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# Control of Fungal Diseases of Tomato (*Lycopersicum esculentum* Mill) Fruits Using Various Plant Extracts

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## Abstract

*Plant harmful fungi induce symptoms of illnesses that significantly impair the quantity and quality of tomato (Lycopersicum esculentum) yields. The high-water composition of tomatoes renders it highly vulnerable to fungal decay. These fungi, responsible for the decay of tomatoes, generate mycotoxins that pose serious risks to human health. Thus, this research goal is to separate, characterize, and classify the fungi linked with the decline of tomato produce sold across four markets in Mkpat Enin and Ikot Abasi Local Government Areas of Akwa Ibom State, Nigeria. Among the fungal pathogens extracted from afflicted tomato fruits were Fusarium moniliformes, Rhizopus stolonifera, and Aspergillus alternata. Different concentrations of the extracts were individually introduced into Potato Dextrose Agar media. The fungal pathogens were at that point individually introduced into the media and left to incubate for 7 days. Findings revealed that $P < 0.05$ suppressed the mycelial evolution of the fungal pathogens at a greater concentration tested, with the extent of inhibition varying among different extracts. The results concerning the proportion inhibition of plant extracts on different fungus indicated that, at concentrations ranging from 40% to 80%, ethanolic extracts derived from Cola acuminata and Zingiber officinale significantly ($p<0.05$) restrained the growth of F. moniliformes, R. stolonifera, and A. alternata. Specifically, the ethanolic leaf extract of C. acuminata demonstrated moderate inhibition on A. alternata at 60%, while the ethanolic extract of Zingiber officinale exhibited low inhibition on F. moniliformes at 40%. At a concentration of 60% in ethanolic extracts, F. moniliformes experienced the greatest inhibition among the plant extracts, whereas A. alternata displayed the least inhibition. Pathogenicity testing indicated that Aspergillus alternata exhibited the largest decay of 25mm in diameter in a healthy tomato fruit, while Rhizopus stolonifera had the smallest decay diameter. Thus, to cover the ledge life of tomato fruits, therefore there's adequate need to implement proper handling techniques and utilize adequate storage facilities.*

**Keywords:** Tomato, Fungi, Spoilage, Plant Extracts, Inhibitory effects.

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## INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill) holds a significant position as a vegetable crop in Nigeria, appreciated across various societal strata and cultural contexts, thus enjoying widespread popularity. Its cultivation spans most regions of the country, where it serves diverse culinary purposes such as soup/stews, ketchups, and salads. Although much of tomato production occurs on a small-scale in-home garden, some commercial fields, particularly in the Northern regions, also contribute to its supply. Despite its adaptability to various climatic conditions, both wet and dry seasons, the consistent availability of tomatoes in the market remains uncertain. This unpredictability primarily stems from the impact of several diseases and the inadequacy of storage facilities (Sabongari and Aliero, 2004).

The crop frequently faces extensive muggings from fungal, bacterial, nematode, and viral pathogens, leading to a significant decrease in yield and shelf life. Field reports have indicated yield losses of up to 80%, with severe incidences of post-harvest diseases documented (Lathe *et al*., 2009). This grim scenario has rendered tomato cultivation challenging in certain regions of the country and during specific times of the year. Common tomato diseases encompass blights, wilts, stem canker, rots, leaf spots, mildew, and damping-off.

Tomato plants (*Lycopersicum esculentum* Mill) typically display a branching growth pattern, dispersal out between 60 to 180 cm (24 to 72 inches), and during fruiting, they may trail somewhat, although some varieties exhibit a more compact, upright form. Their leaves, often hairy, emit a strong odor and are pinnately compound, reaching lengths approximately 45cm (18 inches). Flowers consists of 5 petals, that are yellow, about 2cm (0.8 inches) in diameter, hangs in a downward cluster. The fruits, i.e berries, differs in length of 1.5 to 7.5 cm (0.6 to 3 inches) or larger in diameter. They commonly appear in shades of red, scarlet, or yellow, although green and purple variations occur. The shapes of the fruits varies from closely sphere-shaped to oval, elongated, or pear-shaped. Each fruit contains not less than two compartments filled with small seeds enclosed with jelly-like pulp (Okey *et al.* 2016).

