Original Research Article

Effects of Neem, Moringa and Synthesized Silvernanoparticles Coating on Postharvest Shelf Life and Quality Retention of Tomato (*Solanum lycopersicum* L.)

ABSTRACT

This study aimed to investigate the effect of *Azadirachta indica* (neem) and *Moringa oleifera* (moringa) leaf extracts on the shelf life and quality retention of tomato (*Solanum lycopersicum* L.) fruits during storage. Thirty-five (35) matured tomato fruits were collected, rinsed and grouped for each treatment with neem and moringa coating: Control (n=5), moringa aqueous leaf extract (MALE) (n=5), neem aqueous leaf extract (NALE) (n=5), 1:9 and 6:4 moringa aqueous leaf extract synthesized silver nanoparticles (MALE-AgNPs) (n=5), 1:9 and 6:4 neem aqueous leaf extract synthesized silver nanoparticles (NALE-AgNPs) (n=5), respectively. The firmness, shelf life, and postharvest decay percentage of the tomato fruits were determined. Additionally, fungi associated with the postharvest deterioration of the fruits were isolated and identified using standard procedures. From the results of this study, tomato fruits coated with either neem or moringa crude extract showed the longest shelf life, as compared to the coating with AgNPs. Additionally, two fungi, namely *Aspergillus niger* and *Aspergillus flavus*, were isolated from the decayed tomato fruits. In conclusion, the neem and moringa leaf extracts are effective in the extension of the shelf life and retention of the quality of tomato fruits.

Keywords: Azadirachta indica, Moringa oleifera, Tomato fruits, Postharvest, Shelflife, Preservatives

1. INTRODUCTION

Crop postharvest shelflife extension and their quality retention remains a key global issue. Since food is a crucial part of human life, it is important to prevent postharvest losses and increase the shelf life of food. Tomato (*Solanum lycopersicum* L.)is widely grown and consumed in Nigeria, serving as a valuable source of vitamins and minerals [1]. Tomato is a commonly consumed fruit that is vital for health, and therefore available fresh or in paste form [2].However, the perishability of tomatoes poses challenges for farmers and consumers, affecting the quality and safety of the fruits. Addressing the shelf life is crucial to meet consumer demands and ensure a stable tomato supply [3].

Plant nutraceuticals are sourced from different plant parts as antioxidant and antimicrobial agents in food preservations due to their excellent source of natural bioactive compounds like polyphenols and terpenoids[4]. The extracts of these plants are increasingly considered natural preservatives, potentially replacing synthetic counterparts such as sodium hypochlorite, sodium metabisulphite, and calcium chloride in various applications [5]. Moringa (*Moringa oleifera*) is recognized for its abundance of bioactive compounds, particularly in its leaves, which are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins [6,7]. The embedded bioactive compounds in its leaves contribute pharmacological properties[8,9,10]. Neem (*Azadirachta indica*)on the other hand, is a widely available plant that contains phytochemicals [11], which can inhibit spoilage-causing micro-organisms in tomatoes, and also preserve its level of nutrients [12]. Various phytoconstituents ofitsleaves contributes to various attributed biological activities including, antioxidant, antidiabetic, antimicrobial, antifungal, anti-inflammatory, anti-tumor, anti-cancer, anti-fertility[13].

Most recently, green-synthesized coatings such as plant-derived silver nanoparticles which contains considerable bioactive compounds have been investigated as a potential option to minimize fruit respiration, spoilage, and microbial growth, and thus promote postharvest shelf life and quality retention of fruits [14, 15]. Here in this study, the various biological activities associated with the abundant bioactive compounds of neem and moringa has informed and necessitated the investigation into their use for silver nanoparticles synthesis and their effects on shelf life and quality retention of tomato fruits during storage.

2. MATERIAL AND METHODS

2.1Coating Materials Preparation and Fruit Samples Collection

Fresh leaves ofneem and moringawere obtained at the back of the Lagos State University sports center and a residential area at Adexson, Lagos State, respectively, and were identified and authenticated at the Herbarium of the Department of Botany, Lagos State University, Ojo, Lagos State, Nigeria. The dried leaves were blended to get the powder. The powder was then sieved and kept in separate air-tight conical flasks.

