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Original article

ISOLATION OF SPOILAGE MICROORGANISMS AND METHODS OF EXTENDING THE SHELF LIFE OF TOMATO (*Solanum lycopersicum*) FRUITS

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ABSTRACT

Tomato (*Solanum lycopersicum*) fruit is one of the widely consumed fresh fruits worldwide due to its contribution to health-balance in diet. However, tomato is a highly perishable fruit because it is easily spoilt by certain microorganisms due to its composition. Thus, this research work was carried out to investigate the microorganisms associated with the spoilage of fresh tomato (*Solanum lycopersicum*) fruits obtained from Ipata market, Ilorin, North Central Nigeria. The preservative methods used for the preservation of the tomato fruits were hot water, aqueous chlorine, aqueous calcium, radiation, *Moringa oleifera* extract, Sodom apple extract, *Moringa oleifera* powder and Sodom apple powder treatments. A total of seven bacterial species were isolated and identified which were *Bacillus subtilis, B. aureus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Proteus mirabilis* with *B. subtilis* being the most prevalent with 33.3% and *Pseudomonas aeruginosa* being the least prevalent with 2.8%. The mean bacterial count ranges from 2.5 x 10^5cfu/g to 1.0 x 10^5cfu/g with *Staphylococcus aureus* having the highest count and *Proteus mirabilis,* the lowest count. The fungal isolates were *Aspergillus niger, Saccharomyces cerevisiae, Penicillum* sp, *Fusarium* sp and *Mucor* sp. *Mucor* sp had the most prevalence of 28.2% while *Fusarium* sp is the least prevalent with 15.5%. The mean fungal count ranges from 2.0 x 10^3cfu/g to 1.3 x 10^3cfu/g with *Aspergillus niger* having the highest count and *Mucor* sp with the lowest count. *Moringa oleifera* extract treatment was the most effective of all the preservative methods used with a preservation rate of 93.3%. Hence, this method could be used in extending the shelf life of tomato fruits.

Key Words*:* tomato fruits, microorganisms, spoilage, prevalence, *Moringa oleifera,*

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INTRODUCTION

Agriculture is an important sector in most developing countries [1]. It contributes more than 30% of the total GDP and provides 50% of the food need of the country [2]. Tomato (*Solanum lycopersicum)* is an annual crop having a weak woody stem covered with glistering reddish yellow glandular hairs [3]. The tomato plant is widely cultivated in many parts of the world including Nigeria. The tomato fruit has a smooth skin, green when immature but becomes bright red or yellow as it ripens. The fruit varies greatly in size and shape [3].

The Tomato fruits may be pair-shaped, elongated, flattened and heart-shaped. They are edible, fleshy and reddish when ripe and vary in their acid composition, with white and yellow ones being less acidic. The fruit can be used as flavour in soups and cooked foods or can be eaten as fruits. It is used in many dishes, salads, sauces and drinks. It can also be dried and ground into pancakes or extracted from the pulp and its residue contains 24% of which is used for salad dressing and in the manufacturing of margarine and soap [1]. The residual pressed cake is used as stock feed as well as fertilizer. Tomatoes also earn foreign exchange to the producer countries due to exportation [1].

Tomato fruits are common vegetables eaten raw as salad or for garnishing various cooked food in Nigeria as well as in many parts of the world. The fruit contains high amount of carbohydrate, fats, organic acid, water, minerals, vitamins and pigments. It is estimated that ripe tomato fruits contain approximately 94% water, 4.3% carbohydrates, 1.6% protein, 0.1%fat, 0.6% fibre and vitamins. The nutrients support the growth of microorganisms such as fungi and bacteria which produce enzymes that degrades the nutrients [4]. Tomato fruits contain a lot of water which make them more susceptible to spoilage by microorganisms. The microorganisms reduce not only the nutritional value but also the market value of the tomato fruits [3].

The consumption of tomatoes throughout the world is believed to be beneficial to the heart and other organs. The richest source of lycopene is tomato and tomato based products. Lycopene has been found to prevent prostate cancer, improve the skin's ability to protect itself against harmful ultra violet rays [5], decrease the risk of breast cancer, lung, stomach, bladder, uterine, head and neck cancers, protect against neuron degenerative diseases, lower urinary tract infection and reduce the cardiovascular risk associated with diabetes [6];[7]. Tomato fruits have serious challenges to their existence, these include changes in climate conditions, pests, inadequate rainfall and microorganisms particularly fungi. One of the limiting factors that influence the economic value of tomato fruits is their relatively short shelf life caused by pathogenic attack.