Tomatoes, scientifically known as *Lycopersicum esculentum* and belonging to the Solanaceae family, are widely enjoyed both in their uncooked and administered procedures (Moneruzzaman *et al*, 2008). They are packed with vital nutrients such as vitamins A and C etc (Talves *et al*, 2009). Notably, tomatoes are rich in lycopene, known for its numerous health benefits. Due to their high water content, however, tomatoes are particularly vulnerable to decay by bacteria (Bai and Lindhout, 2006), despite their low content of sugar compared to different variation of fruits. Tomatoes come in various shapes, such as flattened and elongated when ripe, they are corpulent and roseate in color. Their acid content varies, such as yellow and varieties that is a little low in acid content. Tomatoes are versatile, serving as savory additions to soups, cooked dishes, or eaten fresh as fruits. Tomatoes are frequently used in a wide array of salads and beverages, which could be dried and ground into powders for various culinary uses (Effiuwevwere, 2000).

Furthermore, the intake of tomatoes is thought to give significant health benefits, particularly due to their lycopene content, which has been associated with preventing various cancers, improving skin protection against UV rays, reducing cardiovascular risks, and guarding against neurodegenerative diseases (Shidfar *et al*., 2010).

However, tomatoes face significant challenges, including climate change, pest infestations, inadequate rainfall, and microbial attacks, particularly by fungi. Fungal spoilage, driven by organisms like Aspergillus, Alternaria, Fusarium, Rhizopus, and others, poses serious health

risks due to the production of mycotoxins, which can cause mycotoxicoses upon ingestion (Baker, 2006). To ensure the economic value of tomatoes and safeguard public health, routine microbiological examination of tomatoes is essential, contributing significantly to economic development and public safety.

Fruits and vegetables naturally carry harmless microorganisms on their surfaces. However, throughout their growth, harvesting, transportation, and processing, they can become polluted through harmful microorganisms from bases such as animals, soil or humans (Sahlin *et al*, 2003; Brackett, 1988 and Colakoglu, 1983). Fresh produce has been linked to numerous outbreaks of foodborne illnesses worldwide especially in European nations. Various fungi, such as Alternaria, Rhizopus etc. which can contaminate food. Therefore, the potential for organic fruits and vegetables to be contaminated by moulds producing mycotoxins must be recognized.

Mould infection of tomatoes typically start in fields. Damage during harvesting and transportation, along with inadequate hygiene in the process of tomato paste creation, directly affects the quality of the paste by promoting the growth of both harmless and harmful moulds. Additionally, initial high levels of contamination make it challenging to achieve proper sterilization during processing (Anonymous, 2002; Aran *et al*., 1987; Brackett, 1988; Colakoglu, 1983; Gurgun and Halkman, 1996; Hasenekoglu, 1991). Due to the menace caused by fungal pathogens on tomato fruits pre harvest and post harvest, the main aim of this study was to isolate and identify the fungal pathogens associated with fruit rot of tomato and the evaluation of ethanolic leaf extracts of *Cola acuminata* and *Zingiber officinale* in controlling tomato fruit rot fungi in vitro. There was significant $P<0.05$ different

## MATERIALS AND METHOD

### Study Area

The study was conducted at the pathology/mycology laboratory of Akwa Ibom State University, Ikot Akpaden.

### Samples Collection

Fifty tomato samples were acquired from various farms and markets across the four regions of Akwa Ibom State, Nigeria: North, South, East, and West. These samples were then transported in sterilized polyethylene bags and moved to microbiological lab at Akwa Ibom State University, Ikot Akpaden, for fungal isolation. After being stored for 1 week to allow decay to develop, then fifty spoiled tomatoes were selected for this studies. Leaves of Cola acuminata and Zingiber officinale were sourced from the botanic garden of Akwa Ibom State University.

