



## **Antimycotic Potential of Alum on Postharvest Deterioration of Tomato**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author LOA designed the study, performed the statistical analysis, part of the literature searches and wrote the protocol and the first draft of the manuscript. Author EJU, RUT and RGB managed the analyses of the study and part of the literature searches. Author LOA and FWN read and approved the final manuscript.*

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### **ABSTRACT**

Tomato (*Solanum lycopersicum*) is one of the most economically attractive and widely consumed vegetables globally. Their high water content, perishability, transport and poor storage system predisposes them to spoilage by a broad spectrum of mycoflora resulting in huge postharvest losses. This study investigates antimycotic potential of alum on postharvest deterioration of tomato. Composite samples of deteriorating tomatoes were subjected to standard mycological analysis from which total fungal colony counts obtained ranged from  $1.64 \times 10^6$ - $5.70 \times 10^9$  CFU/g, and the following species were identified; *Aspergillus niger*, *A. flavus*, *Fusarium* sp, *Penicillium* sp, *Rhizopus stolonifer*, *Geotrichum candidum* and *Saccharomyces cerevisiae*. *In vitro* antimycotic activity of

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alum (1% (w/v) concentration) was determined on some of the isolates by agar well method (AWM) and diameter of inhibition zone (DIZ) measured using a metre rule. *G. candidum* had the highest DIZ (9.0mm (29.0%) followed by *A. niger* (8.0 mm (25.8%) and 7.0mm (Σ 22.6%) for *Fusarium* and *Penicillium* species respectively. *R. stolonifer* showed no inhibition or zero. pH values increased from 4.35-4.52 whereas TTA values decreased from 0.13-0.07 within 2days of analysis. However, these results indicate that treatment of postharvest deteriorating tomatoes with alum prior to consumption would enhance food safety as some of these fungi are known to be spoilage, toxigenic or opportunistic pathogens. So, their presence raises concern on storability as well as public health risks associated with consumption of these fruits. Therefore, production of tomato requires an integrated and multidisciplinary research approach not only to reduce economic loss but also create consumers' awareness on potential public health hazards of consuming relatively cheaper and pathogen contaminated deteriorating tomatoes.

**Keywords:** Postharvest; alum; mycotoxin; tomato; inhibition; antimycotic; deterioration.

## 1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is the third most cultivated vegetable crop globally and consumed as food, nutrient supplement, flavouring ingredient, medicine, lowers the risk of a variety of cancers such as prostate and cervical cancers and cardiovascular disorders, detoxificant, human system cleanser (blood and urinary tract) and enhances fertility in men [1-8]. It is a major horticultural crop with an estimated total world production of 152.9-173 million tonnes and contributes to the economy of many nations [8,9].

Nigeria is the second largest producer of tomato fruits in Africa and 13th largest in the world [10,11]. The production of tomatoes in Nigeria in 2010 was 1.8 million metric tonnes whereas national demand is about 2-3million metric tonnes annually, this deficit gap resulted in importation of 105,000 metric tonnes of tomato paste valued over ₦ 16 billion between 2009 and 2010 making Nigeria one of the primary importers of tomato globally [12,13,14]. Fresh tomatoes are displayed and sold in baskets, plates, on benches/tables and bare ground covered with polyethylene to consumers, making them vulnerable to contamination, spoilage and susceptible to mycotoxin producing and opportunistic pathogenic fungi [15,16,17,18]. Tomatoes contribute to a healthy, well-balanced diet as they are rich in minerals, vitamins, essential amino acids, sugars, dietary fibres, vitamin B and C, iron and phosphorus [19,20,4,21]. Tomato is eaten raw or with minimal processing into paste/purée, ketchup, jam, juice and other sauces [13,22,23] and could pose a serious threat to food safety, particularly on ingestion of toxigenic and opportunistic pathogens resulting in pathologic conditions such

as diarrhoea, gastroenteritis, respiratory infections and meningitis in animals and humans [24,25,26].

