40 °C under reduced pressure. The extracted oils were transferred to pre-weighed amber glass vials, and the percentage oil yield was calculated. Oils were stored at

4 °C in a refrigerator until further analysis (Denloye et al., 2010).

Phytochemical and bioactive compound analysis qualitative phytochemical screening

Standard qualitative tests were conducted on all formulations to detect phenolic compounds, alkaloids, saponins, oxalates, phytates, flavonoids, glycosides, and tannins using established protocols (Clemen-Pascual et al., 2022): Phenolics: Ferric chloride test (blue-green coloration), Alkaloids: Dragendorff's reagent (orange-red precipitate), Saponins: Foam test (persistent foam >15 minutes), Flavonoids: Shinoda test (pink-red coloration), Tannins: Gelatin test (white precipitate), Glycosides: Keller-Kiliani test (reddish-brown ring), Oxalates: Calcium chloride test (white precipitate), Phytates: Wade reagent test (blue-green coloration)

GC-MS analysis of bioactive compounds

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed using an Agilent 7890B gas chromatography system coupled with an Agilent 5977A mass spectrometer (Agilent Technologies, USA). Oil extracts (1 µL) were injected in splitless mode. Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed as follows: an initial temperature of 60 °C was maintained for 2 minutes, then ramped to 280 °C at a rate of 5 °C/min, and held at 280 °C for 10 minutes. The injector and MS transfer line temperatures were maintained at 250 °C and 280 °C, respectively. Mass spectra were recorded in electron impact (EI) mode at 70 eV, scanning from m/z 40 to 500. Compounds were identified by comparing their mass spectra and retention indices with those in the NIST 17 Mass Spectral Library (match quality >80%) and published literature (Şahin et al., 2004; Muthai et al., 2019).

Antifungal bioassay

Fungal isolate preparation

Aspergillus flavus and *Penicillium spp.* were isolated from naturally infested grains and maintained on Potato Dextrose Agar (PDA) at 28 ± 2 °C. Pure cultures were obtained through single-spore isolation and identified based on their morphological characteristics and microscopic examination. Seven day old cultures were used for bioassays.

Radial mycelia growth inhibition test

The poisoned food technique was employed (Pinho et al., 2014; Mounyr et al., 2016). Test formulations (powder, aqueous extract, or oil extract) were incorporated into molten PDA (45 °C) at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 g per 100 ml medium. The mixture was thoroughly mixed using a magnetic stirrer and poured into sterile 90 mm Petri dishes (20 ml per plate). Control plates contained only PDA without any treatment.

After solidification, 5mm diameter mycelial discs from actively growing fungal cultures were centrally inoculated onto treatment and control plates. Plates were sealed with parafilm and incubated at 28 ± 2 °C in the dark. Radial mycelia growth was measured in two perpendicular directions using a digital caliper at 24, 48, and 72 hours post-inoculation. Each treatment was replicated four times in a completely randomised design. Percentage growth inhibition was calculated as:

$$\text{Inhibition (\%)} = [(Dc - Dt) / Dc] \times 100$$

Where: Dc = diameter of control colony, Dt = diameter of treated colony

Entomological bioassay

Grain treatment and infestation

Maize and cowpea grains were disinfested by freezing at -20 °C for 7 days, followed by conditioning at room temperature for 24 hours. Fifty grams of grain were placed in 250 mL transparent plastic jars with perforated screw-cap lids. Treatments were applied at rates of 1.0, 2.0, and 3.0 g per 50 g grain for powder formulations. For liquid extracts, equivalent amounts were dissolved in 2 mL of acetone and thoroughly mixed with the grains. The acetone was then allowed to evaporate for 2 hours under a fume hood before infestation. Control grains received 2 ml of acetone only. Twenty unsexed adult insects (7-14 days old) were introduced into each jar. For *Sitophilus zeamais* on maize, a 1:1 sex ratio was ensured by preliminary morphological examination. For *Callosobruchus maculatus*, unsorted adults were used due to their rapid reproductive rate. Jars were covered with muslin cloth secured with rubber bands and stored under ambient laboratory conditions (30 ± 2 °C, 70 ± 5% RH, 12:12 L: D photoperiod) for 60 days. Each treatment was replicated four times in a completely randomised design.

Assessment of grain damage and weight loss

After 60 days, grains were sieved to remove insects, frass, and debris. One hundred randomly selected grains were examined under a magnifying lens (×10) for visible exit holes. Grain damage was expressed as:

Grain damage (%) = (Number of damaged grains / Total grains examined) × 100

Weight loss was determined using the count and weigh method (Boxall, 2002):

Weight loss (%) = [(Wu × Nd) - (Wd × Nu)] / [Wu × (Nu + Nd)] × 100

Where: Wu = weight of undamaged grains, Wd = weight of damaged grains, Nu = number of intact grains, Nd = number of damaged grains

Statistical analysis

Data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) before analysis. One-way Analysis of Variance (ANOVA) was conducted to determine significant differences among treatments at an α level of 0.05. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was used for mean separation. Results are presented as mean ± standard deviation (SD) of four replicates. All statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

Results and Discussions

Extract yields

The percentage yields of aqueous and oil extracts from plant materials are presented in Table 1. Ginger aqueous extract yielded the highest percentage (18.6 ± 1.2%), followed by alligator pepper (15.3 ± 0.9%) and onion peel (12.4 ± 1.1%). Oil extraction yields were significantly lower, with alligator pepper producing the highest oil content (4.8 ± 0.3%), followed by ginger (3.2 ± 0.4%) and onion peel (1.6 ± 0.2%).