### Samples Processing

One gram from each spoiled tomato was meticulously cut using a disinfected blade which was then placed into Sabouraud dextrose potage for enrichment, where it was incubated for 24hours. Following this, sequential reductions of the illustrations were conducted in tenfold increments.

### Isolation of fungal pathogens and Morphological Identification

Fungal disease-causing organisms utilized for this study were obtained from contaminated tomato fruits. To separate these disease-causing organisms i.e pathogens, segments of the fruit that were affected were outwardly treated using 70% a solution of sodium hypochlorite (commonly known as bleach) in one minute. Following this, they were promptly rinsed in

three variations of disinfected purified water, dried using a Whatman No.1 sieve paper, then transferred to Petri dishes containing Potato Dextrose Agar (PDA). Each Petri dish accommodated four sections for inoculation. The dishes were then kept at a temperature of maintained at 28 ± 1 degree Celsius until noticeable fungal growth appeared. At the past 5 days, individual detaches were transferred onto newly prepared Potato Dextrose Agar to acquire uncontaminated cultures. Microscopic examination (using an Olympus optical microscope) was conducted to identify the isolated fungi, reference the documentation procedures provided by (Barnett & Hunter, 1998);( Dugan, 2006) wherever possible.

**Pathogenicity test**

Pathogenicity tests were conducted following the methods outlined by (Okigbo *et al*., 2009). Tomatoes that were in good conditions underwent washing disinfected purified water and were then treated using a 1% sodium hypochlorite solution for sterilization. Using a 5mm thickness cork punch, fruit discs were divided (three portions of fruit each), then transferred from isolate discs were inserted into the hovels, replacing those detached discs. These inoculated fruits were left up to 24 to 48 hours. Symptoms of infection became evident during day 2, after which tissue pieces from the affected fruits were extracted and grown on freshly prepared Potato Dextrose Agar. The cultures were then incubated at a temperature of 28 ± $1^0$c in a duration of 7days.

**Preparation of Extracts and Phytochemical Studies**

The leaves of *Cola acuminata* and Zingiber officinale underwent sun drying for a period of 72 hours (equivalent to 3 days) and were subsequently weighed. A quantity of fifty grams (50g) of these leaves was then crushed using a pestle and mortar and macerated in 96% ethanol. The resulting extract was sifted and dissolved using a rotating evaporator in a condition of 45$^o$C until reaching a consistent weight. Later, an exhort extraction machine was also employed in this process. The remainder produces were recorded, and percentage of the residue allocated for plant chemical analysis, following the procedure by (Trease & Evans 1989).

**Antifungal Susceptibility Test**

The samples were diluted using ethanol solvent to create concentrations of 20%, 40%, 60%, 80%, and 100%.

**Dilution Test Procedure**

Initially, 1ml was measured out dispensed separately petri plates using disinfected plungers. Pasteurized PDA was then added to these dishes comprising the solvent excerpts, and the plates were softly rotated to guarantee thorough mixing. Once the medium was solidified, a 5.5 millimeter diameter disc of fully developed culture was obtained using a purified No. 2 cork thump and placed in the middle of each dish. These plates were then placed in an incubator set to room temperature approximately 28$^0$c. For regulation, plates were inoculated with a mixture of purified water and agar rather than solvent extracts and agar combination. Two control plates were created for each solvent extract type. Additionally, a positive regulation was included where no purified water-agar mix was added to the plates. The mycelial diameter was measured for a period of 7 days period (Udo *et al.,* 2001). There was significant difference $p<0.05$ on the effect of the various plant extracts on tomatoe fungal pathogen.

## RESULTS

### Fungal isolation

The fungal disease agents responsible for causing fruit rot in tomatoes, as identified and isolated in this research, include *Fusarium moniliformes, Rhizopus stolonifera,* and Aspergillus alternata.

