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The Potency of the Use of Single and Combined Extracts of *Allium sativum* (Garlic) and *Syzygium aromaticum* (Cloves) against Fungi Causing Tomato Post-Harvest Spoilage

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Abstract

Tomato is high in moisture, low in pH and contain nutrients that makes it very susceptible to attack by microorganisms particularly fungi causing more than 20% post-harvest losses. The study investigated the sensitivity of fourteen isolated fungi from spoiled tomato samples collected from six different markets within Maiduguri metropolis to extracts of garlic, and cloves singly and in combination. The sensitivity of the isolated microbes was tested (using Agar well diffusion method) with aqueous and methanol extracts of garlic, cloves and their combination. The methanol extracts of cloves and combination with garlic completely inhibited the growth of all the fungi isolated at 1.0 and 2.0 mg/mL concentrations. At 45% concentration, the aqueous garlic and combined extracts showed strong antifungal activity against all the fungi except for *Aspergillus terreus*, *Penicillium spp*, *Zygosaccharomyces bailii*, *Candida tropicalis*, and *Aspergillus ustus*. *Penicillium spp* and *Saccharomyces cerevisiae* were shown to be the most sensitive against aqueous extract of garlic with 3.4 mm and 3.2 mm zones of inhibition respectively at 100% concentration, while *Aspergillus parasitucus*, *Aspergillus oryzae*, and *Saccharomyces cerevisiae* are the most sensitive of the combined extract at the same concentration. However, all the tested fungal isolates resisted the aqueous extract of cloves at all the tested concentrations. The combined extract, therefore, exhibited the highest level of inhibition against the growth of the tested fungi at all concentrations and may therefore have potential to arrest post-harvest tomato spoilage.

Keywords: Tomato spoilage, fungi, garlic, cloves, fungal sensitivity

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Introduction

Allium sativum L. commonly called garlic and locally called 'tafarnuwa' in Hausa (Nigeria) is a spice from the family of Liliaceae. It is an herbaceous plant with a strong odour and pungent taste. The sulphur compounds in garlic are what give it its potent aroma and its health benefits. Garlic's antimicrobial properties have been acknowledged for a long time (Avwunugbe *et al*., 2024, Goli, 2024, Indira *et al*., 2024,). In a study conducted by Sallam *et al*. (2004), the impact of fresh garlic, garlic powder, and garlic oil on microorganisms in raw chicken sausages was investigated. The fresh garlic was found to have a better activity over the other two. Another study assessed the antimicrobial effects of dried garlic powders made using different drying methods on specific pathogenic microorganisms. Fresh garlic exhibited the highest activity, followed by freeze-dried powder (Rahman *et al*., 2006). Additionally, chopped garlic at various concentrations was found to inhibit the growth of microorganisms isolated from ground beef and raw meatballs (Aydin *et al*., 2007).

Syzygium aromaticum, commonly called cloves is a common spice used in almost all parts of Nigeria (Saeed and Tariq, 2008) and its anti-microbials are well reported (Majhi *et al*, 2024, Verma *et al*., 2024, Zhao *et al*., 2024). Cloves act as a carminative, boosting the production of hydrochloric acid in the stomach and aiding peristalsis (Saeed and Tariq, 2008). In dentistry, clove essential oil is used as a pain reliever for dental emergencies (Gislene *et al*., 2000). In an in vitro study by Nassan *et a*l. (2015) using the agar well diffusion method, clove extract demonstrated antibacterial activity against Staphylococcus aureus. Additionally, an *in vivo* study with 40 adult male albino rats confirmed the effectiveness of clove extract as a natural antimicrobial agent. Microorganisms consisting of *E. coli, S. aureus, B. thermosphacta, lactobacillus* and *P. fluorescens* growth were inhibited using clove powder (Kuang *et al.,* 2011). Clove has the potential to break down the cell walls and membranes of microorganisms, penetrate the cytoplasmic membranes or enter the cells, and subsequently inhibit the normal synthesis of DNA and proteins (Xu *et al*., 2016). Eugenol, the primary component of clove, can suppress the production of amylase and proteases in *Bacillus cereus*, and it also has the capability to deteriorate cell walls and cause cell lysis (Burt, 2004).