Spoilage of tomato fruits occur as a result of adverse changes in the quality of tomatoes that are brought about by the action of predominantly biological and physical factors. These may be changes in the taste, smell, appearance, or texture of the fruits. The major cause of spoilage of tomato fruits are fungi which include; *Aspergillus phoenicis, Absidia sp, Trichoderma sp, Alterneria alternota, Fusarium oxysporum, Fusarium moniliformis, Aspergillius niger, Mucor sp, Rhizopus stolonifer, Pencillium sp, Geotrichum sp* and *Phytophthora.*

Consumers are becoming increasingly concerned about the incidence of disease in tomato fruits and intensive research is being undertaking to comprehend the measure which can be taken to effect some radical control [8]. Fungal spoilage of tomato fruits has been recognized as a source of potential health hazard to humans and animals due to the fact that they produce mycotoxins which are capable of causing mycotoxicoses in man following ingestion and inhalation [9]. Meanwhile, due to the fact that tomato fruits contain large amount of fluid, the mycotoxins are mostly, not limited to their areas of infections but diffuse rapidly throughout the fruits, contaminating all parts and making them unfit for consumption.

The importance of tomato in the food industry and its nutritional benefit cannot be over-emphasized, as it is even an important part of Nigerian diet [10]. Tomatoes are not only good source of vitamins A and C, they are also a good source of other vitamins and minerals. Tomatoes contain high level of minerals, phosphorus and potassium, they also contain folate and high levels of the antioxidant beta-carotene and lycopene. One medium tomato fruit has 552mcg of beta-carotene and 3,165mcg of lycopene which can help boost immune system by fighting the damaging effects of substances called free radicals [11]. Tomatoes contain important nutrients such as niacin, folate and vitamin B6 that are associated with the reduction of heart disease. One study showed that women who eat 7 to 10 serving of tomatoes products per week had a 29% lower risk of cardio-vascular disease than women who consumed less than a serving and a half of tomatoes product per week [10].

Susceptibility of tomato fruits to microbial colonization is due to its differential chemical composition such as high level of sugar, low PH (4.9-6.5) and its high water activity ($p>0.99$) which favours the growth of microorganisms in tomato fruits [12]. The contamination of tomato fruits by microorganism could also be as a result of poor handling practices in the tomato production practices and transportation [12].

This study was undertaken to isolate and identify microorganisms that are associated or responsible for the spoilage of ripened tomato fruit and its preservation and storage method of increasing its shelf life as it is important to the economy of individual homes, farmers and the country considering the vital role that tomatoes play in the health of people and food security. Most of the usual

preservative methods like drying, freezing and canning often lead to the loss of the freshness of the tomatoes. Little or no attempt has been made in use of plant parts extracts to preserve the fruit. We therefore, seek in this study to evaluate the preservative potentials of the powder of *Moringa oleifera* plant parts, Sodom apple plant part extracts, use of hot water treatment as well as radiation.

MATERIALS AND METHODS

Sample collection

The tomato fruits used for this research work were purchased from Ipata market, Ilorin, North Central Nigeria, in a clean air-tight container and brought into the Microbiology laboratory of Kwara State Polytechnic, Ilorin for isolation of microbes responsible for the spoilage. Fresh leaves, fruits, stem and roots of *Moringa oleifera* and *Calotropis procera* were collected from different locations in Ilorin metropolis. A cutlass was used to cut the roots and the branches, while the leaves and fruits were handpicked and put in clean polythene bags and taken into the laboratory. In the laboratory, each of the plant parts (leaves, stem, roots and fruits) were first pre-washed carefully under a gentle stream of tap water for one to two minutes to remove surface dirt .This was followed by washing for thirty seconds in sterile distilled water containing 1% sodium hypochloride. The plant parts were then removed and rinsed in three successions of sterile distilled water.

Preparation of *Moringa Olifera* Plant parts powder

Seeds were removed from the fruits and the disinfected plant parts (leaves, stem, roots and seeds) were air dried on the laboratory bench for 10 to 12 days, after which they were ground together into fine powder, first with mortal and pestle and then, with a blender in equal ratio of 100g each. The powder of the plant parts were stored in a well-covered clean plastic container and kept in a dust-free locker.