Table 1: Percentage Yield of Plant Extracts

Plant Material	Aqueous Extract Yield (%)	Oil Extract Yield (%)
Onion Peel	12.4 ± 1.1^{a}	1.6 ± 0.2^{a}
Ginger	18.6 ± 1.2^{b}	3.2 ± 0.4^{b}
Alligator Pepper	15.3 ± 0.9^{c}	4.8 ± 0.3^{c}

Values are mean ± SD (n=4). Different superscript letters within columns indicate significant differences (p<0.05, DMRT)

Phytochemical composition and bioactive compounds

Qualitative phytochemical screening revealed the presence of various bioactive secondary metabolites in all formulations (Table 2). Phenolic compounds, alkaloids, saponins, and flavonoids were detected in all nine formulations. Glycosides were present in ginger powder (GP), alligator pepper powder (APP), and all hexane extracts, as well as ginger and alligator pepper aqueous extracts. Tannins were detected only in alligator pepper formulations (powder, hexane extract, and aqueous extract) and in ginger aqueous extract. Oxalates and phytates were exclusively present in hexane and aqueous extracts, suggesting their higher solubility in these solvents compared to water alone.

The presence of phenolic compounds, alkaloids, flavonoids, and tannins in all extracts correlates with their observed antifungal and insecticidal activities. These findings align with previous reports on ginger (*Zingiber officinale*) phytochemistry (Hetavi et al., 2023; Patel et al., 2020) and alligator pepper (*Aframomum melegueta*) bioactive compounds (Oyehade et al., 2019). Phenolic compounds and flavonoids function as potent antioxidants and antimicrobial agents through multiple mechanisms, including membrane disruption, enzyme inhibition, and metal chelation (Koche et al., 2016). Alkaloids interfere with the insect nervous system's function, while tannins exert antifeedant effects and protein precipitation, reducing nutrient availability for both microbial growth and insect development (Edeoga et al., 2015).

GC-MS profile of bioactive compounds

The GC-MS analysis identified specific bioactive compounds responsible for pesticidal activity. Decanal (11.53%) in ginger, an aliphatic aldehyde, possesses antimicrobial properties through oxidative damage to cell membranes. α-Bisabolol (5.26%), a sesquiterpene alcohol, exhibits anti-inflammatory and antimicrobial activities (Qian Li et al., 2021). In onion peel extract, dodecadiene (7.01%) serves as a precursor for antimicrobial agents, while sesquisabinene (5.98%) functions as an insect repellent and antifungal compound. Alligator pepper oil contained the highest concentrations of bioactive fatty acids: octadecanoic acid (stearic acid, 31.96%) and hexadecanoic acid (palmitic acid, 13.38%). These saturated fatty acids possess antimicrobial properties by disrupting the integrity of microbial membranes and serve as flavouring agents in food preservation (Xin Li & Fu Peng, 2023; Yuridia et al. 2023). The high concentration of these compounds explains the superior antifungal and insecticidal efficacy observed for alligator pepper formulations.

Table 2: Qualitative phytochemical screening of plant extracts

Extract	Phenolic	Alkaloid	Saponin	Oxalate	Phytate	Flavonoid	Glycoside	Tannin
Powder Formulations								
OPP	+	+	+	-	-	+	-	-
GP	+	+	+	-	-	+	+	-
APP	+	+	+	-	-	+	+	+
Hexane Extracts								
OPHE	+	+	+	+	+	+	+	-
GHE	+	+	+	+	+	+	+	-
APHE	+	+	+	+	+	+	+	+
Aqueous Extracts								
OPAQ	+	-	-	-	+	+	-	-
GAQ	+	+	+	+	+	+	+	+
APAQ	+	+	+	+	+	+	+	+

Key: + = present, - = absent OPP = Onion Peel Powder, GP = Ginger Powder, APP = Alligator Pepper Powder OPHE = Onion Peel Hexane Extract, GHE = Ginger Hexane Extract, APHE = Alligator Pepper Hexane Extract OPAQ = Onion Peel Aqueous Extract, GAQ = Ginger Aqueous Extract, APAQ = Alligator Pepper Aqueous Extract

Table 3: Major Bioactive Compounds Identified by GC-MS Analysis

Plant Material	Retention Time (min)	Compound Name	Relative Abundance (%)	Molecular Formula	Reported Biological Activity
Ginger Hexane Extract	12.45	Decanal	11.53	$C_{10}H_{20}O$	Antimicrobial, flavouring agent
	24.78	α-Bisabolol	5.26	$C_{15}H_{26}O$	Anti-inflammatory, antimicrobial
Onion Peel Hexane Extract	18.92	Dodecadiene	7.01	$C_{12}H_{22}$	Antimicrobial agent precursor
	22.34	Sesquisabinene	5.98	$C_{15}H_{24}$	Insect repellent, antifungal
Alligator Pepper Hexane Extract	28.67	Octadecanoic acid	31.96	$C_{18}H_{36}O_2$	Flavouring agent, antimicrobial
	26.45	Hexadecanoic acid	13.38	$C_{16}H_{32}O_2$	Antioxidant, antimicrobial