Various synthetic fungicides have been effectively employed to manage postharvest decay in fruits and vegetables. Nonetheless, significant concerns remain. These concerns encompass growing consumer unease about toxic and carcinogenic pesticide residues on food, the prevalence of fungicide-resistant fungal strains due to overuse, and environmental pollution. Consequently, new and effective methods for controlling post-harvest diseases are needed, which pose fewer risks to human health and the environment. We recently reported the isolation and pathogenicity of fungi causing post-harvest spoilage of tomatoes within Maiduguri metropolis (Danaski *et al*., 2022), therefore, this study investigated the antimicrobial potentials of *Allium sativum* and *Syzygium aromaticum* singly and in combination on the isolated tomato post-harvested pathogenic fungi.

Materials and Methods

Fungal Isolates

In Danaski et al. (2022) we reported the isolation and identification of fourteen (14) fungi from rotten tomato samples collected from six (6) different collection sites within Maiduguri metropolis. The fungi are *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus terreus*, *Aspergillus ustus*, *Candida tropicalis*, *Saccharomyces cerevesiae*, *Thielavia terricola*, *Cladosporium spp, Zygosaccharomyces bailii*, *Aspergillus oryzae* and *Penicillium spp*. This study therefore reports on the antifungal potentials of *Allium sativum* and *Syzigium aromaticum* singly and in combination on these isolated fungi.

Collection of Garlic and Cloves

Fresh garlic (*Allium sativum*) *L*. and cloves (*Syzygium aromaticum*) were purchased from Monday Market, Maiduguri, Nigeria. They were identified and authenticated at the Organic Chemistry Laboratory of the Department of Pure and Applied Chemistry, University of Maiduguri, where voucher specimen samples were deposited. The voucher specimen number for the garlic sample is CHM/20.007 and that of cloves is CHM/20/004. The cloves were briefly washed with water, shade dried and processed into powder using electric blender, while the garlic was ground freshly using metallic pestle and mortar to produce a paste.

Preparation of Methanol Extracts (ME)

To prepare the methanol extracts of garlic, cloves, and their combination, 300 g of each were soaked in separate round bottom flasks. For the combination, 150 g of garlic and 150 g of cloves were used. Each plant material was immersed in 500 mL of 85% methanol in water, ensuring complete coverage. The mixtures were shaken vigorously and left at room temperature, with shaking every 6 hours for three days. The extracts were then filtered through muslin cloth and Whatman No. 2 filter paper, and the filtrates were transferred to sterile universal bottles. These were concentrated using a vacuum evaporator at 40°C for 60 minutes to evaporate the solvent. The concentrated extracts were dried in an oven at 40°C for two days until a powder formed at the bottom of the bottles. The labelled bottles containing the methanol extracts were stored in a refrigerator at 4°C.

Preparation of Aqueous Extracts (AE)

Another form of extraction used in this study was using squeezed garlic extract, cloves extract and clove in squeezed garlic extract. Twenty gram (20 g) of fresh garlic were peeled to remove the peels, then ground and squeezed to get 100% garlic juice. Preparations of 80%, 65%, 45% and 20% were then prepared using sterilized distilled water. For aqueous cloves extract, 20 g of clove was macerated in 30 mL of sterile distilled water. The extracts were filtered, concentrated and evaporated to obtain clove extracts. Concentration of 2 g/mL was prepared and used as 100% clove formulation. Combined clove and garlic extract was prepared by combining the squeezed garlic juice with the clove extract (500 µL each of 100% garlic juice and clove extract) and percentages of 80%, 65%, 45% and 20% prepared using sterilized distilled water.

Phytochemical Screening of Extracts

Secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, saponins, anthraquinones and terpenoids present in the different extracts were screened using methods describe by Sofowora, (1993) and Trease and Evans, (2002).

Antifungal Activity Test

The antifungal activity of both aqueous and methanol extracts of cloves, garlic and their combination were determined using Agar well diffusion method as described by Abdallah, *et al*. (2017). Briefly, bottles containing 20 mL of Sabouraud dextrose agar were sterilized by autoclaving, then poured hot into sterile petri dishes (90 mm in diameter) and allowed to solidify at room temperature. A 100 µL inoculum of each selected fungal strain from broth cultures was spread over the agar plates using a sterile cotton swab. Six holes were made in each plate using a sterile 5 mm cork borer. Methanol extracts at concentrations of 0.125 g/mL, 0.25 g/mL, 0.50 g/mL, 1.0 g/mL, and 2.0 g/mL were added to five of the wells, while sterile distilled water was added to the sixth well as a control. The extracts were allowed to diffuse into the agar for an hour, then incubated at 37°C for 18-24 hours. After incubation, the inhibition zones were measured in millimetres as the diameter of the clear zones around the holes using a transparent ruler. All tests were performed in triplicate, and the results were recorded as mean ± SD. The same procedure was used for the aqueous extracts but using the following percentages 20%, 45%, 65%, 80% and 100%.