### Koch's postulates and Pathogenicity test

Pathogenicity tests which was conducted illustrate that the fungi such as *Rhizopus stolonifera*, *Fusarium*, *moniliformes* and *Aspergillus alternata* were accountable for the decay after harvesting of tomato fruits collected in Akwa Ibom State. Pathogenicity evident within 12 to 24 hours subsequently inoculation. The isolates of *R. stolonifer, A. alternata* and *F. moniliformes*, displayed pathogenic behavior on tomato fruits utilize in the respective test. Signs of deterioration induced by *F. moniliformes* were characterized by soft black rot, while *R. stolonifer* and *A. alternata* resulted in soft rot symptoms. Upon re-separation, all three separates exhibited a growing forms consistent with those experiential in the unique separates.

### Disease Incidence

With rate of *Solanum lycopersicum* postharvest fungal in the four markets in Mkpat Enin is shown in Fig. 1. High disease incident (45 cfu/ml) was observed in Ukam market while the market with the least disease incidence (28 cfu/ml) was Akpaden market (Fig. 1)

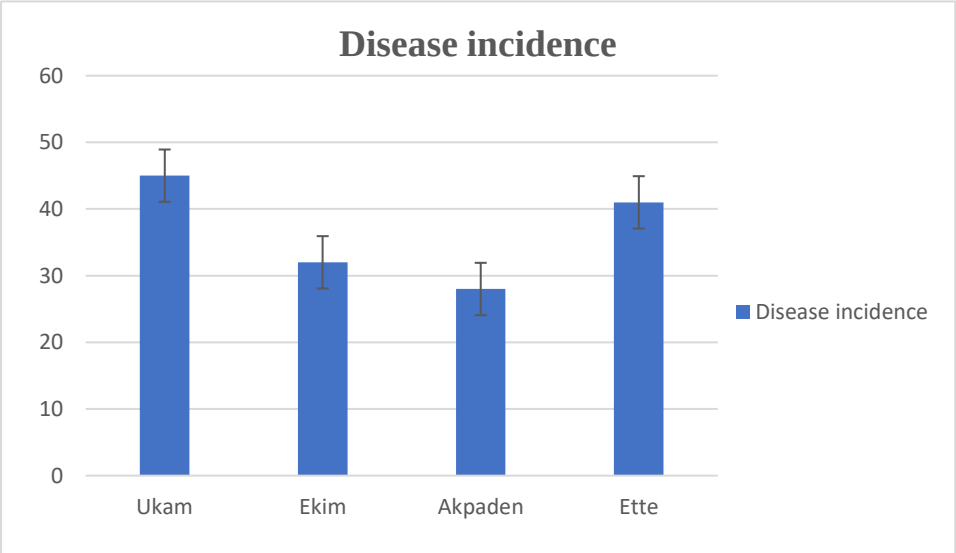


**Fig 1**: Percentage infection of *Solanum lycopersicum* fungal rot in four market area of Mkpat Enin.

### Phytochemical Constituents in Plants Extracts

The qualitative assessment of extracts-based phytochemicals from *Cola acuminata* and *Zingiber officinale* plants showed the occurrence of certain subordinate metabolites including alkaloids, saponins, tannins, cardiac glycosides, anthraquinones, and terpenes across different concentrations. However, phlobatannins and cyanogenetic glycosides were not detected which shown in Table 1.

