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Comparative efficacy of aqueous and ethanolic botanical extracts for control of seed-borne fungal pathogens and preservation of sorghum seed quality during storage in Tanzania

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ABSTRACT

Seed-borne fungal pathogens are major constraints to sorghum seed quality, reducing germination, seedling vigor, and storage longevity. Although synthetic fungicides effectively control pathogens, their continued use raises concerns about environmental contamination, pathogen resistance, and human health. This study evaluated the efficacy of aqueous and ethanolic extracts of *Syzygium aromaticum* and *Zingiber officinale*, applied individually and in combination, against *Fusarium* spp. and *Alternaria* spp. and their potential to preserve sorghum seed quality during storage in Tanzania. Laboratory experiments were conducted using a Completely Randomized Design with three independent replicates. Botanical extracts prepared using distilled water and 70% ethanol were evaluated at 0.5, 1.0, and 1.5 mL/100 mL PDA using the poisoned-food technique, with Metalaxyl + Carbendazim and untreated medium serving as positive and negative controls, respectively. The most effective treatment was subsequently evaluated for seed quality preservation during nine months of ambient storage using four replicates of 100 seeds per treatment. Significant differences ($P \leq 0.05$) were observed among extraction solvents, botanical treatments, and concentrations. Ethanolic extracts demonstrated greater antifungal activity than aqueous extracts and performed comparably to the commercial fungicide. Ethanolic *S. aromaticum* extracts achieved 100% inhibition of both pathogens, while ethanolic *Z. officinale* extracts achieved 96-99% inhibition. Combined ethanolic extracts provided complete pathogen suppression at 1.5 mL/100 mL and maintained 96-98% germination after three months of storage. These findings demonstrate the potential of combined ethanolic extracts as sustainable alternatives to synthetic fungicides. Further studies should validate their efficacy under field conditions and optimize formulation for commercial application.

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INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the world's most important cereal crops and serves as a major staple food for millions of people living in arid and semi-arid regions because of its exceptional tolerance to drought, high temperatures, and poor soil fertility (Andronoiu and Nistor, 2025). Globally, annual sorghum production exceeds 60 million tonnes, with Africa contributing nearly 40% of total production (Mundia *et al.*, 2019). In Tanzania, sorghum is predominantly cultivated by smallholder farmers in semi-arid regions such as Dodoma, Singida, Shinyanga, Simiyu, Tabora, and Mwanza, where it contributes substantially to household food security, livestock feed, and income generation (Ng'ombe *et al.*, 2023).

Despite its adaptability, sorghum productivity remains constrained by several biotic stresses, among which seed-borne fungal pathogens are particularly important (Petkova *et al.*, 2026). Fungi associated with sorghum seeds reduce seed viability, impair germination, decrease seedling vigor, and lower grain quality during storage and after planting (Martín *et al.*, 2022). Among these pathogens, *Fusarium* spp. and *Alternaria* spp. are considered two of the most destructive because they cause seed rot, seed discoloration, seedling blight, poor crop establishment, and, in some cases, mycotoxin contamination (Golosna *et al.*, 2026). Under favorable environmental conditions, these pathogens can cause substantial reductions in seed quality and grain yield, especially where untreated farmer-saved seed is used for subsequent planting seasons (Martín *et al.*, 2022).

Management of seed-borne fungal pathogens in sorghum relies mainly on synthetic fungicides (Palmieri *et al.*, 2022). Although these chemicals effectively suppress fungal infections, their prolonged use has raised increasing concerns regarding environmental contamination, adverse effects on beneficial microorganisms, development of fungicide-resistant pathogen populations, and potential risks to human health (Gikas *et al.*, 2022). Commercial fungicides remain expensive and are often inaccessible to many smallholder farmers in developing countries, including Tanzania (Onyancha *et al.*, 2026). Consequently, there is increasing interest in identifying affordable, environmentally friendly, and locally available alternatives that can provide effective disease management while reducing dependence on synthetic pesticides (Shang *et al.*, 2024).

Botanical extracts have emerged as promising alternatives because they contain diverse bioactive phytochemicals with antifungal activity (Dantas *et al.*, 2025). Numerous studies have demonstrated that plant-derived compounds such as phenolics, flavonoids, terpenoids, and essential oils inhibit fungal growth through multiple mechanisms, including disruption of fungal cell membranes, inhibition of enzyme activity, interference with cellular respiration, and suppression of spore germination (Jalendiran *et al.*, 2026). Among medicinal plants with demonstrated antifungal potential, *Syzygium aromaticum* (clove) and *Zingiber officinale* (ginger) have received considerable attention owing to their high concentrations of eugenol, gingerols, shogaols, and other biologically active compounds that exhibit broad-spectrum antimicrobial properties (Jalendiran *et al.*, 2026).

Previous studies have reported the antifungal activity of clove and ginger extracts against several postharvest and seed-borne fungal pathogens (Jeewon *et al.*, 2024). However, most investigations have evaluated individual plant extracts, focused on a single extraction solvent, or assessed only in vitro fungal inhibition (Amenu *et al.*, 2024). Limited information is available on the comparative efficacy of aqueous and ethanolic extracts applied individually and in combination for simultaneous suppression of *Fusarium* spp. and *Alternaria* spp. while preserving sorghum seed quality during prolonged storage under tropical conditions. Furthermore, comparable information under Tanzanian conditions remains scarce.