Statistical Analysis

All determinations were performed in triplicates and presented as mean ± standard deviation. Differences between two means were analysed using the student's t-test and considered significant at $P<0.05$.

Results

Phytochemical Constituents

The qualitative phytochemical screening of the methanol extracts of cloves (MEC), Garlic (MEG) and its combination (MEGC) revealed the presence of carbohydrates, cardiac glycosides, terpenoids and alkaloids in all the three extracts while anthraquinones were absent in all the three extracts. Saponins, tannins and flavonoids were present in cloves and in combination but absent in garlic extract. (Table 1).

The phytochemicals detected in the three aqueous extracts are shown in Table 2. The results also revealed the presence of carbohydrates, cardiac glycosides, terpenoids and alkaloids in all the three (3) extracts as was observed with the methanol extract. Anthraquinones were absent in all the extracts, whereas tannins, saponins, and flavonoids were found in cloves and the combination, but not in garlic.

Table 1: Some Phytochemical Constituents of Methanol Extracts of Garlic, Cloves and their Combination

S/No.	Phytochemicals	MEG	MEGC	MEC
1.	Carbohydrates	+	+	+
2.	Tannins	_	+	+
3.	Cardiac glycosides	+	+	+
4.	Saponins	_	+	+
5.	Flavonoids	_	+	+
6.	Alkaloids	+	+	+
7.	Free Anthraquinones	_	_	_
8.	Terpenoids	+	+	+

Key: + = present; - = absent; MEG=Methanol Extract of Garlic; MEGC= Methanol Extract of Cloves and Garlic; MEC=Methanol Extract of Cloves.

Table 2: Some Phytochemical Constituents of Aqueous extract of Garlic, Cloves and their Combination

S/No.	Phytochemicals	AEG	AEGC	AEC
1.	Carbohydrates	+	+	+
2.	Tannins	_	+	+
3.	Cardiac glycosides	+	+	+
4.	Saponins	_	+	+
5.	Flavonoids	_	+	+
6.	Alkaloids	+	+	+
7.	Free Anthraquinones	_	_	_
8.	Terpenoids	+	+	+

Key: + = present; - = absent; AEG=Methanol Extract of Garlic; MEGC= Methanol Extract of Cloves and Garlic; MEC=Methanol Extract of Cloves.

Anti-Fungal Potentials of Extracts of Garlic, Cloves and their Combination

Methanol Extracts

The extracts at 0.125 g/mL and 0.25 g/mL concentrations did not inhibit the growth of the different fungal isolates. At 0.5 g/mL concentration, the clove methanol extract inhibited the growth of 9 of the 14 isolated fungi

(Fig.1) with zones of inhibition ranging from 1.9 - 3.4 mm. Five of the fungi (*T. thericola*, *A. oryzae*, *Penecillium, Z. balli* and *Fusarium)* completely resisted the clove methanol extract at this concentration. The garlic and combined extracts did not affect the growth of any of the 14 isolated fungi at the 0.5 g/mL concentration. Significant ($p<0.05$) growth inhibitions were seen for all the isolated fungi at 1.0 and 2.0 g/mL concentrations (Figures. 2 and 3). In the clove extract *S. cerevisiae* and *A. niger* were the most sensitive at 1.0 and 2.0 g/mL respectively with zones of inhibition of 4.0 mm and 4.5 mm respectively. As for the combined extract, *A. parasitucus* and *A. terrus* were the most sensitive at the 1.0 g/mL and 2.0 g/mL concentrations but were not statistically ($p>0.5$) different from the resistance of the remaining fungi. The garlic extract did not show any fungal inhibition for all the concentrations.