Thirty-five (35) red matured, firm, smooth, and healthy tomato fruits were obtained from a local food and fruits market, Iyana Iba market, Ojo, Lagos State, Nigeria. The tomatoes were divided into five for each treatment and control groups. The tomatoes were procured based on their firmness and reddish matured colour before being stored at room temperature. The tomato fruits were washed under running tap water and air-dried at room temperature. Neem/moringa aqueous leaf extracts were prepared by dissolving 70g of neem/ moringa leaf powder in 350 mL distilled water separately.

2.2 Silver nanoparticles (AgNPs) Synthesis

The leaf powder of neem and moringa (100 g each) were dissolved in 1000 mL distilled water, filtered, and stored separately.The silver nanoparticles were prepared according to the methods of [16, 17]with some modifications.A freshly prepared 2 mM silver nitrate solution was mixed with neem and moringa aqueous leaf extract separately in ratios 1:9 and 6:4, respectively. The colour change indicated silver nanoparticle synthesis, and wasfurther confirmed by observingthe absorption peak between 400 – 450 nm using UV-visible spectrophotometer.

2.3 Treatment of Tomato with NALE, MALE, NALE-AgNPs and MALE-AgNPs

The tomato samples were immersed in neem and moringa aqueous leaf extract separately before being arranged in a clean container, and kept at room temperature on the Laboratory table. Changes were observed and data were recorded for the 15 days of treatment to ascertain the effects of the extracts. Conversely, another set of tomatoes were immersed in each AgNPs solution for 2 hours before being placed in a clean container in the laboratory at 25°C. Changes were also observed and data were recorded for the 15 days of treatment to ascertain the effects of the synthesized AgNPs.

2.4 Data collection

The post-harvest decay percentage (PDP), marketability, shelf-life, and firmness of the tomato fruits were calculated [18] using:

Post-harvest decay percentage (PDP) $= \frac{number\ \ of\ decaying\ \ fruits}{total\ number\ \ of\ fruits} \times 100$

Marketability $= \frac{number\ \ of\ Marketable\ \ fruits}{total\ number\ \ of\ fruits} \times 100$

Firmness = rating scale 1 - 5

Where 1is very poor, 2 is poor, 3 is acceptable, 4 is good, and 5 is excellent.

2.5 Isolation and Identification of fungi causing spoilage of Tomato fruits during storage

Potato dextrose agar was used for the isolation of fungi from the tomato fruits and the preparation of pure culture. Thirty-nine grams (39g) of potato dextrose agar was dissolved in 1000 mL of distilled water in a sterile conical flask covered with cotton wool and aluminum foil paper. The mixture was shaken thoroughly and autoclaved at 121°C for 15 minutes under a pressure of 15 pounds per square inch (15lb/inch^2). The medium was cooled after autoclaving to 45°C and then dispensed aseptically into a sterile Petri dish. Chloramphenicol was added to the medium to prevent the growth medium.

The workbench was disinfected, and a sterilized cork-borer was used to extract pieces from a diseased tomato, which were placed into the medium. After 5 days of incubation at 37°C, mixed cultures were re-isolated until obtaining a pure culture. Identification was based on morphological features and microscopic examination using lactophenol cotton blue solution, following the modified procedures of[19].

2.6 Statistical Analysis

The daily weight of the coated tomatoes wasrecorded triplicates and the data were subjected univariate statistical analysis such as mean and standard deviation (SD) using Statistix 10 software. The means were separated using analysis of variance and comparison were made Least Significance Difference (LSD) at 95% confidence level.

3. RESULTS

The 15-day experiment showed that the control group tomatoes spoiled by the 8thday, losing its firmness from the 5thday. However, tomatoes coated with Neem aqueous leaf extract synthesized silver nanoparticles (NALE-AgNPs) solution (6:4) lasted 12 days, with significant weight loss from day 6. Another variant (1:9) lasted 14 days before significant weight loss led to complete deterioration by day 15. Tomatoes coated with neem aqueous leaf extract had the longest shelf life, losing firmness at day 8 and deteriorating completely by day 15.