Therefore, this study evaluated the comparative efficacy of aqueous and ethanolic extracts of *Syzygium aromaticum* and *Zingiber officinale*, applied individually and in combination, for controlling *Fusarium* spp. and *Alternaria* spp. and for maintaining germination, seedling vigor, and overall seed quality of stored sorghum seeds. The findings provide scientific evidence supporting the development of environmentally sustainable botanical bio-fungicides as alternatives to synthetic fungicides for sorghum seed protection in Tanzania.

MATERIALS AND METHODS

Experimental site

The study was conducted in Arusha Region, Tanzania. Laboratory-based procedures for pathogen identification and evaluation of fungicidal activity were carried out at the Nelson Mandela African Institution of Science and Technology (NM-AIST), Botanical extract preparation conducted at the Tanzania Plant Health and Pesticide Authority (TPHPA). The study ran from October 2024 to November 2025.

Collection of sorghum seeds and botanical materials

Approximately 20 kg of untreated sorghum (*Sorghum bicolor* L. Moench) seeds of the MACIA variety were collected from smallholder farmers' storage facilities in Mkalama District (Singida Region), Tanzania, during the 2024/2025 cropping season. Seed samples were collected before chemical treatment to ensure natural contamination by seed-borne fungal pathogens. Fresh flower buds of *Syzygium aromaticum* (clove) were collected from Kilindi District, Tanga Region, while mature rhizomes of *Zingiber officinale* (ginger) were obtained from Same District, Kilimanjaro Region. Plant materials were transported to the laboratory in clean labelled polyethylene bags for extraction and further processing.

Isolation and identification of seed-borne fungal pathogens

Seed-borne fungal pathogens were detected using the standard blotter method described by the International Seed Testing Association (ISTA, 2025). Seeds were surface-disinfected by immersion in 1% sodium hypochlorite solution for 2 min, rinsed three times with sterile distilled water, and air-dried under aseptic conditions.

Four hundred seeds were examined using four replicates of 100 seeds each. Seeds were placed on sterile moistened blotter paper in 90-mm Petri dishes and incubated at 25 ± 2°C under alternating 12-h light and 12-h dark photoperiods for seven days under controlled laboratory conditions.

Following incubation, fungal colonies emerging from infected seeds were examined using stereoscopic and compound microscopes. Preliminary identification was based on colony morphology, pigmentation, growth characteristics, conidial morphology, and hyphal structures using standard taxonomic identification keys.

Representative colonies of *Fusarium* spp. and *Alternaria* spp. were aseptically transferred onto Potato Dextrose Agar (PDA) for purification. Pure cultures were obtained by repeated hyphal-tip subculturing until uniform colonies free from contamination were established.

The purified fungal isolates were further confirmed by molecular identification. Genomic DNA was extracted using the CTAB method, and the internal transcribed spacer (ITS) region was amplified using the universal primers ITS1 and ITS4 (The primer used for *Fusarium* spp was ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATFC-3'); *Alternaria* spp was ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (TCCTCCGCTTATTGATATGC-3') PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet illumination. The expected ITS amplicons confirmed the identity of *Fusarium* spp. and *Alternaria* spp.

Preparation of botanical extracts

Flower buds of *S. aromaticum* and rhizomes of *Z. officinale* were thoroughly washed with tap water,

rinsed with distilled water, and shade-dried at ambient temperature (25–28°C) for two weeks to minimize degradation of heat-sensitive phytochemicals. The dried materials were ground separately into fine powder using a disinfected electric grinder and sieved through a 1-mm mesh.

For each plant species, 5, 10, and 15 g of powdered material were separately extracted in 100 mL of sterile distilled water or 70% ethanol to obtain extract concentrations of 5%, 10%, and 15% (w/v), respectively. The mixtures were kept at room temperature for 24 h with intermittent shaking to facilitate extraction of bioactive compounds.

Extracts were filtered through double-layer sterile muslin cloth followed by Whatman No. 1 filter paper. Ethanolic filtrates were concentrated under reduced pressure using a rotary evaporator to remove the solvent and subsequently reconstituted with sterile distilled water to restore their original concentrations. Fresh aqueous extracts and reconstituted ethanolic extracts were stored at 4°C until use.

In vitro evaluation of antifungal activity

The antifungal activity of aqueous and ethanolic extracts of *S. aromaticum* and *Z. officinale*, applied individually and in combination, was evaluated against *Fusarium* spp. and *Alternaria* spp. using the poisoned-food technique with slight modifications of previously described procedures.

Molten Potato Dextrose Agar cooled to approximately 45°C was amended with each botanical extract at the respective concentrations before pouring approximately 15 mL into sterile 90-mm Petri dishes under aseptic laboratory conditions throughout. After solidification, a 5-mm mycelial disc excised from the actively growing margin of a fourteen-day-old fungal culture was placed at the centre of each plate.

Potato Dextrose Agar amended with Metalaxyl + Carbendazim served as the positive control, whereas unamended PDA served as the negative control throughout the antifungal evaluation. Treatments were arranged in a Completely Randomized Design (CRD) with three independent replicates.

Plates were incubated at 25 ± 2°C until fungal colonies in the untreated control completely covered the Petri dish. Colony diameter was measured in two perpendicular directions, and percentage inhibition of mycelial growth was calculated as;

The percentage inhibition of fungal growth was calculated using the formula:

$$\text{Antifungal activity (Inhibition (\%))} = \frac{(C-T)}{C} \times 100$$

where:

C= colony diameter of fungal growth in the control plate (mm)

T= colony diameter of fungal growth in the treated plate (mm)

Seed treatment and storage

The botanical treatment producing the highest antifungal activity during the in vitro bioassay was selected for seed treatment. Sorghum seeds were uniformly coated with the selected botanical extract under sterile laboratory conditions, thoroughly mixed to ensure complete coverage, and air-dried at room temperature before storage.