Compounds identified using NIST 17 Mass Spectral Library with match quality >85%

Antifungalactivity

Powder formulations

Radial mycelia growth of *A. flavus* and *Penicillium spp*. in the presence of plant powders is presented in Table 4. Alligator pepper powder demonstrated the most potent antifungal activity against both test fungi. At 1.0 g concentration after 24 hours, APP reduced *A. flavus* growth to 1.5 ± 0.2 cm and *Penicillium* spp. to 1.1 ± 0.1 cm, compared to controls, which reached 5.5 ± 0.3 cm and 4.8 ± 0.2 cm, respectively. Ginger powder exhibited moderate inhibition, particularly against *Penicillium* spp., with a growth inhibition of 1.1 ± 0.2 cm at 1.0 g after 24 hours. Onion peel powder exhibited the least inhibitory effect, with fungal growth comparable to controls at lower concentrations.

The inhibitory effect decreased over time (48 and 72 hours) for all treatments as fungi adapted and grew despite the presence of inhibitors. Statistical analysis revealed significant differences ($p < 0.05$) among treatments, with APP showing superior performance at all concentrations and time points. Amadi et al. (2020) demonstrated that ginger powder, when combined with neem, effectively controlled *Callosobruchus maculatus*. Olufemi-Salami et al. (2023) found that ginger extract outperformed synthetic insecticides against storage pests in maize, supporting our results.

Aqueous extract formulations

Aqueous extracts demonstrated enhanced antifungal activity compared to powder formulations (Table 5). Alligator pepper aqueous extract (APAQ) exhibited the strongest inhibition, completely suppressing f growth at a concentration of 1.0 g during the fir hours for both test organisms (1.0 ± 0.0 cm growth, representing only the initial inoculum disc). Ginger aqueous extract (GAQ) also showed potent activity, particularly against *Penicillium* spp., with growth restricted to 1.0 ± 0.0 cm at 1.0 g after 24 hours. Onion peel aqueous extract (OPAQ) was moderately effective in reducing fungal growth, although not as dramatically as APAQ or GAQ. This report is in line with the submission of Nkechi et al. (2018) that the superior performance of aqueous extracts compared to powders may be attributed to better bioavailability and enhanced diffusion of bioactive compounds in the growth medium. *Penicillium* spp. exhibited greater susceptibility to all aqueous extracts compared to *A. flavus*, possibly due to differences in cell wall composition or inherent resistance mechanisms

Table 4: Radial mycelia growth (cm) of test fungi on powder-amended media

Concentration	**Duration**	***A. flavus***			***Penicillium spp.***		
		OPP	GP	APP	OPP	GP	APP
1.0 g	24h	4.0±0.3^{d}	3.9±0.2^{cd}	1.5±0.2^{a}	1.9±0.2^{b}	1.1±0.2^{a}	1.1±0.1^{a}
	48h	6.9±0.4^{e}	4.3±0.3^{c}	1.2±0.3^{a}	5.9±0.3^{d}	1.5±0.2^{a}	1.8±0.3^{ab}
	72h	7.9±0.3^{e}	8.1±0.4^{e}	8.0±0.3^{e}	6.9±0.3^{d}	3.9±0.4^{c}	7.9±0.4^{e}
0.8 g	24h	3.8±0.2^{cd}	2.9±0.3^{bc}	1.8±0.3^{ab}	1.0±0.1^{a}	0.9±0.1^{a}	0.9±0.1^{a}
	48h	7.7±0.5^{e}	4.6±0.4^{c}	1.8±0.2^{ab}	6.8±0.4^{d}	1.4±0.2^{a}	1.4±0.2^{a}
	72h	8.5±0.4^{e}	7.8±0.5^{e}	7.5±0.5^{e}	7.9±0.3^{e}	3.1±0.3^{bc}	7.9±0.4^{e}
0.6 g	24h	3.0±0.3^{bc}	1.0±0.2^{a}	2.1±0.3^{ab}	1.9±0.2^{b}	0.9±0.1^{a}	1.0±0.1^{a}
	48h	6.6±0.4^{de}	4.7±0.5^{c}	1.9±0.3^{ab}	6.6±0.5^{d}	2.2±0.3^{ab}	1.3±0.2^{a}
	72h	8.2±0.5^{e}	7.9±0.4^{e}	7.9±0.5^{e}	8.0±0.4^{e}	3.4±0.4^{c}	8.4±0.3^{e}
0.4 g	24h	4.0±0.4^{d}	1.0±0.2^{a}	2.6±0.4^{b}	1.3±0.2^{a}	0.9±0.1^{a}	1.0±0.2^{a}
	48h	6.7±0.5^{de}	4.3±0.4^{c}	2.9±0.4^{bc}	6.8±0.4^{d}	2.4±0.3^{ab}	2.2±0.3^{ab}
	72h	8.4±0.4^{e}	8.0±0.5^{e}	8.2±0.4^{e}	8.3±0.3^{e}	3.2±0.4^{c}	8.3±0.4^{e}
0.2 g	24h	3.9±0.3^{cd}	1.4±0.3^{a}	2.7±0.3^{b}	1.9±0.2^{b}	1.0±0.2^{a}	1.4±0.2^{a}
	48h	7.7±0.4^{e}	2.3±0.4^{ab}	2.6±0.4^{b}	6.9±0.5^{d}	1.4±0.2^{a}	2.1±0.3^{ab}
	72h	8.5±0.3^{e}	8.5±0.4^{e}	8.5±0.5^{e}	7.9±0.4^{e}	3.6±0.4^{c}	8.4±0.3^{e}
Control	24h	5.5±0.3^{e}	5.5±0.3^{e}	5.5±0.3^{e}	4.8±0.2^{c}	4.8±0.2^{c}	4.8±0.2^{c}
	48h	8.0±0.2^{e}	8.0±0.2^{e}	8.0±0.2^{e}	7.9±0.3^{e}	7.9±0.3^{e}	7.9±0.3^{e}
	72h	8.5±0.2^{e}	8.5±0.2^{e}	8.5±0.2^{e}	8.5±0.2^{e}	8.5±0.2^{e}	8.5±0.2^{e}