Table 1: Qualitative Phytochemical Screening of *Cola acuminata* and *Zingiber officinale*

| Test | *Cola acuminata* | *Zingiber officinale* |
|---|---|---|
| **Alkaloids** | | |
| i. Wagners test | + | ++ |
| ii. Mayer's reagent | ++ | ++ |
| **Saponins** | | |
| Foaming test | ++ | + |
| **Tannins** | | |
| i. Ferreous chloride test. | +++ | ++ |
| ii. Bromine water test | +++ | ++ |
| **Flavonoids** | | |
| Shinoda reduction test | - | + |
| **Cardiac glycoside** | | |
| Salkowski's test | ++ | + |
| **Anthraquinones** | | |
| i. Borntrager's test | +++ | ++ |
| ii. Combined anthraquinone | +++ | ++ |
| **Phlobatannins** | | |
| Hydrochloric acid test | - | - |
| **Cyanogenetic glycosides** | | |
| Pirate paper test | + | - |
| **Terpenes** | | |
| Chloroform and Conc. Sulphuric acid | ++ | +++ |

**Key:**
**+++ =** High concentration, **++ =** Moderate concentration, **+ =** Trace concentration
**-=** Not detected

**Antifungal Effect Of *Cola acuminata* And *Zingiber officinale* Extracts On** *Fusarium moniliformes, Rhizopus stolonifera And Aspergillus alternata* **At Different Concentrations**

Figures 2 and 3 illustrate the effects of *Cola acuminata* and *Zingiber officinale* on the inaccessible pathogens. The effectiveness of these plant excerpts contrary to fungi causing tomato fruit rot was assessed in vitro. Findings demonstrated substantial ($P < 0.05$) inhibition of fungal mycelial growth at higher concentrations of the extracts, with variations in inhibition rates between the two extracts. Analysis of percentage inhibition revealed that ethanolic extracts of *Cola acuminata* and *Zingiber officinale* substantial ($p<0.05$) reserved the progression of *F. moniliformes, R. stolonifera*, and *A. alternata* at concentrations ranging from 40% to 80%. Notably, at a 60% concentration, the ethanolic leaf extract of Cola acuminata exhibited moderate inhibition against *Aspergillus alternata* (Figure 2), while the ethanolic extract of *Zingiber officinale* at 40% concentration showed lower inhibition against *F. moniliformes* (Figure 3). Across the board, *F. moniliformes* showed the highest susceptibility to the plant extracts at a 60% concentration, whereas *Aspergillus alternata* was the smallest reserved (Figure 2). Nevertheless, the proportion reserve of mycelial progression followed a consistent pattern across all tested pathogens and plant extracts. Enhanced antifungal activity corresponded with increasing concentrations of the plant extracts (Figures 2 and 3). The observed differences in fungitoxic potency among the effectiveness of these plant extracts could be linked to how each fungal pathogen responds differently to various concentrations of the extracts.

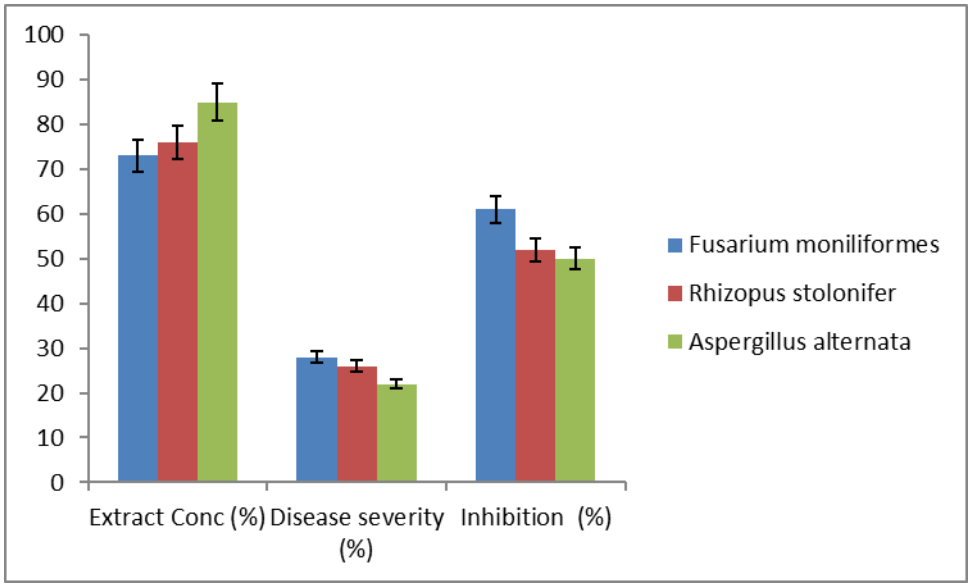


**Figure 2:** Effect of *Cola acuminata* ethanol leaf extract on mycelia progression of fungal separates at diverse absorption