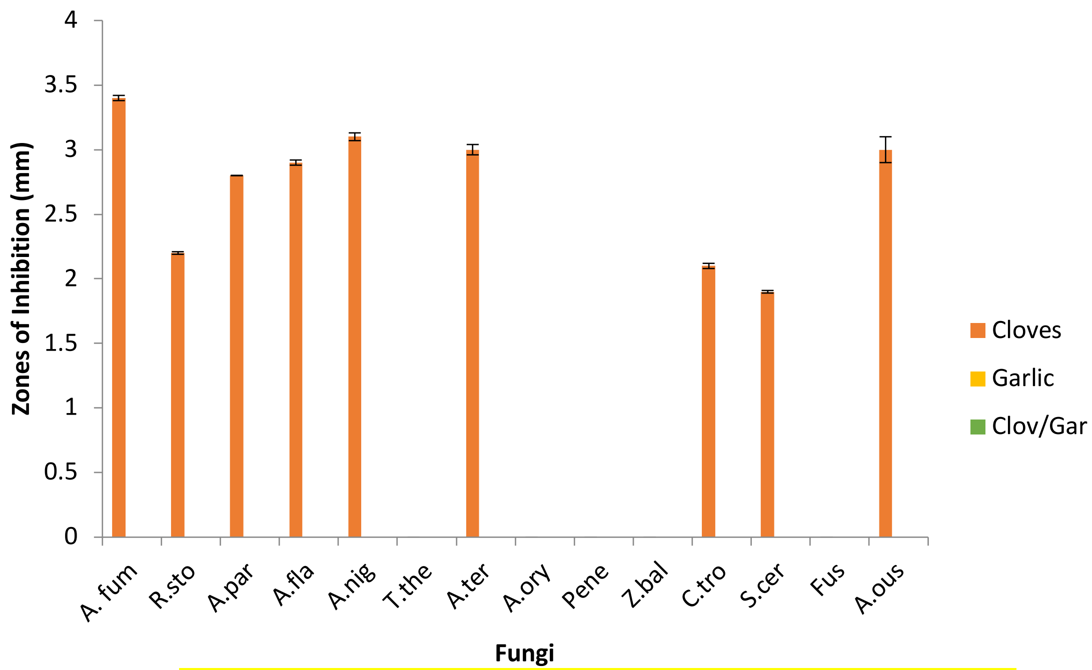

Fig.1: Efficacy of Methanol Extracts of Garlic, Cloves and their Combination on Growth of different Fungi at 0.50 g/mL Concentration

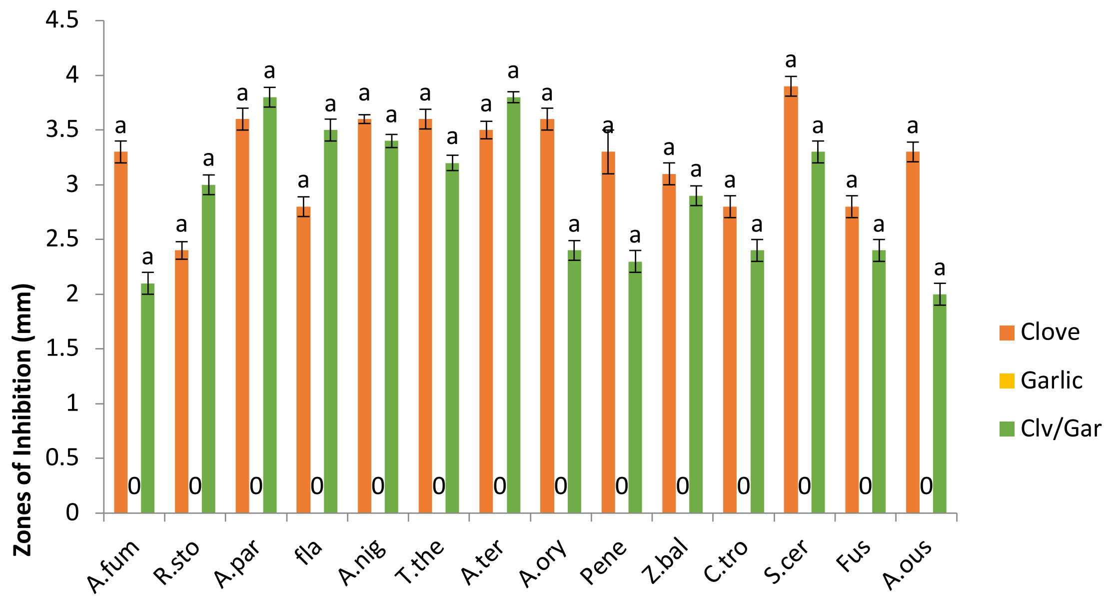

Fig. 2: Efficacy of Methanol Extracts of Garlic, Cloves and their Combination on Growth of different Fungi at 1.0 g/mL Concentration
Different letters on the same isolate indicate diference at p<0.5