Seeds treated with Metalaxyl + Carbendazim at the manufacturer's recommended rate served as the positive control, whereas untreated seeds served as the negative control.

All seed samples were packed in labelled paper bags and stored under ambient laboratory conditions (25 ± 2°C and 60–85% relative humidity) for nine months. Seed quality was evaluated after 3, 6, and 9 months of storage.

Germination test

Seed germination was evaluated according to ISTA (2025) using the between-paper method. Four replicates of 100 seeds were prepared for each treatment. Seed rolls were incubated in a germination chamber maintained at 25 ± 2°C and 90 ± 5% relative humidity.

Final count was recorded on day ten. Only normal seedlings were considered for determination of germination percentage.

Measurement of seedling growth and vigor

Ten normal seedlings were randomly selected from each treatment on the tenth day after germination. Shoot length was measured from the collar region to the tip of the shoot, whereas root length was measured from the collar to the tip of the primary root. The same seedlings were dried in a forced-air oven at 70 ± 1°C for 24 h to determine seedling dry weight.

Seedling Vigor Index-I (SVI-I) and Seedling Vigor Index-II (SVI-II) were calculated according to Abdul-Baki and Anderson (1973) using the following equations:

SVI-I = Germination (%) × (Shoot length + Root length)

SVI-II = Germination (%) × Seedling dry weight

Statistical analysis

The experiment was conducted using a Completely Randomized Design (CRD). Data were subjected to one-way analysis of variance (ANOVA) using Statistica version 8.0 software. Treatment means were separated using Fisher's Least Significant Difference (LSD) test at the 5% probability level. Data are presented as mean ± standard error (SE), and statistical significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Morphological and molecular identification of seed-borne fungi

Morphological examination of naturally infected sorghum seeds revealed *Fusarium* spp. and *Alternaria* spp. as the predominant seed-borne fungi. *Fusarium* isolates produced abundant white-to-pink aerial mycelia and sickle-shaped macroconidia, whereas *Alternaria* isolates formed dark olive-green colonies with multicellular, septate and beaked conidia (Fig. 1a). These diagnostic features are consistent with the characteristic morphology of the two genera and indicate that both pathogens were present in the sampled seed lots (Zida *et al.*, 2008).

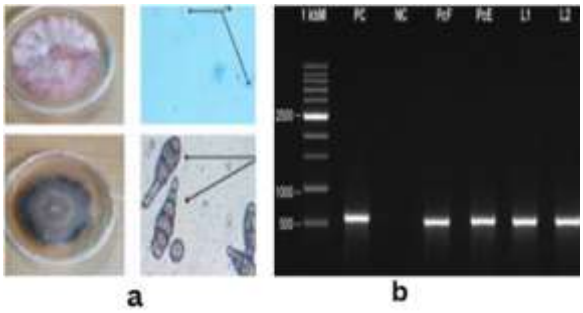


Fig. 1. Morphological and molecular identification of seed-borne fungi: (a) colony and conidial characteristics and (b) ITS-PCR products of approximately 750 bp.

PCR amplification of the internal transcribed spacer region using ITS1 and ITS4 primers generated clear products of approximately 750 bp for the representative isolates (Fig. 1b), supporting the morphological identification. The agreement between morphological and molecular observations strengthened confidence in the identification of the isolates used in the subsequent antifungal experiments. The occurrence of *Fusarium* spp. and *Alternaria* spp. is agronomically important because infected seeds can serve as sources of fungal inoculum, reduce germination and seedling establishment, and contribute to seed deterioration and potential mycotoxin contamination(Ferrigo *et al.*, 2016; Fous and Bránayik, 2026).

These findings highlight the importance of controlling seed-borne fungal pathogens before planting rather than waiting until the crop has already emerged and diseases have developed. Maintaining seed health is especially important for farmers who save and reuse their seeds for the next planting season, as infected seeds can carry fungal pathogens from one season to another. Therefore, this study provides useful information on the potential of botanical extracts to control seed-borne fungi while maintaining the quality of sorghum seeds during storage. However, the identification of Fusarium and Alternaria does not mean that these were the only fungal genera present in the sampled seeds, as other fungal species may also have been associated with them.

Table 1. Inhibition effect of ethanolic extracts of *S. aromaticum, Z. officinale*, and their combination against *Fusarium* spp. grown at different incubation periods under *in vitro* conditions

Plant extract	Solvent for extraction	Concentration (% v/v; mL extract/ 100 mL PDA)	Day 5	Day 6	Day 9	Day 10	Day 14
S. aromaticum	Ethanol	0.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	95.03 ± 0.00e
	Ethanol	1	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	96.27 ± 0.00d
	Ethanol	1.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	98.89 ± 0.13ab	97.51 ± 0.00c
Z. officinale	Ethanol	0.5	88.62 ± 0.48b	83.34 ± 0.69b	72.96± 0.52d	66.22± 1.20f	56.24 ± 0.40g
	Ethanol	1	100.00 ± 0.00a	100.00 ± 0.00a	90.29 ± 0.53c	86.63 ± 0.37e	60.66 ± 0.50f
	Ethanol	1.5	100.00 ± 0.00a	100.00 ± 0.00a	97.01 ± 0.29b	92.66± 0.30d	88.26 ± 0.36e
S. aromaticum + *Z. officinale*	Ethanol	0.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	97.41 ± 0.00c	96.27± 0.00d
	Ethanol	1	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	99.89 ± 0.00a	99.86 ± 0.00a
	Ethanol	1.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Chemical fungicide (Positive control)			100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Untreated (Negative control)			0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00e	0.00 ± 0.00g	0.00 ± 0.00h
F-statistics			42244.00	20468.00	15220.00	5424.20	18320.00
P-value			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P=0.05 from each other using Fisher's Least Significant Difference (LSD) test.