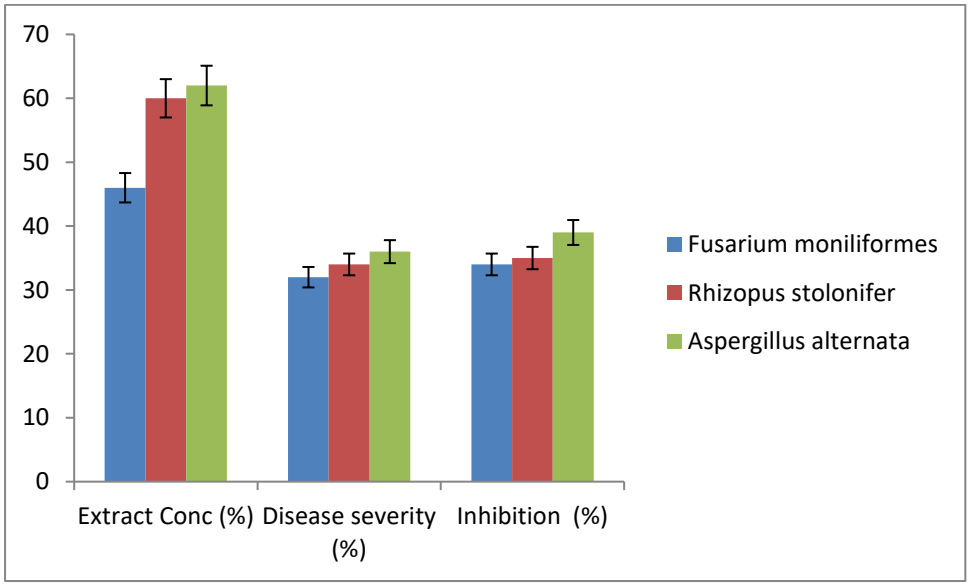


**Figure 3**: Effect of *Zingiber officinale* ethanol leaf extract on mycelia progression of fungal separates at diverse absorption.

## DISCUSSION

This study examined the fungi accountable for the decay of harvested tomato fruits spoilage in four markets which included Ukam, Ekim, Akpaden in Mkpat Enin, and Ette in Ikot Abasi Local Government Areas, Akwa Ibom State, Nigeria. Findings indicated that tomato fruits from Ukam market exhibited the highest incidence of spoilage, whereas Akpaden market had the lowest (Figure 1). The identified fungal isolates included *Fusarium moniliforme, Rhizopus stolonifer*, and *Aspergillus alternata* ( Ibrahim *et al,* 2011) emphasized Aspergillus niger as a key fungus involved in the production of volatile compounds in decaying tomatoes. Similarly, Baker (2006) isolated *Aspergillus niger* from rotten tomatoes and classified it as pathogenic. Akinmusire (2011) observed the link between Rhizopus species and tomato spoilage. Wogu & Ofuase (2014) identified Aspergillus spp, Penicillium spp, Fusarium spp, and Saccharomyces spp from spoiled tomatoes. Additionally, Mbajiuka & Enya (2014) reported finding Aspergillus spp, Penicillium spp, and Saccharomyces cerevisiae in spoiled tomatoes, while Fatih *et al,* (2005) documented *Alternaria alternata* and Fusarium oxysporum.