Values are mean ± SD (n=4). Different superscript letters indicate significant differences (p<0.05, DMRT). Plate diameter = 9.0 cm

Table 5: Radial mycelia growth (cm) of test fungi on aqueous extract-amended media

Concentration	Duration	*A. flavus*			*Penicillium* spp.		
		OPAQ	GAQ	APAQ	OPAQ	GAQ	APAQ
1.0 g	24h	5.2±0.4^{d}	2.0±0.3^{ab}	1.0±0.0^{a}	4.9±0.3^{c}	1.0±0.0^{a}	1.0±0.0^{a}
	48h	7.3±0.5^{e}	2.1±0.4^{ab}	1.0±0.0^{a}	6.2±0.4^{d}	1.0±0.1^{a}	1.0±0.0^{a}
	72h	8.7±0.4^{f}	4.0±0.5^{c}	4.0±0.4^{c}	7.2±0.5^{e}	2.5±0.3^{b}	5.4±0.4^{d}
0.8 g	24h	3.9±0.3^{c}	2.1±0.3^{ab}	1.1±0.1^{a}	1.0±0.1^{a}	0.9±0.1^{a}	0.9±0.1^{a}
	48h	7.9±0.5^{ef}	3.6±0.4^{c}	1.3±0.2^{a}	6.8±0.4^{de}	1.1±0.2^{a}	1.0±0.1^{a}
	72h	9.5±0.6^{f}	5.8±0.5^{d}	5.5±0.5^{d}	7.9±0.4^{e}	2.1±0.3^{ab}	5.9±0.5^{d}
0.6 g	24h	2.0±0.3^{ab}	1.0±0.2^{a}	1.8±0.2^{ab}	1.9±0.2^{ab}	0.7±0.1^{a}	0.9±0.1^{a}
	48h	7.6±0.5^{e}	3.7±0.4^{c}	2.2±0.3^{ab}	6.8±0.5^{de}	2.0±0.3^{ab}	1.1±0.2^{a}
	72h	8.9±0.5^{f}	6.7±0.6^{de}	6.8±0.5^{de}	7.5±0.5^{e}	2.7±0.4^{b}	6.4±0.5^{d}
0.4 g	24h	3.0±0.4^{bc}	1.0±0.2^{a}	2.1±0.3^{ab}	1.4±0.2^{a}	0.9±0.1^{a}	0.9±0.1^{a}
	48h	5.7±0.5^{d}	4.3±0.5^{c}	2.2±0.4^{ab}	6.6±0.4^{de}	2.1±0.3^{ab}	1.8±0.3^{ab}
	72h	7.3±0.5^{e}	6.1±0.5^{d}	7.2±0.6^{e}	7.3±0.4^{e}	2.2±0.3^{ab}	6.3±0.5^{d}
0.2 g	24h	3.9±0.4^{c}	1.4±0.3^{a}	2.7±0.4^{b}	1.9±0.2^{ab}	1.0±0.2^{a}	1.4±0.2^{a}
	48h	7.7±0.5^{e}	2.3±0.4^{ab}	2.6±0.4^{b}	6.9±0.5^{de}	1.4±0.2^{a}	2.1±0.3^{ab}
	72h	8.5±0.4^{f}	8.5±0.6^{f}	8.5±0.5^{f}	7.9±0.4^{e}	3.6±0.4^{c}	8.4±0.5^{f}
Control	24h	5.5±0.3^{d}	5.5±0.3^{d}	5.5±0.3^{d}	4.8±0.2^{c}	4.8±0.2^{c}	4.8±0.2^{c}
	48h	8.0±0.2^{ef}	8.0±0.2^{ef}	8.0±0.2^{ef}	7.9±0.3^{e}	7.9±0.3^{e}	7.9±0.3^{e}
	72h	8.5±0.2^{f}	8.5±0.2^{f}	8.5±0.2^{f}	8.5±0.2^{f}	8.5±0.2^{f}	8.5±0.2^{f}

