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***Research Article***

# COMPARISON BETWEEN WATER WASHING AND DETERGENT WASHING ON REDUCTION OF POST HARVEST LOSSES OF TOMATO (*LYCOPERSICUM ESCULENTUM*)

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## ABSTRACT
Research was carried out on tomato on sell at two major vegetables markets areas of Kano state to determine the more effective way of increasing shelf life of tomato. In the research comparison was made between the two method of treatment used by marketers at both Tarauni and Yankaba markets washing with water and washing with detergent. Result of the study showed that four fungal colonies were isolated from samples of tomato collected from the two locations. The four fungal colonies have the following composition *A.niger* 140(38.6%), *A.flavus* 124(34.1%), *A.fumigatus* 21(5.8%) and *R.stolonifer* 78 (21.5%). A total of 375 colonies was counted from the tomato samples in the two location out of which water washed tomato recorded the high number of colonies count 253(67.5%), while low colonies were isolated from detergent wash tomato collected from the two locations 122(32.5%). Therefore, this study establish that the lower colony counted from detergent wash tomato sample from the two locations is an indication that washing of tomato with detergent is more effective in reducing post-harvest losses by washing away the surface microorganisms.

***Keywords:*** *Aspergillus, Detergent, Tomato, Water*

## INTRODUCTION
Tomato *(Lycopersicon esculentum Mill. Syn. Solanum lycopersicon)* is a widely grown fruit the world over, It is native to South America (Nonneoke, 1989), but was introduced into West Africa by Portuguese traders and freed slaves from West Indies. Nigeria is second largest producer of tomato in Africa with over one million hecters of land used annually for cultivation of tomato and second only to Egypt and 13th in the world, and produces over six million tonnes of tomato annually (Erinle, 1989). Tomato (*Lycopersicon esculentum*) is one of the most important vegetable crops grown in Nigeria. Tomato has been in cultivation in Nigeria for a very long time. It is an important condiment in most diets and a very cheap source of vitamins A and C. The major tomato producing areas in Nigeria lie between latitudes 7.5 °N and 13 °N, and within a temperature range of 25 – 34 °C (Villareal, 1980; Denton and Swarup, 1983) these areas include most States in Northern Nigeria.
Tomato is rich in vitamins, minerals and lycopene, an excellent antioxidant that helps to reduce the risk of prostate and breast cancer (Giovannucci, 1999). Global production is about 89.8 million metric tonnes from an area of about 3,170.000 ha. Tomato accounts for about 18% of the average daily consumption of vegetables in Nigeria and may be pressed into pastes or purse which is used for cooking and in the production of fruit drinks (Babalola *et al.*, 2010). In Nigeria there is a glut of production during the season and scarcity at off season due to poor post-harvest storage facilities (Yahaya, 2006).
Fungi are the most important and prevalent pathogens that infect a wide range of host plants, causing destruction and economic loss in tomato either in the field, storage or transportation. The worldwide postharvest losses of tomato are as high as 30-40% (Kader, 1992; Agrios, 2005), but this is much higher in developing countries like Nigeria due to improper handling methods (Kader, 1986; Prigojin *et al.*, 2005). The quality and nutritional value of freshly produced tomato fruits is affected by pre- and postharvest diseases due to improper handling and other conditions.
In 1987, Opadokun reported that 21% of tomato harvested in Nigeria is lost to rot in the field and additional 20% to poor storage system, transportation and marketing. While Kutama *et al.*, (2007) estimated total loss in Nigeria due to these constraints as about 60%. Fresh tomato fruits are highly

perishable with a short shelf-life and high susceptibility to fungal diseases. They are difficult to store for long periods without incurring losses and as the fruits ripen, they become more susceptible to microbial infections (Moss, 2002; Rehman, 2007; Ewekeye *et al*., 2013).