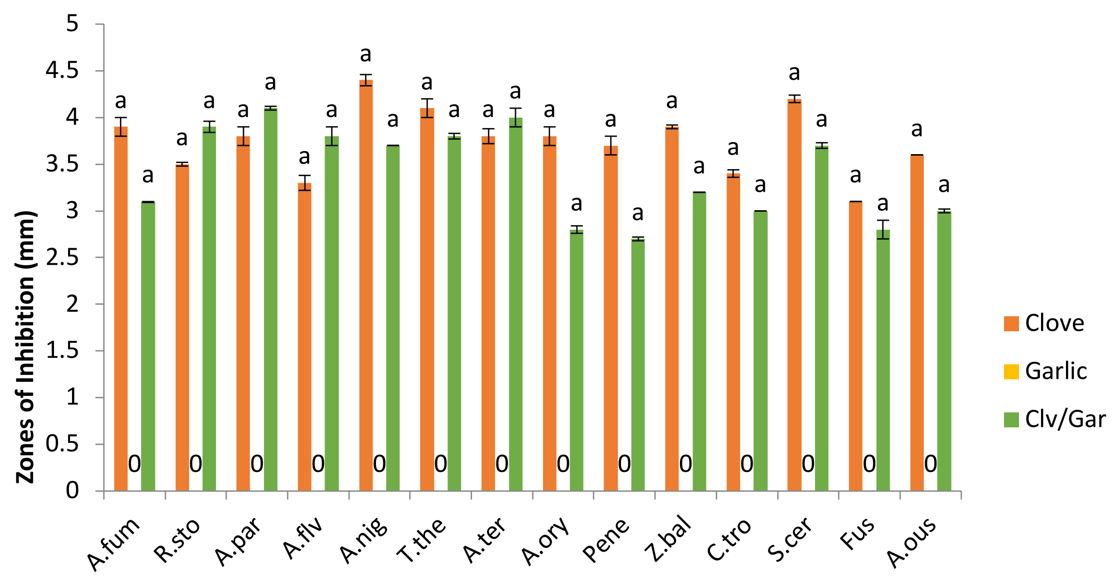

Fig. 3: Efficacy of Methanol Extracts of Garlic, Cloves and their Combination on Growth of different Fungi at 2.0 g/mL Concentration
Different letters on the same isolate indicate diference at p<0.5

Aqueous Extracts

The antifungal potentials of the aqueous extracts of garlic, clove and combination against the 14 different fungal isolates from the rotten tomatoes are presented in figures 4 to 7. At 45% concentration, the garlic and combined extracts showed strong antifungal activity against all the fungi except for *A. terrus*, *Penicillium*, *Z. balli*, *C. tropicalis*, *Fusarium* and *A. oustus*. The minimum effect of aqueous extracts of garlic was 0.7mm zone of inhibition, while for the combined aqueous extract, *A. fumigatus* and *T. stolonifer* showed the least zone of inhibition (1.3 mm). *A. penicillium* and *S. cerevisiae* were shown to be the most sensitive against aqueous extract of garlic with 3.4 mm and 3.2 mm zones of inhibition respectively, while *A. parasitucus*, *A. oryzae*, *S. cerevesae* and F*usarium* are the most sensitive to the combined aqueous extract. However, all the tested fungal isolates were resistant to the aqueous clove extract. *A. terrus, Penicillium*, *Z. balli*, *C. tropicalis*, *A. austus* and *Fusarium* were resistant to all the 3 extracts at 45% concentration (Figure 6) but were sensitive to the aqueous and the combined extracts of garlic at higher concentrations of 65%, 80% and 100% (Figures 5 to 7). The combined extract, therefore, exhibited the highest level of inhibition against all the sensitive fungi at all the concentrations tested and for some of the fungi the differences were significant (p<0.5).