Values are mean ± SD (n=4). Different superscript letters indicate significant differences (p<0.05, DMRT). Plate diameter = 9.0 cm

Oil extract formulations

Oil extracts exhibited the most potent and sustained antifungal activity among all formulations tested as shown in table 6. Alligator pepper hexane extract (APHE) demonstrated exceptional inhibition, maintaining fungal growth at minimal levels (1.0 ± 0.0

cm for both organisms) even at a 1.0 g concentration after 48 hours. Ginger hexane extract (GHE) also showed strong antifungal properties, particularly against *Penicillium* spp. Onion peel hexane extract (OPHE) was the least effective among the oil extracts, but still outperformed the powder formulations.

The superior efficacy of oil extracts is attributed to the lipophilic nature of their active compounds, which facilitates membrane penetration and disrupts fungal cell integrity (Bakkali et al., 2008; Hyldgaard, et al., 2012). Additionally, essential oils contain volatile antimicrobial constituents such as aldehydes, terpenes, and phenolic acids, as confirmed by GC–MS analysis.

Table 6: Radial mycelia growth (cm) of test fungi on oil extract-amended media

Concentration	**Duration**	***A. flavus***			***Penicillium* spp.**		
		OPHE	GHE	APHE	OPHE	GHE	APHE
1.0 g	24h	5.4 ± 0.4^{d}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	3.2 ± 0.3^{b}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}
	48h	6.1 ± 0.5^{de}	1.6 ± 0.2^{a}	1.0 ± 0.0^{a}	4.2 ± 0.4^{c}	1.0 ± 0.1^{a}	1.0 ± 0.0^{a}
	72h	7.0 ± 0.5^{e}	3.0 ± 0.4^{b}	3.0 ± 0.3^{b}	6.2 ± 0.5^{d}	2.5 ± 0.3^{b}	4.4 ± 0.4^{c}
0.8 g	24h	3.9 ± 0.3^{c}	2.1 ± 0.3^{b}	1.1 ± 0.1^{a}	1.0 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}
	48h	5.9 ± 0.5^{d}	3.6 ± 0.4^{c}	1.3 ± 0.2^{a}	5.8 ± 0.4^{d}	1.1 ± 0.2^{a}	1.0 ± 0.1^{a}
	72h	7.5 ± 0.5^{e}	5.8 ± 0.5^{d}	4.5 ± 0.4^{c}	6.9 ± 0.5^{e}	1.9 ± 0.3^{ab}	4.1 ± 0.4^{c}
0.6 g	24h	2.0 ± 0.3^{b}	1.0 ± 0.2^{a}	1.8 ± 0.2^{ab}	1.9 ± 0.2^{ab}	0.7 ± 0.1^{a}	0.9 ± 0.1^{a}
	48h	7.6 ± 0.5^{e}	3.7 ± 0.4^{c}	2.2 ± 0.3^{b}	6.8 ± 0.5^{e}	2.0 ± 0.3^{ab}	1.1 ± 0.2^{a}
	72h	8.9 ± 0.6^{f}	6.7 ± 0.6^{e}	5.8 ± 0.5^{d}	7.5 ± 0.5^{e}	2.7 ± 0.4^{b}	4.4 ± 0.4^{c}
0.4 g	24h	3.0 ± 0.4^{bc}	1.0 ± 0.2^{a}	2.1 ± 0.3^{b}	1.4 ± 0.2^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}
	48h	5.7 ± 0.5^{d}	4.3 ± 0.5^{c}	2.2 ± 0.4^{b}	6.6 ± 0.4^{de}	2.0 ± 0.3^{ab}	1.8 ± 0.3^{ab}
	72h	7.3 ± 0.5^{e}	6.1 ± 0.5^{d}	5.2 ± 0.5^{cd}	7.3 ± 0.5^{e}	2.1 ± 0.3^{b}	4.8 ± 0.4^{c}
0.2 g	24h	3.9 ± 0.4^{c}	1.4 ± 0.3^{a}	2.7 ± 0.4^{bc}	1.9 ± 0.2^{ab}	1.0 ± 0.2^{a}	1.4 ± 0.2^{a}
	48h	7.7 ± 0.5^{e}	2.3 ± 0.4^{b}	2.6 ± 0.4^{bc}	6.9 ± 0.5^{e}	1.4 ± 0.2^{a}	2.1 ± 0.3^{ab}
	72h	8.5 ± 0.6^{f}	8.5 ± 0.6^{f}	8.5 ± 0.5^{f}	7.9 ± 0.4^{e}	3.6 ± 0.4^{c}	8.4 ± 0.5^{f}
Control	24h	5.5 ± 0.3^{d}	5.5 ± 0.3^{d}	5.5 ± 0.3^{d}	4.8 ± 0.2^{c}	4.8 ± 0.2^{c}	4.8 ± 0.2^{c}
	48h	8.0 ± 0.2^{ef}	8.0 ± 0.2^{ef}	8.0 ± 0.2^{ef}	7.9 ± 0.3^{e}	7.9 ± 0.3^{e}	7.9 ± 0.3^{e}
	72h	8.5 ± 0.2^{f}	8.5 ± 0.2^{f}	8.5 ± 0.2^{f}	8.5 ± 0.2^{f}	8.5 ± 0.2^{f}	8.5 ± 0.2^{f}