Table 1. Weight of Effects of *Solanum lycopersicum* after coating with NALE and NALE-AgNPs

Groups/Days	Control	NALE	Nano 1	Nano 2
IW1	91.5±4.64^{a}	74.57±12.26^{ab}	80.87±4.92^{ab}	83.23± 8.14^{a}
IW2	91.50± 4.64^{a}	84.3± 12.50^{a}	81.97± 4.88^{a}	84.80± 8.41^{a}
DAT 1	89.60± 4,32 ^{a}	79.03 ± 12.08^{ab}	80.83± 3.85^{abc}	83.00± 8.29^{a}
DAT 2	88.07 ± 4.16^{ab}	76.70± 12.08^{ab}	78.83± 3.23^{abc}	81.47± 8.11^{a}
DAT 3	82.33 ± 3.61^{bc}	75.33± 13.25^{ab}	76.13± 4.37^{abcd}	80.23± 7.88^{a}
DAT 4	79,30 ± 6.67^{c}	75.30± 11.98^{ab}	72.57± 5.50^{bcde}	78.37±7.04^{ab}
DAT 5	75.80 ±6.05^{cd}	73.37±13.20^{ab}	73.30±4.03^{cde}	78.83± 8.34^{ab}
DAT 6	70.30± 4.21^{de}	72.60 ±13.1^{ab}	71.10± 4.33^{def}	76.50±7.02^{abc}
DAT 7	66.57 ± 4.10^{de}	71,87± 12.64^{ab}	68.57± 5.31^{defg}	74.27± 6.12^{abcd}
DAT 8	61.93 ± 4.73^{f}	71.40 ± 12.47^{ab}	67.27 ± 5.14^{efg}	69.20± 4.50^{bcd}
DAT 9	0	70.93 ± 12.14^{ab}	65.97± 4.97^{efg}	67.40 ± 2.94^{cde}
DAT 10	0	67.60± 11.00^{ab}	64.73 ± 4.73^{fg}	65.00 ±1.39^{de}
DAT 11	0	64.33 ± 11.75^{ab}	63.83± 4.39^{fg}	57.33± 0.75^{ef}
DAT 12	0	62.87 ± 12.24^{b}	62.87 ± 4.27^{g}	49.40± 0.35^{f}

Mean ± SEM values with the same alphabet in the same row are not significantly different from each other at $p<0.05$, where, NALE= Neem aqueous leaf extract, Nano 1 = Neem aqueous leaf extract dissolved in $AgNO_3$ at 1:9, and Nano 2 = Neem aqueous leaf extract dissolved in $AgNO_3$ at 6:4, IW1= Initial weight before Coating, IW2= Initial weight after coating. DAT= Days after treatment.

The 15-day experiment revealed that all fruit samples deteriorated by the 15th day, with observable decay starting from the 5th day. White mold appeared from the 3rd day before decay. Tomato fruits coated with Moringa aqueous leaf extracts deteriorated from the 8th day, but some lasted until the 15th day. However, those coated with Moringa aqueous leaf extract synthesized silver nanoparticles (MALE-AgNPs) showed preservation until the 8th day (in a 6:4) and some lasting until the 10th day (in a 1:9). Significant differences in weight loss were observed on specific days between moringa-coated and control fruits, as well as between silver nanoparticle-treated and control fruits.

Table 2. Weight of Effects of *Solanum lycopersicum* after coating with MALE and MALE-AgNPs