Table 2. Inhibition effect of aqueous extracts of *S. aromaticum*, *Z. officinale*, and their combination against *Fusarium* spp. grown at different incubation periods under *in vitro* conditions

Plant extract	Solvent for extraction	Concentration (% v/v; mL extract/ 100 mL PDA)	Day 5	Day 6	Day 9	Day 10	Day 14
Syzygium aromaticum	Aqueous	0.5	32.94 ± 1.07e	31.25 ± 0.61i	30.09 ± 0.38h	26.98 ± 0.57f	25.46 ± 0.00e
	Aqueous	1	46.53± 0.00c	42.50 ± 0.42e	41.67 ± 0.44f	35.31 ± 0.00d	31.67 ± 0.00d
	Aqueous	1.5	47.95± 1.60c	51.67 ± 0.47c	51.61 ± 0.00c	41.78 ± 0.00c	39.13± 0.00c
Zingiber officinale	Aqueous	0.5	20.84 ± 2.08g	34.83 ± 1.03h	23.38± 0.38i	22.38± 0.00g	21.60± 0.63f
	Aqueous	1	32.21 ± 0.76e	43.43 ± 1.40d	36.08± 0.50g	26.83 ± 0.57ef	25.46 ± 0.00e
	Aqueous	1.5	53.31 ± 0.76b	51.49± 0.39c	43.24± 0.76d	34.74± 0.31d	31.67± 0.00d
S. aromaticum + *Z. officinale*	Aqueous	0.5	24.71 ± 0.64f	35.56 ± 0.64g	35.31 ± 0.00h	27.84 ± 0.41e	25.46± 0.00e
	Aqueous	1	42.14 ± 0.64d	41.60± 0.48f	42.80 ± 1.83e	35.31 ± 0.00d	31.67± 0.00d
	Aqueous	1.5	52.55 ± 0.94b	58.81± 0.47b	55.64 ± 0.67b	46.95 ± 0.57b	40.37± 0.00b
Chemical fungicide (Positive control)			100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Untreated (Negative control)			0.00 ± 0.00h	0.00 ± 0.00j	0.00 ± 0.00j	0.00 ± 0.00h	0.00 ± 0.00g
F-statistics			633.13	1303.42	1277.83h	5206.20	16214.50
P-value			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P=0.05 from each other using Fisher's Least Significant Difference (LSD) test.

Antifungal activity against *Fusarium* spp.: Influence of extraction solvent and concentration

Antifungal activity against *Fusarium* spp. differed significantly among botanical species, extraction solvents, concentrations and incubation periods ($P < 0.001$; Tables 1 and 2). Ethanolic extracts consistently produced greater inhibition than aqueous extracts. On day 14, ethanolic *S. aromaticum* inhibited mycelial growth by 95.03–97.51%, while *Z. officinale* produced 56.24–88.26% inhibition. The combined ethanolic extract maintained 96.27% inhibition at 0.5 mL/100 mL and achieved 99.86–100% inhibition at 1.0 and 1.5 mL/100 mL, respectively. The highest combined concentration was statistically comparable to Metalaxyl + Carbendazim and remained fully inhibitory throughout the 14-day incubation period.

In contrast, aqueous extracts produced moderate and less persistent inhibition. At day 14, inhibition ranged from 25.46 to 39.13% for *S. aromaticum*, 21.60 to 31.67% for *Z. officinale*, and 25.46 to 40.37% for the combined extract. Although all aqueous treatments were superior to the untreated control, fungal growth increased with incubation time, showing that their inhibitory effect was less durable than that of the ethanolic formulations.

The strong solvent effect may reflect differences in the types and quantities of bioactive compounds recovered

by the extraction media. Ethanol and water differ in polarity and may therefore recover different proportions of plant constituents. However, the extent to which these differences explain antifungal activity depends on the plant material and extraction procedure (El-Saadony *et al.*, 2025; Lee *et al.*, 2024). In the present study, eugenol, gingerols, shogaols and other individual phytochemicals were not quantified. Consequently, the greater efficacy of ethanolic extracts demonstrates the importance of extraction solvent under the tested conditions but does not establish which compounds were responsible for the observed inhibition.

A concentration-dependent response was evident, particularly for *Z. officinale* and the combined extracts. Higher concentrations generally maintained stronger inhibition as incubation progressed. The superior performance of *S. aromaticum* may be associated with eugenol and other constituents reported to possess antifungal activity. Previous research has demonstrated antifungal activity of clove-derived preparations against *Fusarium oxysporum*, supporting the potential of clove as a source of antifungal compounds (Muñoz Castellanos *et al.*, 2020). Ginger extracts have also been reported to inhibit the growth of plant-pathogenic fungi, including *Fusarium solani*, although the magnitude of inhibition depends on the fungus and treatment conditions(El-Beltagi *et al.*, 2025). These findings provide relevant context for the activity observed in the present study, but they do not establish the specific biochemical mechanisms responsible for inhibition by the extracts tested here.