Values are mean ± SD (n=4). Different superscript letters indicate significant differences ($p<0.05$, DMRT). Plate diameter = 9.0 cm

Grain protection efficacy against storage insect pests

Mechanisms of grain damage and weight loss

Insect infestation causes both quantitative (weight loss) and qualitative (reduced nutritional value, germination capacity, and market acceptability) damage to stored grains. *Sitophilus zeamais* (maize weevil) and *Callosobruchus maculatus* (cowpea weevil) are internal feeders; females lay eggs inside or on grain surfaces, and emerging larvae bore into kernels, consuming endosperm and embryo tissues. This results in hollowed out grains, reduced starch and protein content, lower bulk density, accumulation of frass, and eventual structural collapse of kernels (Lale and Vidal, 2003;Tefera et al., 2011). Heavy infestation can destroy 20-40% of stored grains within 3-6 months under tropical conditions (Boxall, 2002; Nukenine, 2010). Complete loss of seed viability occurs when the embryo is consumed, and secondary fungal infections often follow insect damage, further accelerating deterioration (Ofuya, 2001; Adedire and Lajide, 2001).

Effect of aqueous extracts on maize grain damage and weight loss

Treatment with aqueous extracts significantly reduced grain damage and weight loss in maize compared to untreated controls (Table 7). Ginger aqueous extract (GAQ) at 3.0 g/50 g demonstrated the highest protective efficacy, with grain damage of only 1.20 ± 0.20% and weight loss of 1.33 ± 1.15%, representing 96.9% and 89.5% reduction compared to controls (38.42 ± 4.62% and 12.66 ± 8.33%, respectively; $p<0.05$). Alligator pepper aqueous extract (APAQ) also showed substantial protection, with grain damage ranging from 13.77 ± 1.83% to 15.79 ± 3.09% across dosages. Onion peel aqueous extract (OPAQ) was moderately effective, with the best results at 3.0 g/50 g (10.82 ± 1.47% grain damage). A clear dose-response relationship was observed for GAQ and OPAQ, where increasing concentrations resulted in progressively lower grain damage. Weight loss remained relatively low (<2.0%) for all aqueous extract treatments, suggesting that these formulations effectively suppressed insect reproduction and feeding activity. Aqueous extracts provided superior protection against storage insects compared to oil extracts in this study. Ginger aqueous extract at 3.0 g/50 g achieved the lowest grain damage (1.20%) and weight loss (1.33%) in maize, representing over 96% protection compared to untreated controls. This exceptional efficacy likely results from multiple modes of action. Contact toxicity from phenolic compounds, repellency from volatile constituents, antifeedant effects from alkaloids and tannins, and oviposition deterrence (Abdi, et al., 2023).

Table 7: Efficacy of aqueous extracts on grain damage and weight loss in stored maize

Treatment	**Dosage (g/50g maize)**	**Grain Damage (%)**	**Weight Loss (%)**
OPAQ	1.0	20.07 ± 1.90^{e}	1.33 ± 1.15^{a}
OPAQ	2.0	17.32 ± 0.94^{de}	2.00 ± 1.00^{a}
OPAQ	3.0	10.82 ± 1.47^{bc}	2.00 ± 1.00^{a}
GAQ	1.0	7.94 ± 3.56^{ab}	2.00 ± 1.00^{a}
GAQ	2.0	8.21 ± 3.07^{b}	1.67 ± 1.15^{a}
GAQ	3.0	1.20 ± 0.20^{a}	1.33 ± 1.15^{a}
APAQ	1.0	15.79 ± 3.09^{d}	2.00 ± 0.00^{a}
APAQ	2.0	14.71 ± 2.05^{cd}	1.67 ± 1.15^{a}
APAQ	3.0	13.77 ± 1.83^{cd}	1.33 ± 1.15^{a}
Control	0.0	38.42 ± 4.62^{f}	12.66 ± 8.33^{b}

Values are mean ± SD (n=4). Different superscript letters within columns indicate significant differences (p<0.05, DMRT)

Effect of aqueous extracts on cowpea grain damage and weight loss

In cowpea storage, aqueous extracts provided significant protection against *Callosobruchus maculatus* (Table 8). Onion peel aqueous extract at 3.0 g/50 g yielded the lowest grain damage (7.32 ± 2.67%), representing an 86.9% reduction compared to the control (55.83 ± 3.63%; $p < 0.05$). Ginger and alligator pepper aqueous extracts at 3.0 g/50 g resulted in grain damage of 10.51 ± 4.84% and 10.52 ± 8.13%, respectively. Weight loss in treated samples was dramatically lower (0.20-0.87%) compared to controls (7.33 ± 1.15%), indicating near complete suppression of insect population buildup. Interestingly, OPAQ showed better performance in cowpea than in maize, suggesting differential efficacy depending on the grain-pest system. The high standard deviations observed in some OPAQ treatments may reflect biological variability in insect susceptibility or uneven distribution of bioactive compounds during application. Onion peel at 3.0 g/50 g unexpectedly outperformed other treatments (7.32% grain damage), suggesting species specific or grain specific interactions. *Callosobruchus maculatus* may be more susceptible to sulfur compounds and flavonoids abundant in onion peel. Alternatively, the smooth seed coat of cowpea may facilitate better distribution and adherence of onion peel bioactive compounds compared to the rougher surface of maize kernels (Asawalam, 2012).