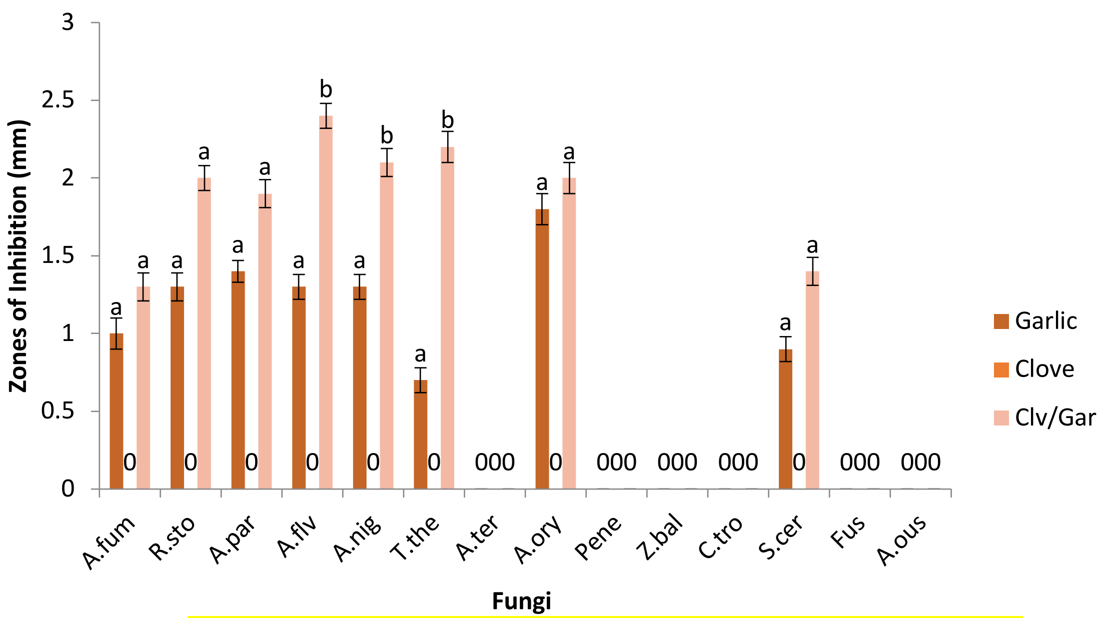

Fig. 4: Efficacy of Aqueous Extracts of Garlic, Cloves and their Combination on Growth of different Fungi at 45% Concentration
Different letters on the same isolate indicate diference at p<0.5

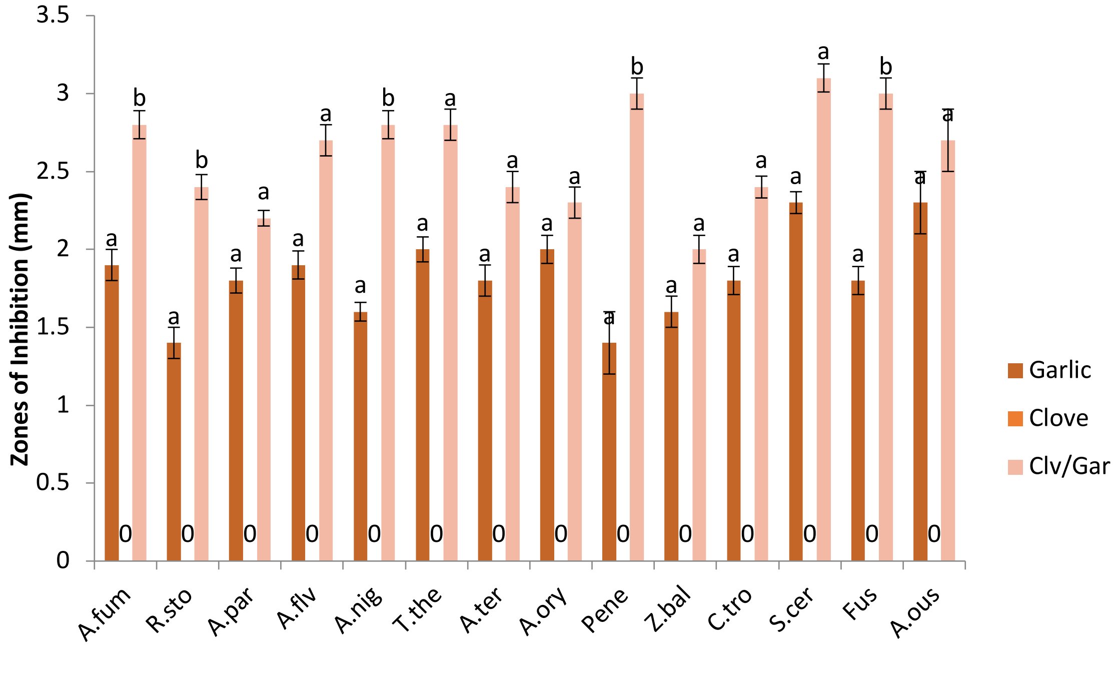

Fig. 5: Efficacy of Aqueous Extracts of Garlic, Cloves and their Combination on Growth of different Fungi at 65% Concentration