Groups/Days	Control	MALE	Nano 3	Nano 4
IW1	91.50 ± 4.64^{a}	81.73 ± 3.90^{abc}	93.77 ± 2.64^{abc}	94.07 ± 6.31^{ab}
IW2	91.50 ± 4.64^{a}	95.50 ± 5.24^{a}	99.30 ± 5.27^{a}	100.73 ± 7.94^{a}
DAT 1	89.53 ± 4.21^{a}	86.93 ± 10.47^{ab}	97.50 ± 5.15^{ab}	98.67 ± 7.72^{a}
DAT 2	88.07 ± 4.16^{ab}	82.90 ± 10.12^{abc}	95.03 ± 4.73^{abc}	95.80 ± 7.01^{ab}
DAT 3	82.33 ± 3.61^{bc}	83.83 ± 11.07^{abc}	89.13 ± 7.90^{abcd}	92.77 ± 6.92^{ab}
DAT 4	80.80 ± 4.08^{cd}	80.73 ± 9.64^{abc}	87.77 ± 7.42^{bcd}	91.50 ± 6.29^{ab}
DAT 5	75.80 ± 6.05^{de}	77.53 ± 7.75^{bcd}	86.57 ± 6.75^{cd}	90.17 ± 5.71^{abc}
DAT 6	72.77 ± 4.25^{ef}	74.13 ± 5.81^{bcd}	87.87 ± 6.12^{bcd}	89.43 ± 6.84^{abc}
DAT 7	68.93 ± 4.19^{fg}	70.67 ± 7.11^{cd}	85.30 ± 4.85^{cd}	88.00 ± 6.76^{abc}
DAT 8	64.67 ± 4.73^{g}	73.87 ± 6.12^{bcd}	82.60 ± 3.12^{d}	85.47 ± 5.77^{bcd}
DAT 9	0.00 ± 0.00^{h}	65.33 ± 11.61^{d}	69.97 ± 4.79^{e}	77.97 ± 7.08^{cde}
DAT 10	0.00 ± 0.00^{h}	64.83 ± 11.56^{d}	62.13 ± 7.04^{e}	74.47 ± 8.31^{de}
DAT 11	0.00 ± 0.00^{h}	64.33 ± 11.51^{d}	49.73 ± 13.28^{f}	68.07 ± 16.17^{e}
DAT 12	0.00 ± 0.00^{h}	63.20 ± 11.01^{d}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}

Mean ± SEM values with the same alphabet in the same row are not significantly different from each other at $p<0.05$, where, MALE= Moringa aqueous leaf extract, Nano 3 = Moringa aqueous leaf extract dissolved in $AgNO_3$ at 1:9, and Nano 4 = Moringa aqueous leaf extract dissolved in $AgNO_3$ at 6:4, IW1= Initial weight before Coating, IW2= Initial weight after coating. DAT= Days after treatment.

Table 3 illustrates the postharvest decay of tomato fruits during storage period of 15 days. The percentage of decay was observed to increase as the days increased. The deterioration of tomato fruits started on the 5th day with only 20 percent of both control and NALE-AgNPs (6:4) observed. Meanwhile, it was observed that, the different concentrations of MALE-AgNPs (1:9 and 6:4) delayed decay up to the 15th day.

Table 3. Post-harvest decay percentage of tomato fruitscoated with NALE, MALE and their synthesized silver nanoparticles at different concentrations

	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
Control	0	20	`60	100	100	100	100
NALE	0	0	20	40	40	60	80
MALE	0	0	40	40	40	60	80
Nano 1	0	0	80	80	80	80	100
Nano 2	0	20	60	60	60	100	100
Nano 3	0	0	40	40	*	*	*
Nano 4	0	0	60	60	*	*	*

** Denotes null set (totally decayed sample).*

Figures 1 and 2 illustrate the firmness of the tomato fruits. It was observed that the tomatoes coated with the aqueous leaf extracts of neem and moringa have the longest shelf life of 15 days compared with 8 days of Moringa and Neem silver nanoparticles and the control.

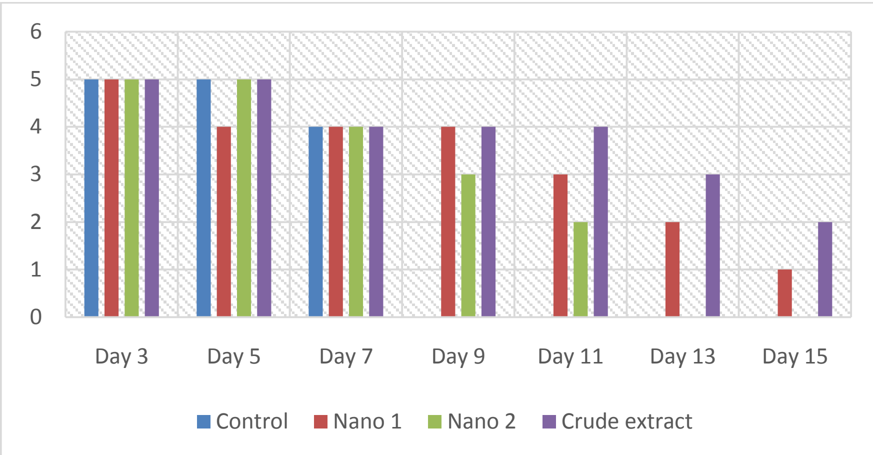

Figure 1. Firmness of Tomatocoated with NALE crude and NALE-AgNPs during storage.