Preparation of *Moringa Olifera* Plant parts extract

100g of the plant parts powder of *Moringa oleifera* was weighed and dissolved in 100ml of distilled water in a beaker and was allowed to soak for4 hours after which it was sieved into a beaker using a muslin cloth.

Extraction concentration

The concentration of the Moringa oleifera plant parts was prepared to give 100g/ml. This was done by dissolving 100g of the plant parts powder in 100ml of sterile distilled water in a beaker.

Application of Moringa olifera plant parts extract on tomato fruits

Four pieces of firm, smooth and healthy tomato fruits were soaked in the Moringa oleifera plant part extract for 5 minutes and were removed and placed in a plastic container and were kept for observation for 30days.

Application of *Moringa oleifera* plant parts powder on tomato fruits

The fruits were covered with the powder from Moringa olifera plant part extract for 5 minutes and were removed and placed in a plastic container and kept for observation for 30days at 25^0C.

ii. Use of Sodom Apple, *(Calotropis procera, i.e. Bomu bomu) Plant Parts*

The method used for *Moringa oleifera* plant parts was also used for this treatment method

iii. Hot Water Treatment

The four pieces of tomatoes used were sorted and cleaned. The tomatoes were covered in hot water at 88^0c containing garlic and ginger for ten minutes, after which the water was drained and the tomatoes were stored in an air-tight container which was put inside hot water (water at 100^0c) for five minutes before it was closed, labeled, and kept for observation for 30days.

iv. Chlorine Treatment

Chlorine solution [Sodium hypochlorite] was obtained from the Chemistry laboratory in Kwara State Polytechnic, Ilorin, Nigeria. The chlorine concentration used was 100 part per million [ppm] [13]. The fruits were dipped in the chlorinated water for 30 secs, drained and kept in a clean air-free container and kept for observation for 30 days.

v. Calcium Treatment

Calcium chloride was obtained from the Chemistry laboratory of the Kwara State Polytechnic, Ilorin, Nigeria. The fruits were dipped in Calcium chloride solution of 0-5% concentration for 30 secs, [14], kept in a clean air-free container for 30days.

vi. UV-C Irradiation

UV-C radiation was provided by fluorescent germicidal lamps (GE 30 W) with peak emission at 254 nm. The fruits were irradiated with a predetermined dose of $3.7kGm^{-2}$ for 10 minutes according to [15]. The tomato fruits were placed in an open plastic container and stored in the dark at 25^0C for observation.

Vii Control

A control sample was set up in which no treatment was administered, four firm pieces of tomato fruits were wash, rinsed and placed in a plastic container for observation at 25^0C.

PRESERVATION RATE

The preservation rate of each preservative method used is calculated by using the following, formula:

$$R\ \% = \frac{NDV}{NTD} \times \frac{100}{1}$$

R = Rate

NDV = Number of days of viability

NTD = Number of days of research

Microbial isolation

The tomato fruits were obtained from Ipata market, Ilorin, and were brought into the laboratory for microbial isolation. Fresh, firm, healthy, ripe tomato fruits were washed, drained of water and kept free from dust and insect at room temperature for 14 days to allow to undergo a natural process of spoilage.

The spoilt fruit samples were grounded using a sterile mortal and pestle and one gram was weight aseptically into a 9mil sterile water and shaken vigorously to have a homogenized solution. Serial dilution of up to 10^{-4} of the homogenate was made in sterile test tube.1ml of the serially diluted tomato fruit sample was pipetted into each serially marked petri dishes.

The total microbial count was carried out on the spoilt tomato fruit samples using the pour plate method. Nutrient agar and MacConkey agar were used for bacteria isolation while Potato Dextrose agar and Sabroud dextrose agar were used for fungi isolation, all media used are prepared according to the manufacturers' prescription. The plates were subsequently incubated at 37^0C for 24hours for bacteria and 25^0C for 72hours for fungi. At the end of incubation, developed colonies were counted and colonies forming units per unit gram of tomato fruit sample were calculated and recorded.

Characterization and identification of bacterial isolates

Discrete colonies that developed after incubation, were subcultured to obtain pure cultures which were stored at 4^oC and used subsequently for microscopic characterization and biochemical analyses. The distinct colonies that developed in the pure culture plates were observed for the morphological and cultural characteristics including the nature of margin, shape, colour, cell type and arrangement. The isolates were further characterized and identified following biochemical procedures as described by [16]. These include Gram staining, motility test, spore formation test, catalase test and coagulase test.