Different letters on the same isolate indicate diference at p<0.5

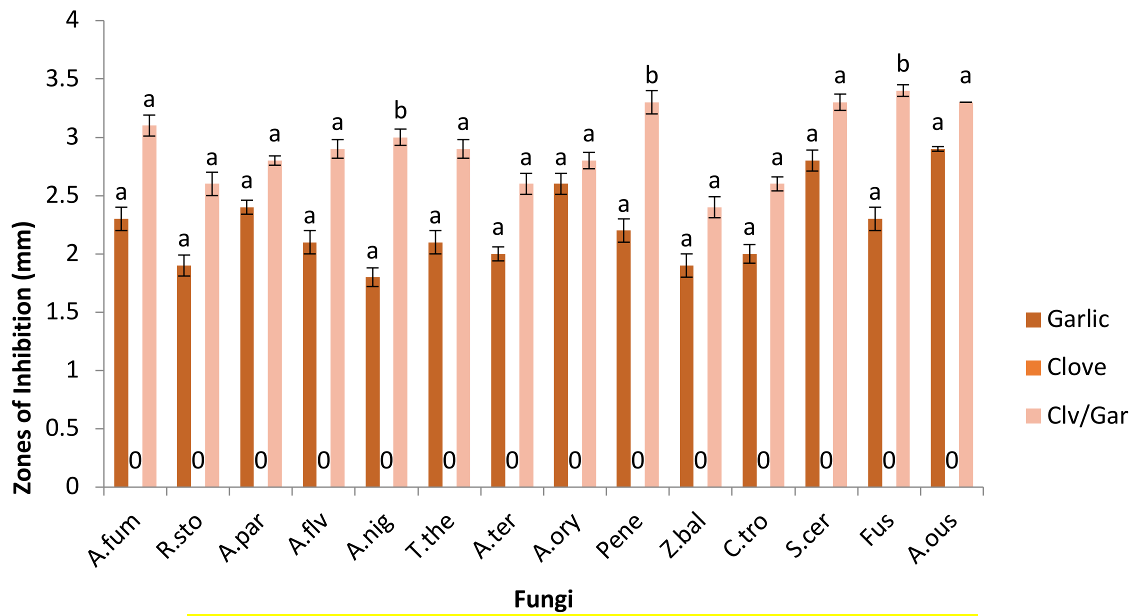

Fig. 6: Efficacy of Aqueous Extracts of Garlic, Cloves and their Combination on Growth of different Fungi at 80% Concentration

Different letters on the same isolate indicate diference at p<0.5

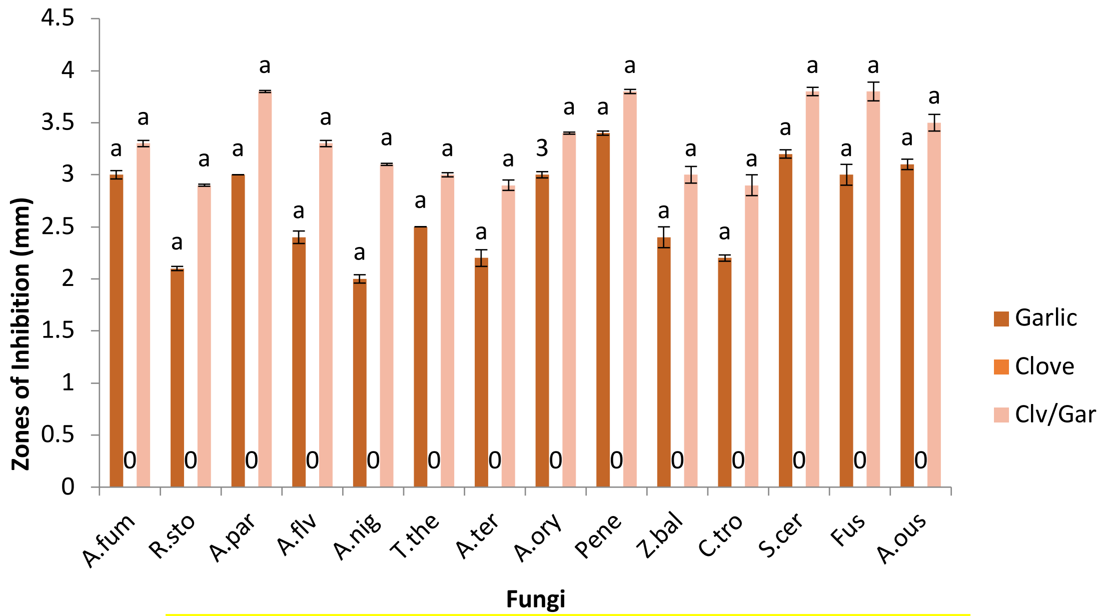

Fig. 7: Efficacy of Aqueous Extracts of Garlic, Cloves and their Combination on Growth of different Fungi at 100% Concentration