The differences between the two botanical extracts suggest that their antifungal performance depended not only on the extraction solvent but also on the botanical material and concentration applied. In particular, the comparatively high and sustained inhibition produced by ethanolic *S. aromaticum* indicates that clove extract was highly active against *Fusarium* spp. under the experimental conditions. By contrast, the greater variation in inhibition observed with ginger extract suggests that its efficacy was more dependent on concentration. These findings highlight the importance of evaluating botanical extracts across multiple concentrations and incubation periods rather than relying on measurements taken at a single time point. Representative cultures in Fig. 2 visually confirm the near-complete suppression produced by ethanolic treatments compared with the continued colony development observed in aqueous treatments.

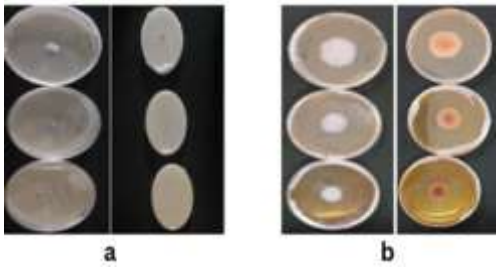


Fig. 2. Representative culture plates illustrating inhibition of *Fusarium* spp. by (a) ethanolic and (b) aqueous extracts

Antifungal activity against *Alternaria* spp. and performance of combined extracts

A similar treatment pattern was observed against *Alternaria* spp., with highly significant differences among treatments ($P < 0.001$; Tables 3 and 4). Ethanolic *S. aromaticum* maintained 96.02–98.67% inhibition by day 14, whereas ethanolic *Z. officinale* ranged from 62.57% at 0.5 mL/100 mL to 99.11% at 1.5 mL/100 mL. The combined ethanolic extract produced 97.34, 98.67 and 100% inhibition at 0.5, 1.0 and 1.5 mL/100 mL, respectively, and the highest concentration matched the chemical fungicide.

Aqueous preparations were less effective, and their activity declined with incubation time. At day 14, *S. aromaticum* inhibited *Alternaria* spp. by 18.50–32.36%, *Z. officinale* by 5.09–21.45%, and the combined extract by 19.54–39.72%. Nevertheless, the combined aqueous formulation consistently outperformed either aqueous extract alone at the higher concentrations, indicating that the combination improved observed antifungal activity even when water was used as the extraction medium. Fig. 3 further illustrates the contrast between the strong suppression obtained with ethanolic extracts and the residual colony growth in aqueous treatments.

Table 3. Inhibition effect of ethanolic extracts of *S. aromaticum*, *Z. officinale*, and their combination against *Alternaria* spp. grown at different incubation periods under *in vitro* conditions

Plant extract	Solvent for extraction	Concentration (% v/v; mL extract/ 100 mL PDA)	Day 5	Day 6	Day 9	Day 10	Day 14
S. aromaticum	Ethanol	0.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	96.02± 0.00
	Ethanol	1	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	97.34 ± 0.00
	Ethanol	1.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	98.67± 0.00
Z. officinale	Ethanol	0.5	90.65± 0.62b	83.66 ± 0.65b	76.80± 0.66c	72.26± 0.47c	62.57± 0.82e
	Ethanol	1	100.00 ± 0.00a	100.00 ± 0.00a	97.81 ± 0.36b	92.43 ± 0.33b	87.03 ± 0.42d
	Ethanol	1.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	99.11 ± 0.22a
S. aromaticum + *Z.officinale*	Ethanol	0.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	97.34 ± 0.00c
	Ethanol	1	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	98.67 ± 0.00b
	Ethanol	1.5	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Chemical fungicide (Positive control)			100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.01a	100.00 ± 0.00a
Untreated (Negative control)			0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00f
F-statistics			25233.00	23269.00	17307.00	29509.00	11139.80
P-value			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P=0.05 from each other using Fisher's Least Significant Difference (LSD) test.

Table 4. Inhibition effect of aqueous extracts of *S. aromaticum*, *Z. officinale*, and their combination against *Alternaria* spp. at different incubation periods under *in vitro* conditions

Plant extract	Solvent for extraction	Concentration (% v/v; mL extract/ 100 mL PDA)	Day 5	Day 6	Day 9	Day 10	Day 14
Syzygium aromaticum	Aqueous	0.5	30.28 ± 0.89g	28.87 ± 1.03g	23.22 ± 0.44g	20.40 ± 0.36f	18.50 ± 0.54g
	Aqueous	1	39.36± 0.66f	38.76 ± 0.43f	36.14 ± 0.58e	31.28 ± 0.88c	24.69 ± 0.48d
	Aqueous	1.5	48.18 ± 1.20e	45.99 ± 0.51d	43.85± 0.64c	42.63 ± 0.34b	32.36 ± 0.49c
Zingiber officinale	Aqueous	0.5	13.46 ± 0.69j	12.20 ± 0.43j	7.88 ± 0.46j	6.06 ± 0.45h	5.09± 0.66h
	Aqueous	1	21.20± 0.66i	17.86± 0.51i	12.92 ± 0.36i	15.99 ± 0.39g	10.84±0.53g
	Aqueous	1.5	28.35 ± 0.35h	27.74± 0.51h	26.15± 0.49h	25.92 ± 1.82d	21.45 ± 0.36e
S. aromaticum + *Z. officinale*	Aqueous	0.5	59.40 ± 0.74d	40.43 ± 0.60e	34.77± 0.58f	23.56± 0.55e	19.54 ± 0.38e
	Aqueous	1	62.60 ± 0.70c	53.15 ± 0.51c	42.17 ± 0.55d	31.12 ± 0.67c	31.91± 1.16c
	Aqueous	1.5	66.61 ± 0.84b	62.30± 0.71b	55.11 ± 0.35b	42.47± 0.34b	39.72± 0.23b
Chemical fungicide (Positive control)			100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
Untreated (negative control)			0.00 ± 0.00k	0.00 ± 0.00k	0.00 ± 0.00k	0.00 ± 0.00i	0.00 ± 0.00i
F-statistics			1611.80	2485.85	3541.84	1374.62	2459.10
P-value			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P=0.05 from each other using Fisher's Least Significant Difference (LSD) test.