The fragility, short shelf span and perishable nature of tomato requires good transportation network, storage and adequate processing facilities. The absence of these infrastructures coupled with microbial infection, poor pest and disease management affects aesthetic, organoleptic and nutritional values with annual postharvest losses of approximately 45-60% of this produce in the country [27,28,29]. High cost of fresh ripened tomatoes during off seasons also tend to lure consumers to patronize vendors of relatively cheaper deteriorating contaminated fruits [7,30] thereby compounding the challenges of food safety and associated foodborne illnesses.

The colonization of postharvest deteriorating tomatoes by fungi is a critical phase which have attracted series of research interests. Such that a variety of genera/species including *Aspergillus niger*, *A. phoenicis*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus stolonifer* and *Saccharomyces cerevisiae* have been identified. These workers maintained that fungi of the genera; *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Mucor*, etc., were associated with most of the tomato disease and spoilage [31-36].

Several authors have reported the use of pretreatment with different compounds either in isolation or in combination with other preservation methods for extension of quality and storage life of fresh harvested produce [37,38,39,40]. In Nigeria, treatments with inorganic compounds alone or in combinations as control agents; sodium metabisulphite,

calcium chloride, boric acid, sodium hypochlorite (Milton) and potassium sorbate to enhance the management of postharvest losses of fruits have been documented [41-44]. However, alum (potassium aluminium sulphate; KAS) the study compound has not been investigated in relation to its efficacy as an antimycotic. Reports indicate that alum is nontoxic, used as medicine and in mouthwashes [45,46], in food processing and preservation, cosmetics, domestic and industrial water treatments, cures canker sores, has delatexing and bioactivities, etc [47-53]. Since fungi has been implicated as principal disease and spoilage microorganisms of postharvest tomatoes [41,54], treatment with alum which inhibits survival and growth would most probably improve shelf life, quality and safety. The benefit of this inorganic salt have not been discovered and/or explored and hence, there is paucity and

dearth of research information on its application and impact on pre- and postharvest tomato spoilage in Nigeria. Therefore, a preliminary investigation was initiated which focused on isolation, identification and antimycotic potential of alum on postharvest deterioration of tomatoes in Port Harcourt, Rivers State.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Sample

Deteriorating tomato samples (Fig. 1) were purchased from Ultramodern Market, Nkpolu-Oroworukwo, Mile 3, Port Harcourt, Rivers State. The samples were packaged in aseptic polyethylene and immediately transported to the Department of Microbiology Laboratory, Rivers State State University for analysis.



Fig. 1. Spoilt/decaying tomatoes (usually purchased at very cheap price by consumers) at the open market

## 2.2 Preparation of Alum

A mass of 1.0 g crystalline solid alum (Analytical grade. Vickers Laboratories, Ltd, England) was reconstituted in 99 ml sterile distilled water to obtain 1.0% (w/v) concentration.

## 2.3 Physico-Chemical Analysis of Tomatoes

From the same samples (tomato) representative 25g were blended with 50 ml distilled water, pH and titratable acidity (TTA) were determined from days 0-2. A digital pH meter (Labtech, Malaysia) which was calibrated using two buffer solutions was used for measurement. Titratable acidity was determined from the same sample using phenolphthalein (Phph) indicator and 0.1N (Molarity) sodium hydroxide (NaOH) standard solution. Ten (10) millilitre of supernatant/filtrate (test sample) was dispensed into 50 ml beaker and 3 drops of indicator (1.0 g of Phph in 99.0 ml of distilled water) added. The burette contained 0.1 N NaOH which was titrated against the contents in the beaker until a pink colour was obtained (endpoint). The reading was taken and repeated as required. The acid content of the tomato sample was calculated using the formula below:

$$TTA = \frac{V \times M \times F}{V}$$

Where

- V = Volume of NaOH used for titration (ml)
- M = Molarity of NaOH solution
- F = Factor of citric acid (0.0064)
- V = Volume of sample (ml)

## 2.4 Mycological Analysis

A composite deteriorated tomato samples was washed with sterile distilled water and drained from which twenty five (25) grammes was blended into a paste in 225 ml of distilled water using electrically powered blender (Waring commercial Torrington, USA). The resulting homogenate was serially diluted in 0.85% (w/v) physiological saline as diluent. An aliquot portion of 0.1 ml of decimal dilution was inoculated onto preprepared solidified Sabouraud's dextrose agar (SDA) supplemented with antibiotics (50 mg tetracycline).