Different letters on the same isolate indicate diference at p<0.5

Discussion

Cloves and garlic are known for their antimicrobial properties (Nassan *et al*., 2015, Sulieman *et al*., 2023, Goli, 2024) and are commonly used as flavour enhancers in food preparations and in herbal medicines. In this study, the potentials of the spices singly and in combination to inhibit the growth of isolated fungi causing tomato spoilage were investigated. The methanol extract of garlic at 0.5 g/mL did not produce any antifungal effect on any of the 14 fungi tested, whereas all the organisms were found to be sensitive to the squeezed garlic extract and was concentration dependent. These results corroborate with the previous studies of Iwalokun *et al*., (2004) and Ankri and Mirelman (1999), where inhibition of *C. albicans* using fresh crushed garlic homogenate were reported. Historically cloves and garlic has been used conventionally and medically as anti-infective agents (Rosslenbroich and Stuebler, 2000). Garlic contains a sulphur compound called allicin, which is believed to be responsible for its antimicrobial activity (Ankri and Mirelman 1999). In addition to the allicin, garlic has at least 33 other sulphur containing compounds, several enzymes, 17 amino acids, and minerals (Newall *et al*., 1996; Salih *et al*., 2016). Other reported antimicrobials in garlic include possessing organic sulphur and phenolic compounds (Johnson *et al*., 2011, Oyedemi and Afolayan, 2011, Salih *et al*., 2016). Dutta *et al*., (2004), had also reported the excellent antifungal property of garlic, further supporting the results from this study, especially the squeezed garlic extract. Anjorin *et al*., (2008) reported that garlic effectively inhibited *Fusarium spp*., while Bhuiyan *et al*., (2008) found that garlic extracts were effective in controlling the growth of *Colletotrichum dematium* at a 20% concentration. Rathore and Kumar (2020) reported that garlic antifungal mechanism includes the inhibition of fungal cell wall synthesis, disruption of membrane integrity, and interference with fungal enzyme activity. The authors highlight the potential of garlic as a natural antifungal agent and suggests its use in combination with conventional antifungal treatments to enhance efficacy and reduce resistance.

All the 14 isolated fungi were found to be sensitive to the methanol extract of cloves, but not to the aqueous extract. The potential of clove as a natural preservative and its ability to extend shelf life have been documented (Kacániová *et al*., 2021). Research has shown that incorporating clove into foods can effectively prolong shelf life while maintaining the original taste, flavour, texture, and appearance (Haro-González *et al*., 2021). Cloves anti-fungal mechanism on the other hand is believed to be associated with disruption of the fungal cell membrane, inhibition of spore germination, and interference with fungal enzyme activity (Prakash and Srinivasan 2014). Therefore, the findings of this study suggest that clove could be used to control post-harvest spoilage in tomatoes.

The methanol and aqueous extracts of the combined extract showed better anti-fungal properties compared with the single extracts of the cloves and garlic and completely inhibited the growth of the fungi tested. The combination of these natural antifungal agents revealed enhanced efficacy than when they are used singly. Rathore and Kumar (2020) had earlier suggested combination of different antifungals to enhance efficacy and reduce resistance.

Several authors have reported the antifungal activity of both alkaloids (Singh *et al*., 2007, Wang *et al*., 2013, Miroslav *et al*., 2015) and terpenoids (Tang *et al*., 2000, Onishi *et a*l., 2000, Rao *et al*., 2010), which are two of the phytochemicals detected in all the extracts. These phytochemicals may possibly be responsible for the antifungal potentials of the spices studied, through mechanisms that include disruption of cell membrane integrity, inhibition of enzyme activity, interference with DNA and RNA synthesis, and modulation of gene expression (Thawabteh *et al.,* 2024; Raj *et a*l., 2024).

Conclusion

The methanol extracts of cloves singly and in combination with garlic completely inhibited the growth of isolated fungi at 1.0 and 2.0 mg/mL concentrations. The aqueous extracts of garlic singly and in combination completely inhibited the growth of fungi isolated at 65, 80 and 100% concentrations. The microbes were however resistant to the aqueous extract of cloves and methanol extract of garlic. Extracts of garlic, cloves and their combination, therefore, have the potentials to significantly reduce tomato post-harvest loss.

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