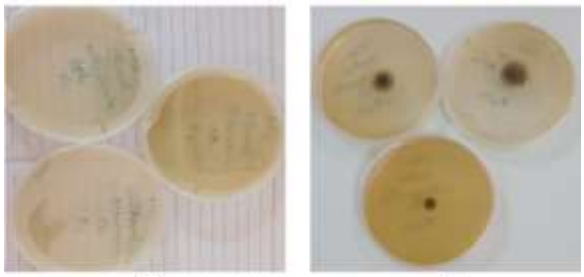


Fig. 3. Representative culture plates illustrating inhibition of *Alternaria* spp. by (a) ethanolic and (b) aqueous extracts

The response of *Alternaria* spp. further demonstrates that the efficacy of botanical extracts varied according to the pathogen, botanical material and concentration. Although ethanolic clove extract maintained consistently high inhibition, ginger extract showed a more pronounced concentration-dependent response, increasing from 62.57% inhibition at the lowest concentration to 99.11% at the highest concentration by day 14. This pattern suggests that the appropriate concentration may be particularly important when ginger extract is used individually (Bunevičius *et al.*, 2026). However, because the study did not investigate the physiological or molecular responses of the fungal isolates, the differences in susceptibility cannot be attributed to a specific resistance or sensitivity mechanism.

The combined ethanolic extract achieved complete inhibition of *Alternaria* spp. at 1.5 mL/100 mL, demonstrating its strong antifungal performance under the tested conditions. The combination also maintained high inhibition at the lower concentrations.

The strong performance of the combined formulation is due to the clove and ginger contain different bioactive constituents that may affect fungal growth through complementary processes (Rajkumar *et al.*, 2025). For example, eugenol has been associated with disruption of fungal membrane integrity, while ginger-derived compounds have been investigated for their effects on cellular metabolism. Such complementary activities could contribute to the observed inhibition. Nevertheless, these mechanisms were not measured directly in the present study and should be regarded as possible explanations rather than demonstrated processes.

Importantly, the complete inhibition observed with the combined ethanolic extract does not, by itself, establish synergism between clove and ginger. The combination may produce additive effects or derive much of its activity from the more potent component. Demonstrating synergy would require an experimental design that evaluates the individual and combined extracts at appropriate concentrations and quantitatively distinguishes synergistic from additive effects. The present findings therefore establish the high efficacy of the combined formulation under the tested conditions, while the nature of the interaction between its constituents remains to be determined.

The highest concentration of the combined ethanolic extract performed comparably to Metalaxyl + Carbendazim in the in vitro assay. This comparison is encouraging because it identifies a botanical formulation that warrants further evaluation as a potential seed-treatment preparation. However, comparable inhibition in vitro should not be interpreted as proof of equivalent effectiveness under farm storage or field conditions. Differences in environmental conditions, formulation stability, seed characteristics and application methods may influence performance outside the laboratory.

Effects on sorghum seed germination and seedling vigour during storage

Storage duration significantly reduced germination, seedling growth, dry matter accumulation and vigour in all treatments, but the magnitude of deterioration differed markedly among treatments (Figs. 4 and 5; Table 5). Seeds treated with the combined ethanolic extract maintained approximately 96%, 93% and 89% germination after three, six and nine months, respectively, and performed closely to the chemical fungicide. The combined aqueous treatment retained lower germination, approximately 89%, 84% and 79% over the same periods, while untreated seed declined to about 58% after nine months.

Table 5. Effect of aqueous and ethanolic extracts in combined *S. aromaticum* and *Z. officinale* (1.5 mL^{-1}) on seedling biomass and vigor indices of Sorghum seeds

Treatments	Storage period (Months)	Seedling dry weight (mg)	Seedling vigor index-I	Seedling vigor index-II
T1-Combined extracts (Aqueous)	3	158.7±0.92f	2608±10.8cd	14124±18.1c
	6	148.1±0.82g	2293±8.6d	12440±27.4d
	9	134.5±0.82h	1959±10.0e	10625±19.4e
T2-Combined extracts (Ethanolic)	3	186.5±0.90b	3379±13.2a	17904±21.5a
	6	175.2±0.79c	3036±10.2b	16118±13.3bc
	9	161.3±0.79e	2666±10.2cd	14194±23.9c
T3-Chemical fungicide (Metalaxyl+Carbendazim)	3	189.4±0.90a	3501±13.1a	18371±20.3a
	6	177.4±0.85c	3143±8.5ab	16498±21.8b
	9	164.2±0.91d	2778±11.6c	16256±15.6bc
T4-Untreated control (Negative control)	3	121.6±0.89i	1708±11.3f	9485±20.1f
	6	102.4±0.89j	1311±8.4g	7066±20.1g
	9	82.5±0.77k	951±9.6h	5423±17.9h
F-statistics		1556.8	6039.4	43999
P-value		< 0.001	< 0.001	< 0.001

Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P=0.05 from each other using Fisher's Least Significant Difference (LSD) test.