## 2.5 Enumeration, Cultural and Morphological Characteristics

Mean heterotrophic fungal colony counts were obtained from aliquots (0.1 ml) of samples from different ten-fold dilutions after incubation at  $25 \pm 2^\circ\text{C}$  for 1-4 days. Viable representative colonies were picked at random, streaked and subcultured for purification and stored in the refrigerator at  $2-4^\circ\text{C}$ . Then, isolates were identified after staining with lactophenol cotton blue and compared with observed cultural and morphological characteristics as described [55-58].

## 2.6 Antimycotic Activity

This was determined using agar well method (AWM) [59] with various identified fungal species (1-2d for fast growing species) after adjustment to 0.5 McFarland turbidity standard. The surface-dried SDA were spread-plated with cultures of 1-2d before wells (6mm diameter) were made aseptically with sterile cork borer, and equidistantly spaced from one another. Then 50 $\mu\text{l}$  of 1% alum solution was dispensed into each well and incubated, diameters of inhibition zones were measured and mean standard deviation determined. Sterilized distilled water was used as the control.

## 2.7 Statistical Analysis

Means of duplicate measurements and standard deviations (SD) were determined for the samples after replication of experiment using Microsoft Excel® 2016.

## 3. RESULTS

Mean fungal counts, pH and titratable acidity of the ripe deteriorating tomato samples from day 0 to day 2 are presented in Table 1. The mean total fungal counts ranged from  $1.64 \times 10^6$  to  $5.70 \times 10^9$  Cfug, pH values ranged from 4.35 to 4.52 whereas the TTA values ranged from 0.07 to 0.13. These results suggest that there was increase in both the fungal colony counts and pH. In contrast, the TTA decreased.

The fungal genera and species identified in deteriorating tomatoes include *Fusarium*, *Aspergillus niger*, *A. flavus*, *Penicillium*, *Geotrichum candidum*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*. Most of these fungi has been implicated in postharvest deterioration of tomatoes (Table 2).

**Table 1. Mean fungal count, pH and TTA of deteriorating tomatoes from days 0-2**

Paramters	Duration(Days)		
	Day 0	Day 1	Day 2
TFC	1.64x10 <sup>6</sup> Cfug	3.00x10 <sup>7</sup> Cfug	5.70x10 <sup>9</sup> Cfug
pH	4.35	4.45	4.52
TTA	0.13	0.09	0.07

Legend: MFC = Mean fungal count; TTA = Titratable acidity

**Table 2. Cultural and morphological characteristics of fungal isolates from tomatoes**

Code	Macroscopic characteristics	Microscopic characteristics	Identified isolates
01	White, soft cottony, pink colour on reverse side	Sickle shaped macroconidia with spores	<i>Fusarium</i> sp
02	Effuse, brownish-black colouration	Septate, vesicle bearing Sterigmata and conidia	<i>Aspergillus niger</i>
03	Greenish colony	Septate conidiophore, vesicle bearing conidia	<i>A. flavus</i>
04	Green-yellowish velvety colony	Phialide with conidia, septate, branching conidiophore	<i>Penicillium</i> sp
05	White creamy colony (surface) reverse side, brown	No conidia, branching hyphae rectangular arthroconidia	<i>Geotrichum candidum</i>
06	White to gray fluffy colony	Aseptate, sporangia on columella, long hyphae, sporulate and turns black	<i>Rhizopus stolonifer</i>
07	Creamy colony, matted, moist/mucoid	Ovoid/spherical, budding cells, with single ascus	<i>Saccharomyces cerevisiae</i>

Antimycotic potential of alum against some of the isolates of postharvest deterioration of tomatoes and percentage inhibition are shown in Table 3. *Geotrichum candidum* exhibited the highest DIZ value of 9.0mm corresponding to (29.0%) inhibition potential followed by *Aspergillus niger* (8.0mm = 25.8%) and *Penicillium* and *Fusarium* species (7.0mm  $\Sigma$  22.6%) respectively. *Rhizopus stolonifer* was not inhibited at concentration dosage used. Previous work has revealed antimycotic activity of alum on *A. flavus* and *S. cerevisiae* [52].