Ghosh (2009) identified *Fusarium oxysporum*, *Aspergillus niger*, and *Rhizopus stolonifer* in spoiled tomatoes. The high spoilage rate in Ukam market may be due to poor sanitation, overcrowding, inadequate storage, and unhygienic handling practices. This aligns with Onuorah and Orji (2015), who reported similar fungi in major markets in Awka, Nigeria. The study also assessed the inhibitory effects of plant extracts on these fungi, finding that at 40-80% concentrations, ethanolic extracts of *Cola acuminata* and *Zingiber officinale* significantly ($p<0.05$) inhibited *F. moniliformes*, *R. stolonifera*, and *A. alternata*. Specifically, a 60% concentration of Cola acuminata extract moderately inhibited *Aspergillus alternata* (Figure 2), while a 40% concentration of Zingiber officinale extract showed low inhibition of *F. moniliformes* (Figure 3). At a 60% concentration of ethanolic extracts, *F. moniliformes* exhibited the highest inhibition, while *Aspergillus alternata* showed the least inhibition (Figure 2). The antifungal effectiveness of the plant extracts improved with higher concentrations (Figures 2 and 3). The varying effectiveness of the plant extracts against fungal pathogens can be attributed to the different levels of susceptibility of the fungi to the extract concentrations. This finding aligns with the research of Amadioha (2000) and Okigbo (2009). Ilondu *et al*, (2001) noted that certain plants comprise of a phenolic compounds and vital oils that inhibit microorganisms. Ahmed and Stoll (1996) also reported that these compounds are responsible for the antifungal properties of the extracts, which are effective against various pests, including fungi, with ginger rhizome extract being particularly noted for its antifungal efficacy. The plant extracts demonstrated significant differences in their ability to inhibit fungal growth, likely due to phytochemical compounds such as alkaloids, saponins, and tannins presented in Chiejina & Ukeh, ( 2013) which was In the findings of Amadioha & Obi ,( 1999) and Udo *et al*,( 2001), who reported that plant extracts with bioactive compounds could control fungal pathogens in plants. The greater efficiency of *Cola acuminata and Zingiber* officinale may be due to their high alkaloid content, as alkaloids are considered highly therapeutic (Okigbo, 2009). This finding aligns with the results of Okey *et al.* (2016), who demonstrated that extracts from Vernonia amygdalina and Cola acuminata effectively controlled fungal-induced fruit rot in tomatoes (*Solanum lycopersicum*) in vitro. Their study revealed notable antifungal activity against pathogen mycelial growth, with a significance level of $P<0.05$.

The pathogenicity test results indicated that the fungi inoculated into vital tomato fruits displayed identical characteristics to those re-isolated from the infected fruits, confirming that these fungi caused the spoilage. The high-water content of tomatoes, along with environmental factors, handling conditions, storage quality, fungal load on handlers, and the overall quality of the tomatoes, all contribute to fungal spoilage. The fungi identified in this study produce potent mycotoxins, which pose significant health risks. For instance, *Aspergillus alternata* produces Ochratoxin, a potent carcinogen, underscoring the importance of discarding spoiled tomatoes to avoid health hazards. It is crucial for farmers and marketers to implement proper measures taken during the garnering, conveyance, storing, and sale of tomatoes to reduce the danger of contact to these damaging contaminants and metabolites.

## CONCLUSION

This study identified *Fusarium moniliformes*, *Rhizopus stolonifera*, and *Aspergillus alternata* as the fungi responsible for soft rot in tomatoes from four different markets. In vitro experiments indicated that plant extracts derived from *Cola acuminata* and *Zingiber officinale* effectively suppressed the mycelial growth of these pathogens at elevated concentrations, with statistical significance ($P < 0.05$), with varying levels of effectiveness between the extracts. Tomatoes, known for their high dietary and nutritional value, suffer economically and pose health risks when spoiled by fungi. Typically transported in conditions that favor fungal growth, tomatoes often travel in locally woven baskets and sacks from production to consumption areas.

Therefore, implementing strict quality control measures during harvesting, transport, handling, and processing is crucial. Regular inspections Food inspectors are also advised to prevent the sale of tainted tomatoes, thereby lowering health risks associated with the mycotoxins produced by these fungi.

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