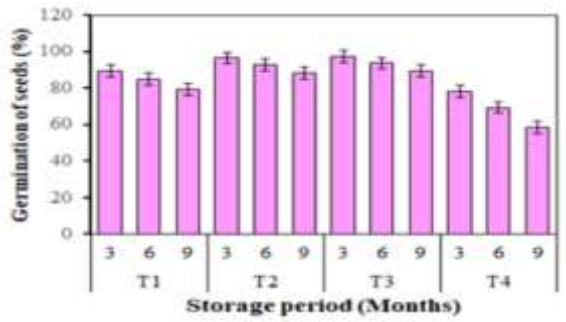


Fig. 4. Effects of combined *S. aromaticum* and *Z. officinale* on sorghum seed germination

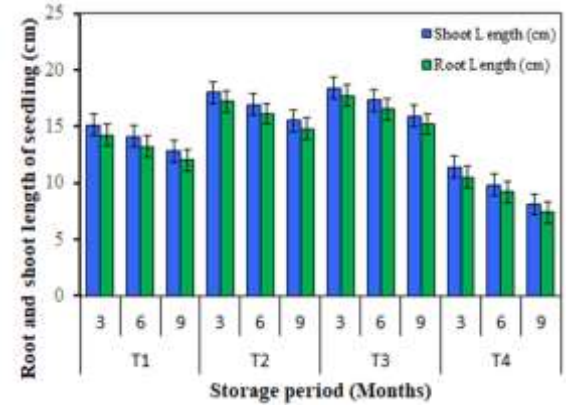


Fig. 5. Effects of combined *S. aromaticum* and *Z. officinale* on sorghum shoot and root lengths

Seedling growth followed the same pattern. The combined ethanolic treatment maintained longer shoots and roots than the aqueous treatment and untreated control throughout storage (Fig. 5). At three months, its seedling dry weight was 186.5 mg, Seedling Vigor Index-I was 3,379 and Seedling Vigor Index-II was 17,904. After nine months, these values remained at 161.3 mg, 2,666 and 14,194, respectively. By comparison, untreated seed declined from 121.6 to 82.5 mg in dry weight, from 1,708 to 951 in Seedling Vigor Index-I, and from 9,485 to 5,423 in Seedling Vigor Index-II (Table 5). The combined ethanolic treatment therefore slowed, but did not completely prevent, physiological ageing.

The findings lie in the relationship between fungal suppression and the preservation of seed performance. The combined ethanolic treatment was associated with both high antifungal activity and better maintenance of germination and seedling vigour during storage. This suggests that the potential benefit of the treatment extends beyond inhibition of fungal growth in culture to the preservation of important indicators of seed quality.

The preservation of seed quality may partly reflect the suppression of seed-associated fungi, which can damage seed tissues and compromise germination. Seed ageing may also involve lipid peroxidation, membrane leakage and enzyme inactivation (Xing *et al.*, 2025). By reducing fungal growth, botanical treatment may help limit pathogen-related damage and preserve the resources required for germination and early seedling development.

Although clove and ginger contain phenolic compounds that have been investigated for antioxidant activity (Ghasemzadeh *et al.*, 2010), the present study did not directly measure antioxidant activity, reactive oxygen species, lipid peroxidation or membrane integrity in treated seeds. It is therefore not possible to conclude that antioxidant protection was responsible for the improved storage performance. The observed differences may be explained, at least in part, by reduced fungal damage, while any additional contribution from antioxidant effects requires experimental confirmation.

The storage results also demonstrate that botanical treatment did not eliminate the effects of seed ageing. Germination, dry weight and vigour indices declined over time, including in seeds treated with the combined ethanolic extract. This observation is important because it shows that the treatment helped preserve seed quality but did not completely prevent deterioration during prolonged storage.

The distinction between slowing deterioration and preventing it completely is particularly relevant to practical seed management. The combined ethanolic formulation should therefore be considered a promising candidate for improving seed-quality retention rather than a means of eliminating all storage-related losses. Storage duration and environmental conditions remain important factors that may affect the longevity of treated seed.

Table 6. Mean antifungal activity (%) of ethanolic extracts of *S. aromaticum*, *Z. officinale*, and their combination against *Fusarium* spp. and *Alternaria* spp.