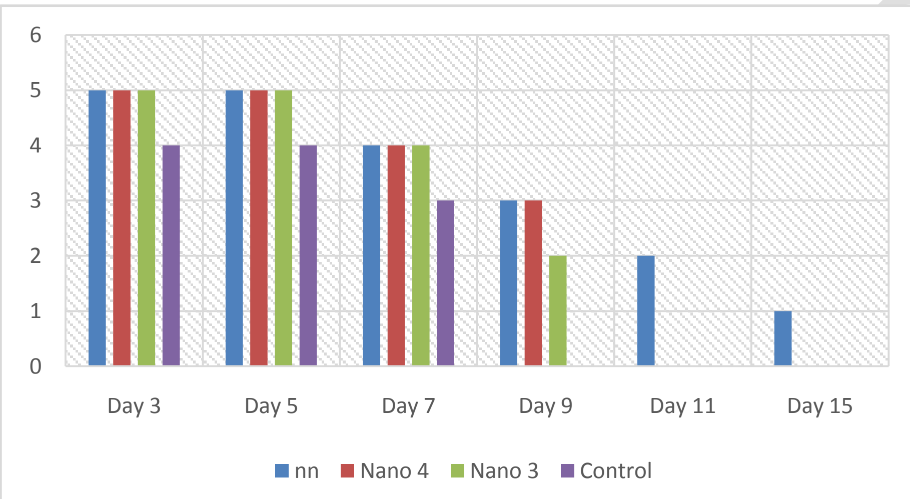

Figure 2. Firmness of Tomato coated with MALE crude (nn) and MALE-AgNPs during storage.

A total of two fungi, *Aspergillus* species,were isolated, identified, and characterized from the deteriorated tomato samples. These were*A. niger* and *A. flavus*.
The conidia of *A. niger*were dark brown to black and spherical having a sporulated surface growth on the culture media, with visible aseptate hyphae (without cross-wall)while, the conidia of*A. flavus*were smooth with green dispersed spores, and septate (cross-walled) hyphae were present with phialides.

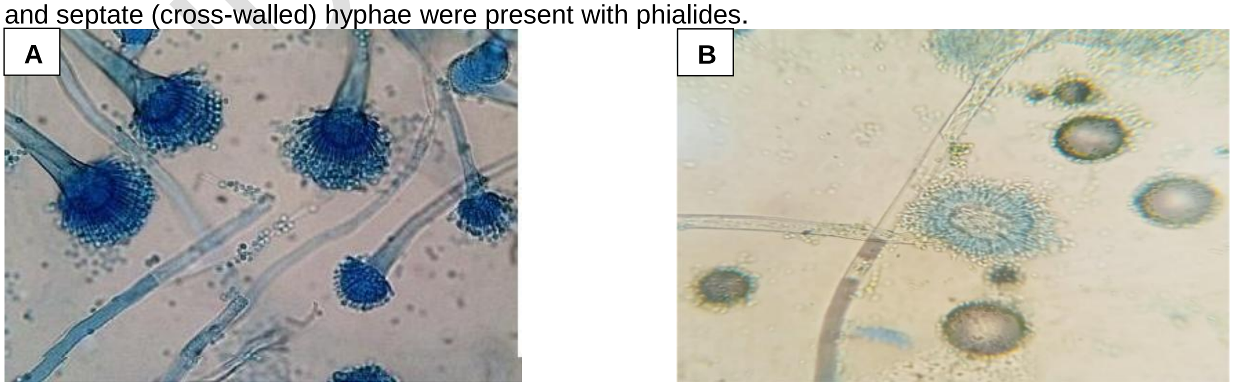

Figure 3. Photomicrograph of (a) *Aspergillus niger*(X400) (b) *Aspergillus flavus*(X400) isolated from deteriorated tomato fruits.