Fungi isolation and characterization

The fungi isolation and characterization was carried out using Sabroud Dextrose Agar and Potato Dextrose Agar and was observed after 5-7 days of incubation. The colonies were observed under the microscope after they were flooded with methylene blue. The isolates were characterised based on their macroscopic and microscopic features.

RESULTS

Preservation methods and rates

The preservative methods employed in this research work show variability in their preservative abilities. Hot water treatment shows 40%, chlorine shows 86%, calcium shows 66.7%, Radiation shows 80%, Moringa extract 93.3%, Sodom apple extract 60%, Moringa powder 66.7%, and Sodom apple powder 46.7% (Table 1).

The microorganisms isolated from spoilt tomato fruits at the end of this research work were characterized and identified based on their cultural, morphological and biochemical characteristics. The bacteria isolated are; *Bacillus subtilis, Staphylococcus aureus, Bacillus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Proteus mirabilis* (Table 2).

Table 1: preservation method and preservation rate (%) of fresh Tomatoes

Time in day	Hot water treatment	Chlorine	Calcium	Radiation	Moringa extract	Sodom apple extract	Moringa powder	Sodom apple powder	Control
2	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs
4	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs
6	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs
8	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs
10	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs
12	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs
14	3pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs
16	2pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	3pcs	2pcs
18	1pcs	4pcs	4pcs	4pcs	4pcs	4pcs	4pcs	2pcs	2pcs
20	0	4pcs	4pcs	4pcs	4pcs	2pcs	4pcs	1pc	1pc
22	0	2pcs	3pcs	4pcs	4pcs	1pc	3pcs	1pc	1pc
24	0	4pcs	2pcs	4pcs	4pcs	1pc	2pcs	0	0
26	0	4pcs	1pc	2pcs	4pcs	0	1pc	0	0
28	0	3pcs	0	0	4pcs	0	0	0	0
30	0	2pcs	0	0	3pcs	0	0	0	0
Mean preservation rate	40%	86.7 %	66.7%	80%	93.3%	60%	66.7%	46.7%	46.7%

Table 2: characterization and identification of bacterial isolates from spoilt tomato fruits

Characteristics	Description of isolates						
CULTURAL							
Margin	Smooth	Smooth	Smooth	Entire	Entire	Smooth	Entire
Colour	Colourless	Yellow	Colorless	Pink	Creamy	Creamy	Creamy
Shape	Small & irregular	Large	Small	Small	Large	Medium	Large
Morphologyl							
Cell type	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Single	Cluster	Single	Single	Single	Single	Single
Gram practical	+	+	+	-	-	-	-
Mortility Test	+	-	+	-	+	-	+
Coagulase	-	+	-	-	-	-	-
Catalase	+	+	+	+	+	-	+
Spore formation	+	-	+	-	-	-	-
Probable microorganism	*Bacillus Subtilis*	*S. aureus*	*B. aureus*	*Escherichia Coli*	*Pseudamanas aeruginosa*	*Salmonella Typhi*	*Proteus mirabilis*

Key: + positive
- negative

The occurrence of the bacterial isolates from spoilt tomato fruit samples revealed that *Bacillus subtilis* was the most prevalent with 33.3% while the least prevalent was *Pseudomonas aeruginosa* with 2.8%. The bacterial isolate that has the highest population was *staphylococcus aureus* with 2.5 x 10^4cfu/g and the isolates with lowest population was *Proteus mirabilis* with 1.0 x10^4cfu/g (Table 3).

The fungal isolates are; *Saccharomyces cerevisiae*, *Fusarium sp*, *Aspergillus niger, Mucor sp, Penicillium sp*. All these fungi are identified based on their macroscopic and microscopic characteristics (Table 4).

The most prevalent fungal isolate was *Mucor sp* with 28.2% while *Fusarium sp* was the least prevalent with 15.4%. The fungal isolate from tomato fruits sample in this research were: *Aspergillus niger* with 2.0 x 10^4cfu/g, *Fusarium sp* with 1.5x10^4cfu/g Saccharomyces *cerevisiae* with 1.8x10^4cfu/g *Penicillium sp* with 1.6x10^4cfu/g and *Mucor sp* with 1.3x10^4cfu/g (Table 5).