#### 4. DISCUSSION

This investigation revealed some spoilage fungi associated with deterioration of postharvest tomatoes which provides a milieu for their proliferation due to low pH, high moisture content and nutrient composition. *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Geotrichum* identified

in this study have not only food relevance but is linked with reduction in the aesthetic, nutritional and economic losses to growers of these vegetables [60,61,22,62,18,30,36,63]. Some of these fungi are not only known phytopathogens in the field and storage but constitutes animal and human health hazards when mycotoxins are produced [64-66]. *Aspergillus* and *Penicillium* species produces potent toxins e.g., ochratoxins and citrinins whereas *Fusarium*; fumonisins, trichothecenes and zearalenone. The beneficial impact of KAS was demonstrated by its ability to inhibit growth of *Geotrichum candidum*, *Aspergillus niger*, *Fusarium* and *Penicillium* species in deteriorating tomatoes and happens to be the first time such a phenomenon has been reported. The susceptibility of these species to KAS may be attributed to low acidity and/or deleterious impacts on fungal cell walls. Such antimycotic activity would probably improve the quality and storage of ripe tomatoes.

**Table 3. Antimycotic activity of alum (1.0%) on some isolates from deteriorating tomato**

Fungal species	Diameter inhibition zone (DIZ)(mm)	% inhibition
<i>Aspergillus niger</i>	8.0	25.8
<i>Penicillium</i> sp	7.0	22.6
<i>Fusarium</i> sp	7.0	22.6
<i>Geotrichum candidum</i>	9.0	29.0
<i>Rhizopus stolonifer</i>	0.0	00.0

However, the emerging interest on the use of KAS alone and in combination treatments with plant extracts or inorganic compounds on microorganisms in foods and clinical samples with enhanced activity had been reported [49,51-53]. Several fungal species are air-borne or present in the environment or in raw materials [61] and their load/counts could drastically increase if they alight or settle on exposed nutrient-rich substances thus, hastening deterioration. The mean fungal load ( $1.64 \times 10^6$  -  $5.70 \times 10^9$  Cfug<sup>-1</sup>) and the associated species in spoiled/deteriorating tomatoes reported presently corroborates earlier investigations [67,68,66] which signals that consumption of such relatively cheap produce could be agents in foodborne illness. Although, prior studies on ripe/deteriorating tomatoes indicate that the pH range from 3.9-4.5 and such increase could be influenced by ripeness level, ratio of sugars, length of storage, total acids [69-71,39,72,73] and in part by fungal colonization as depicted in this study. Decrease in TTA values may be attributed to changes in citric acid alone or both citric and malic acid or to the ratio of malic and citric acids which may be linked to the conversion of malic to citric acid due to advancement in maturity and/or storage [39,9,73-76] characteristic of deteriorating over ripe tomatoes. While it is unlikely that a single strategy will be successful in eradicating contamination and postharvest losses of fresh produce by human and phytopathogenic fungi, a multi-pronged approach including sound regulatory policies with adequate enforcement, good agricultural practice (GAP) in seed production, adherence to good manufacturing practices (GMP) during minimal processing, poor harvesting and storage as well as antimycotic intervention treatments may reduce the risks of outbreak of foodborne illnesses associated with fresh produce and vegetables.

## 5. CONCLUSION

The study revealed that the pH and mycoflora increased whereas the TTA decreased in deteriorating tomato fruits. KAS has proved to be a good and reliable antimycotic agent by inhibiting a wide spectrum of fungal genera. The dynamics of these factors (pH, TTA, fungal load and inhibition by KAS) in fresh produce would underscore the safety and rate of deterioration.

However, implementation of effective food safety management systems in the fresh produce

industry is of utmost importance to ensure product safety for consumers. It is hereby suggested that KAS/alum could be used as one of the tomato sanitizers in the field and in postharvest storage to reduce microbial levels and ensure food safety.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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