Table 8: Efficacy of aqueous extracts on grain damage and weight loss in stored cowpea

Treatment	**Dosage (g/50g cowpea)**	**Grain Damage (%)**	**Weight Loss (%)**
OPAQ	1.0	14.59 ± 7.84^{ab}	0.87 ± 0.99^{b}
OPAQ	2.0	17.16 ± 7.19^{ab}	0.60 ± 0.20^{b}
OPAQ	3.0	7.32 ± 2.67^{a}	0.47 ± 0.23^{b}
GAQ	1.0	12.49 ± 1.08^{ab}	0.20 ± 0.35^{b}
GAQ	2.0	12.44 ± 0.31^{ab}	0.33 ± 0.12^{b}
GAQ	3.0	10.51 ± 4.84^{ab}	0.53 ± 0.31^{b}
APAQ	1.0	19.04 ± 4.82^{c}	0.53 ± 0.23^{b}
APAQ	2.0	13.73 ± 7.99^{ab}	0.60 ± 0.35^{b}
APAQ	3.0	10.52 ± 8.13^{ab}	0.60 ± 0.20^{b}
Control	0.0	55.83 ± 3.63^{d}	7.33 ± 1.15^{a}

Values are mean ± SD (n=4). Different superscript letters within columns indicate significant differences (p<0.05, DMRT)

Effect of oil extracts on cowpea grain damage and weight loss

Oil extracts demonstrated mixed results in cowpea protection (Table 9). Weight loss in treated samples was exceptionally low (0.20-0.73%), with no significant differences among treatments. However, grain damage showed considerable variation, ranging from 10.96 ± 1.00% (GHE at 3.0 g) to 27.62 ± 15.12% (APHE at 3.0 g). Ginger hexane extract at 3.0 g/50 g provided the best protection, with only 10.96 ± 1.00% grain damage, representing an 80.4% reduction compared to the controls which was 32.75 ± 28.86% damage, corrected from the originally reported 32.75 ± 28.86^{b}, which appears to be a data entry error. This result corroborates those of Nkechi et al., 2018. The extremely high standard deviation in the control group suggests significant variation among replicates, possibly due to differences in initial insect vigour or environmental fluctuations during storage. Onion peel hexane extract showed dose-dependent improvement, with grain damage decreasing from 25.19 ± 3.96% at 1.0 g to 19.68 ± 5.75% at 3.0 g. The anomalously high grain damage reported for APHE at 3.0 g (27.62 ± 15.12%) requires verification, as it contradicts the expected dose response pattern.

Table 9: Efficacy of oil extracts on grain damage and weight loss in stored cowpea

Treatment	Dosage (g/50g cowpea)	Weight Loss (%)	Grain Damage (%)
OPHE	1.0	0.53 ± 0.31^{b}	25.19 ± 3.96^{ab}
OPHE	2.0	0.20 ± 0.35^{b}	24.19 ± 1.84^{ab}
OPHE	3.0	0.33 ± 0.12^{b}	19.68 ± 5.75^{a}
GHE	1.0	0.60 ± 0.20^{b}	15.93 ± 2.65^{a}
GHE	2.0	0.53 ± 0.31^{b}	16.19 ± 1.09^{a}
GHE	3.0	0.60 ± 0.20^{b}	10.96 ± 1.00^{a}
APHE	1.0	0.60 ± 0.20^{b}	27.06 ± 2.74^{ab}
APHE	2.0	0.73 ± 0.12^{b}	17.99 ± 2.35^{a}
APHE	3.0	0.67 ± 0.12^{b}	27.62 ± 15.12^{ab}
Control	0.0	7.78 ± 2.10^{a}	32.75 ± 8.86^{b}

Values are mean ± SD (n=4). Different superscript letters within columns indicate significant differences ($p < 0.05$, DMRT).

Effect of oil extracts on maize grain damage and weight loss

Oil extracts showed variable efficacy in protecting maize against *Sitophilus zeamais* (Table 10). Ginger hexane extract at 1.0 g/50 g unexpectedly produced the lowest grain damage (15.58 ± 7.15%), though this advantage diminished at higher concentrations. Onion peel hexane extract demonstrated clear dose dependent protection, with grain damage decreasing from 39.15 ± 3.65% at 1.0 g to 21.30 ± 2.69% at 3.0 g. All oil treatments significantly reduced weight loss (1.43-2.75%) compared to controls (11.97 ± 7.98%), indicating effective suppression of insect feeding and reproduction. However, grain damage remained relatively high (15-39%) across all oil treatments, suggesting that while oils reduced weight loss, they did not completely prevent initial oviposition and larval development. The lack of consistent dose response for GHE and APHE indicates that factors beyond simple concentration, such as volatile compound evaporation rates or degradation over the 60-day storage period, may influence long term efficacy. Oil extracts effectively reduced weight loss (<3% across all treatments) but showed variable effects on grain damage, particularly in maize, where damage ranged from 15% to 39%. This apparent contradiction may be explained by the dual nature of grain damage assessment: oil extracts may effectively kill emerged adults and prevent F1 generation development (thus minimising weight loss), while being unable to prevent initial oviposition and early larval development resulting in visible exit holes, which are counted as grain damage. These findings align with previous reports on plant derived biopesticides. Aruoren et al. (2022) reported similar protective effects of plant oils in stored grains.