Table 3: Occurrence of Bacterial Isolates and Average bacterial counts in samples of spoilt tomato fruits

Bacterial Isolates	Numbers of Occurrence	Percentage of Occurrence (%)	Average bacterial counts (x10^4cfu/g)
Bacillus subtilis	12	33.3	1.7
Staphylococcus aureus	3	8.3	2.5
Bacillus aureus	8	22.2	2.0
Eschericia coli	7	19.4	2.2
Pseudomonas aeruginosa	1	2.8	1.2
Salmonella typhi	3	8-4	1.6
Proteus mirabilis	2	5.6	1.0
TOTAL	36	100	

Table 4: Isolation and characterization of fungal isolates from tomato samples

Fungal	Macroscopy	Microscopy
Saccharomyces Cerevisiae	The colonies are flat, moist, smooth, glistering or dull and are cream in colour	Unicellular, globulous, and ellipsoids to elongate shape
Fusarium sp	White and cottony at the initial stage but develop pink center with a higher periphery	Conidiosphore bear conidia singly or cluster, septate hyphae with canoe-shaped micro conidia.
Aspergillus niger	Green filamentous with a large proliferation of black spores	Septate hyphae, branched conidiosphore with secondary branches. The conidiosphore is broad at the tip forming rounding Vesicle like chains
Mucor sp	Grows fast and cover agar surface with white fluff that turns grey later, reverse side is white	Sporangiosphores are long, often branched and bear terminal spore filled sporangia. Hyphae non-septate
Penicillium sp	Flat and rapid growing filament and valvety, woolly, or cottony in texture	Chains of single celled conidia (ameroconidia) are produced in biseptal succession from a specialized conidiogenous cell called phialide

Table 5: Occurrence of Fungal Isolates and Average fungal counts in samples of spoilt tomato fruits

Fungal Isolates	Numbers of Occurrence	Percentage of Occurrence (%)	Average fungal counts ($x10^4$ cfu/g)
Saccharomyces cerevisiae	7	17.9	1.8
Fusarium sp	6	15.4	1.5
Aspergillus niger	7	17.9	2.0
Mucor sp	11	28.3	1.3
Penicillium sp	8	20.5	1.6
TOTAL	39	100	

DISCUSSION

Several treatments and techniques are applied during the post-harvest preservation of fresh fruits for the control of microorganisms and pests, ripening and senescence and prevention or delay of some physiological disorder. In this work, hot water, Aqueous chlorine, Aqeous calcium, Radiation, *Moringa oleifera* extract, Sodom apple extract, *Moringa oleifera* powder and Sodom apple powder treatments were employed to preserve tomato fruits.

The bacteria isolated from this research work is similar to the findings reported by [17].

The presence of *Escherichia coli* shows that the fruit has been contaminated with faecal matter either on the field or during post-harvest handling. Also the presence of *Bacillus aureus* shows that it is contaminated during post-harvest handling.

The fungal isolated from this work is similar to the findings reported by [17].

The presence of *Saccharomyces cerevisae* and *Aspergillus niger* shows that the tomato fruits had also been contaminated before harvesting right from the field by the field soil microbes. This is because *Saccharomyces cerevisae* and *Aspergillus niger* are soil microbes.

[17] reported the presence of *Klebsilla aerogenes* and *Bacillus cereus* in his research about spoilage microbes of tomato in which were not isolated in this research work. The specie of Bacillus that was detected in this work was *Bacillus aureus* which differs from that of [17] and [18]. [18] reported the presence of *Bacillus coagulans* and *Bacillus stearothermophilus* in spoilt tomato. Similarly, [19] also isolated *Bacillus megaterium* and *Bacillus laterosporus* from tomato fruit sample. However, the isolation of *Bacillus subtilus*, *B. aureus, Staphylococcus aureus, E-coli*,

Salmonella typhi and *Proteus mirabilis* in this study is similar to the report of [17] and [20].

The occurrence of the bacterial isolates from spoilt tomato fruit samples revealed that *Bacillus subtilis* was the most prevalent with 33.3% while the least prevalent was *Pseudomonas aeruginosa* with 2.8%, this is similar to the report of [3] that *Bacillus subtilis* was the most prevalent bacteria isolated.

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