4. DISCUSSION

The prevention of fruit spoilage by pathogenic bacteria and preservation of fruit freshness poses a serious challenge in the fruit industry. Concerns over the use of synthetic preservatives have led to a shift towards exploring plant-based alternatives. The effectiveness of neem and moringa aqueous leaf extracts in reducing tomato decay observed in this study suggests that it could be a viable alternative for combating pathogen-related decay in tomatoes. This observation aligns with a study reported by [20], who reported that treating various kinds of fruits with chitosan and guava leaf extract significantly increased the shelf life of the fruits. Similarly, our findings are consistent with the report of [21], who highlighted the effectiveness of extracts from medicinal plants like *Allium sativum*, *Azadirachta indica*, *Mentha arvensis*, and *Psoralia corlylifolia* in preserving fruits from thepathogenic and environmental factors. Moreover, the extract of neem increases the shelf life of the fruits by reducing the fungal and bacterial spoilage during storage.

Tomatoes coated with moringa and neem aqueous leaf extracts exhibited reduced post-harvest decay, reflected in the lower number of decayed fruits compared to both control and NALE and MALE-synthesized silver nanoparticles.These plants also showed higher marketability with a greater number of marketable fruits in both categories. While control fruits lasted for only 8 days, most of the treated tomato fruits still retained their color and number, and became completely rotten on the 15th day. Among all, the tomato fruits with coated with MALE-AgNPs at different concentrations low postharvest decay percentage. This implied that coating with MALE-AgNPs could help tomato fruits resist environmental and pathogenic attack better than other treatments. The observed progressive weight loss in neem-coated tomatoes and control aligns with the report of [22], who stated that post-harvest weight change in fruits is typically linked to temperature and storage time, often attributed to water loss through transpiration. Thus, the higher the temperature, the higher the respiratory rate of the fruits and the higher its metabolic activity, which may lead to an increase in weight loss during storage. The higher decrease in the firmness of the control tomato fruits compared to the treated fruits may be attributed to a higher rate of metabolic activities and activity of cell wall degrading enzymes that loosens the fruit skin which result in higher permeability of the cell for higher rate of moisture loss.

Another possible reason why the incorporation of silver nanoparticles showed lower preservative effects on tomatoes compared to the leaf extracts of Neem and Moringa could be attributed to the complex interactions between silver nanoparticles and the tomato surface, as well as the distinct antimicrobial properties of the leaf extracts. AgNPs exert antimicrobial activity primarily through the release of silver ions that disrupt microbial cell membranes and inhibit cellular processes [23], the antimicrobial mechanisms of neem and moringa leaf extracts are multifaceted and may involve the inhibition of microbial enzymes, disruption of cell membranes, which interferes with microbial metabolism [24]. This diversity in antimicrobial mechanisms could contribute to the superior preservation effects of the leaf extracts on tomatoes compared to AgNPs.

In addition, the findings of this study also revealed some of the fungi associated with the post-harvest decay of the tomato fruits in storage. These fungiare*Aspergillus niger* and *A. flavus* which have previously been reported as pathogens of tomato fruits by [25,26]. Theyhave also been found in other crops including orange, Sour-sop, and garri (fried mashed fermented cassava) [3]. Association of these fungi with these fruits/ foods may suggest their omnipresent, non-host specific and non-geographical-specific nature.

CONCLUSION

Our findings from this study demonstrated that neem (*Azadirachta indica*) and moringa (*Moringa oleifera*)leaf powder can effectively prolong the shelf life and also preserve the quality of tomato fruits beyond their typical limits. This offers a valuable information on plant leaves' potential in post-harvest preservation in addition to their known nutraceutical properties. Future studies may explore the phytochemical composition, *in vitro* and *in vivo* potentials of the leaf extract powder of the plants in preventing disease development in tomato fruits, which may possibly explicate their postharvest shelf life and quality retention potentials on tomato fruits.

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