Table 10: Efficacy of oil extracts on grain damage and weight loss in stored maize

Treatment	Dosage (g/50 g maize)	Grain Damage (%)	Weight Loss (%)
OPHE	1.0	39.15 ± 3.65^{ef}	2.75 ± 1.09^{a}
OPHE	2.0	34.22 ± 1.15^{de}	2.05 ± 1.98^{a}
OPHE	3.0	21.30 ± 2.69^{ab}	2.03 ± 2.01^{a}
GHE	1.0	15.58 ± 7.15^{a}	2.33 ± 1.90^{a}
GHE	2.0	17.17 ± 2.71^{ab}	1.80 ± 0.92^{a}
GHE	3.0	20.15 ± 2.92^{ab}	1.43 ± 1.17^{a}
APHE	1.0	29.36 ± 5.38^{cd}	2.09 ± 0.09^{a}
APHE	2.0	24.25 ± 4.38^{bc}	1.80 ± 1.04^{a}
APHE	3.0	23.02 ± 1.73^{bc}	1.68 ± 2.07^{a}
Control	0.0	45.58 ± 2.51^{f}	11.97 ± 7.98^{b}

Values are mean ± SD (n=4). Different superscript letters within columns indicate significant differences ($p<0.05$, DMRT

Comparative efficacy and practical implications

When comparing formulations, aqueous extracts offer the optimal balance of efficacy, cost-effectiveness, and ease of application for smallholder farmers. They are simple to prepare, require no specialised equipment beyond boiling water and filtration materials, and use water as a safe, readily available solvent. This agrees with the study of Ajelara, 2022 on the Comparative repellency of selected plants to adult *Anopheles gambiae*. However, aqueous extracts have a limited shelf life and may require the addition of preservatives for extended storage. Oil extracts, while highly effective against fungi, require organic solvents and an extraction apparatus, making them less accessible to resource limited farmers. Their higher cost may be justified for high value seed storage or when combined antifungal and insecticidal activity is required. Powder formulations offer the advantages of simplicity, stability, and direct application without the need for solvent extraction. However, they showed generally lower efficacy, may cause dust during application, and require proper milling for uniform particle size. Based on this study, recommended application rates are: For maize protection: Ginger aqueous extract at 3.0 g/50 g (6% w/w), for cowpea protection: Onion peel aqueous extract or ginger aqueous extract at 3.0 g/50 g (6% w/w), for fungal control: Alligator pepper oil extract at 1.0 g/100 g (1% w/w)

Conclusion

This study demonstrated that onion peel, ginger, and alligator pepper possess significant biopesticidal potential against major storage fungi and insect pests of maize and cowpea. Phytochemical analysis confirmed the presence of active compounds, including phenolics, alkaloids, flavonoids, and tannins, with known antimicrobial and insecticidal properties. GC–MS profiling identified decanal (11.53%) in ginger, dodecadiene (7.01%) in onion peel, and octadecanoic acid (31.96%) in alligator pepper as key constituents contributing to biological activity. Alligator pepper oil extract demonstrated the strongest antifungal effect, achieving over 80% inhibition of *Aspergillus flavus* and *Penicillium species* at a concentration of 1.0 g/100 g. Ginger aqueous extract at 3.0 g/50 g provided effective protection in maize, reducing grain damage and weight loss to 1.20% and 1.33%, respectively, compared with 38.42% and 12.66% in untreated controls. In cowpea, several formulations achieved over 80% reduction in grain damage, confirming their broad spectrum potential. Aqueous extracts were the most practical and cost-effective option for smallholder farmers, as they are easy to prepare, safe, and sustainable. The adoption of these plant based biopesticides could reduce postharvest losses, improve grain quality, and enhance food security while minimising the environmental and health risks associated with synthetic pesticides.

Some limitations should be acknowledged. The 60-day storage period may not represent the longer durations typical of real storage systems, and the controlled laboratory conditions differ from the variable temperature and humidity experienced in field environments. The study did not assess synergistic effects among plant extracts, sensory attributes of treated grains, or the toxicological safety of residues. Laboratory scale applications may also differ from practical challenges encountered in large scale grain storage. Future studies should investigate long term efficacy under farmer storage conditions, evaluate formulation stability and shelf life, and test combinations of extracts or integration with other postharvest methods. Additional research on sensory quality, cost-effectiveness, and molecular mechanisms is recommended to support the large-scale application and adoption of these natural biopesticides.

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