Fungicidal plant	Solvent for extraction	Concentration(% v/v; mL extract/ 100 mL PDA)	*Fusarium* spp. (Mean ± SE)	*Alternaria* spp. (Mean ± SE)
S. aromaticum	Ethanol	0.5	98.70± 0.00d	97.56 ± 0.03b
	Ethanol	1	98.40± 0.00c	98.80± 0.00ab
	Ethanol	1.5	99.77± 0.02ab	99.97± 0.02a
Z. officinale	Ethanol	0.5	77.91 ± 0.31f	80.97 ± 0.32d
	Ethanol	1	89.58 ± 0.15e	96.22 ± 0.13c
	Ethanol	1.5	96.33± 0.09b	99.86± 0.03ab
S. aromaticum + *Z. officinale*	Ethanol	0.5	99.60 ± 0.00bc	99.60± 0.00b
	Ethanol	1	100.00± 0.00a	100.00± 0.00a
	Ethanol	1.5	100.00± 0.00a	100.0± 0.00a
Chemical fungicide (Positive control)			100.00 ± 0.00a	100.00 ± 0.00a
Untreated (Negative control)			0.00 ± 0.00g	0.00 ± 0.00e
F-statistics			73285.00	77035.00
P-value			< 0.001	< 0.001

Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P=0.05 from each other using Fisher's Least Significant Difference (LSD) test.

Table 7. Mean antifungal activity (%) of aqueous extracts of *S. aromaticum*, *Z. officinale*, and their combination against *Fusarium* spp. and *Alternaria* spp.

Fungicidal plant	Solvent for extraction	Concentration (% v/v; mL extract/ 100 mL PDA)	*Fusarium* spp. (Mean ± SE)	*Alternaria* spp. (Mean ± SE)
Syzygium aromaticum	Aqueous	0.5	24.31 ± 0.23i	26.94± 0.28g
	Aqueous	1	36.24± 0.19f	36.62± 0.23f
	Aqueous	1.5	46.05± 0.20c	45.00± 0.29d
Zingiber officinale	Aqueous	0.5	22.28 ± 0.35j	9.23± 0.23j
	Aqueous	1	34.06 ± 0.18g	16.47± 0.25i
	Aqueous	1.5	44.64± 0.28d	25.32± 0.34h
S. aromaticum + *Z.officinale*	Aqueous	0.5	26.04± 1.24h	38.83± 0.40e
	Aqueous	1	38.37± 0.55e	47.23 ± 0.37c
	Aqueous	1.5	53.36± 0.29b	56.43± 0.25b
Chemical fungicide (Positive control)			100.00± 0.00a	100.00 ± 0.00a
Untreated (Negative control)			0.00 ± 0.00k	0.00 ± 0.00k
F-statistics			2972.73	9683.90
P-value			< 0.001	< 0.001

Each value is a mean ± standard error of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P=0.05 from each other using Fisher's Least Significant Difference (LSD) test.

The combined ethanolic treatment performed closely to the commercial fungicide in several seed-quality assessments.

However, the interpretation of this comparison should be based on the statistical results for each measured outcome and storage period. Numerical similarity or a nonsignificant difference does not, on its own, establish formal equivalence between the botanical formulation and the commercial fungicide. Nevertheless, the sustained germination and higher seedling vigour observed in the combined ethanolic treatment provide a strong justification for further investigation under practical storage conditions.

Overall treatment performance, practical implications and future research

The average comparisons confirmed that extraction solvent and botanical combination influenced antifungal efficacy (Tables 6 and 7; Fig. 6). At the highest concentration, ethanolic treatments achieved 96.33–100% average inhibition, whereas the best aqueous combination achieved 53.36% inhibition of *Fusarium* spp. and 56.43% inhibition of *Alternaria*

spp. Although *S. aromaticum* was generally more effective than *Z. officinale* individually, their combination provided the most consistent suppression of both pathogens.

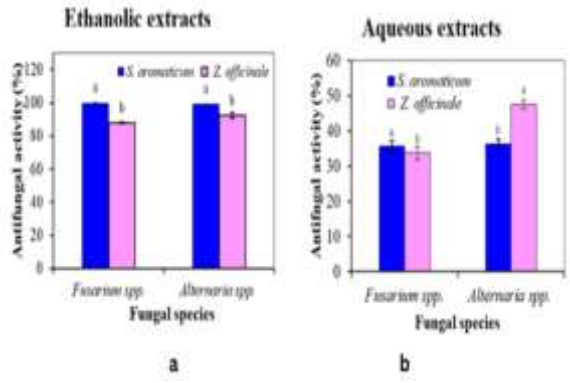


Fig. 6. Susceptibility of *Fusarium* spp. and *Alternaria* spp. to *S. aromaticum* and *Z. officinale* extracts: (a) ethanolic extracts and (b) aqueous extracts

Overall, the combined ethanolic extract at 1.5 mL/100 mL was the most promising treatment, achieving complete fungal inhibition in vitro and helping preserve sorghum seed germination and vigour during nine months of ambient storage. However, its practical effectiveness remains to be established under farm storage and field conditions.

Further research should quantify the extracts' bioactive constituents, assess formulation stability and seed safety, and determine whether the combined extracts act additively or synergistically. Multi-location field trials are also needed to evaluate seedling emergence, crop establishment and yield. These findings support further development of the combined botanical formulation as a potential sorghum seed treatment in Tanzania, pending validation of its safety, consistency and effectiveness under practical conditions.

CONCLUSION

The combined ethanolic extract of *Syzygium aromaticum* and *Zingiber officinale* at 1.5 mL/100 mL completely inhibited *Fusarium spp*. and *Alternaria* spp., performed comparably to Metalaxyl + Carbendazim, and substantially preserved sorghum seed germination and vigor during nine months of storage. Its superior activity over aqueous and individual extracts demonstrates the importance of solvent selection, concentration and complementary phytochemical interactions. The formulation is therefore a promising botanical seed treatment for sustainable management of sorghum seed-borne fungi, subject to field validation, formulation optimization, safety evaluation and quality standardization.

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