Although a large amount of this vegetable is produced annually, its availability all year round is limited due to the high incidence of pest and diseases as well as poor storage facilities (Kutama *et al.*, 2007). This huge loss has prompted the search for simple, effective and economical methods to control pre- and post-harvest diseases and other losses in tomato (Wilson and Wisniewski, 1989). The practice of washing tomato is highly significance in reducing contamination at post-harvest losses by washing away the surface microorganism that causes spoilage thereby reducing post-harvest losses of the commodity (Hernandez-Brenes, 2002a). In an effort to determining the better washing methods that will reduce losses due to fungal infection of the tomato which causes spoilage of tomato. Therefore, two hypotheses were tested. First washing tomato with detergent is more effective in reducing post-harvest losses than washing with water. Second to isolate and identify the common pathogenic fungi that are associated with postharvest spoilage of tomato on sale at Yankaba and Tarauni market Kano, Nigeria.

## MATERIALS AND METHODS

### *Study Site*

**Yankaba market**: is located at Nassarawa local government area of Kano state. It is one of the largest vegetable markets in Kano state. There are no vegetables grown in Kano state that are not found at the market. Despite being one of the largest markets in Kano state, however, there are no adequate storage facilities in the market. Some marketers store their vegetables on the floor of the stores, while others kept their vegetables packed in baskets. Marketers hardly used chemicals on their vegetables. They however washed them either with water or detergents.

**Tarauni market:** is located at Tarauni local government area of Kano state. It is one of the vegetable markets in Kano state. There are no vegetables grown in Kano state that are not found at Tarauni market. Despite being one of the major vegetable markets in Kano state, there are no good storage facilities in the market. Some marketers store their vegetables on the floor of the stores, while others kept their vegetables packed in baskets. Marketers hardly used chemicals on their vegetables. They however washed them either with water or detergents.

### *Experimental Procedure*

In this study an investigation was carried out to provide information on fungal colonies responsible for post-harvest losses of tomato. The investigation period span from November 2014 to January 2015. This coincides with glut period of tomato.

### *Isolation and Identification of Postharvest Fungi*

This involved the isolation and identification of fungi associated with losses of quality and quantity of Tomato respectively. The methodology used in this research follows the one used by Yahaya (2006) and is described below:

### *Sample Collection and Collection Site*

Twenty samples of fresh tomato were obtained twice a week Monday and Thursday directly from Tarauni and Yankaba markets. The samples were separately placed in polyethylene bags and transported to laboratory at Biology Department Kano University of Science and Technology, Wudil.

### *Isolation Media*

Potato dextrose agar was used for isolation of post- harvest fungi. The medium has the following constituents per liter:

Potato 4.0g
Dextrose 20.0g
Agar 15.0g
Water 1litre

Thirty nine grams of PDA were weighed using weight balance and suspended in 1litre of distilled water. The contents were sterilized by autoclaving machine at $121^0$c for 15 minutes. These were allowed to cool

for 5 minutes on a laboratory bench until the temperature fell down. Then 1ml of lactic acid was added to inhibit bacterial growth. The media was dispensed into sterilized Petri dishes of 9cm diameter.

***Sample Handling***

Ten tomato fruit each from Tarauni and Yankaba markets were surface sterilized by washing with distilled water while the remaining ten samples each from Tarauni and Yankaba market were sterilized with detergent and allowed to dry. Portions (2mm) were cut with a sterilized scalpel. Cut pieces were placed on PDA and incubated at 25.7 ±2c for 3 days.

***Colony Count and Subculture***

Each week, growth of fungal colonies was monitored and the number of colonies that appeared was counted and recorded. Each distinct colony was sub cultured into fresh PDA.

***Pathogenicity Test***

Pathogenicity tests were conducted to prove Koch postulate. All fresh samples were separately washed in 10% (v/v) sodium hypochlorite solution and rinsed in 3 changes of running tap water and allowed to dry. A ruler was used to mark a (2mm) diameter circle on each sample; a sterilized needle was used to streaked fungal hyphae on marked portions.

Controls were inoculated with sterile distill water. Materials were placed on the laboratory bench. Sterilized forceps were used to remove portions from the diseased areas on the $4^{th}$ day and placed on freshly prepared PDA plates and incubated at 25.7 ±2oc for 3 days. Fungal growth that appeared was recorded.

***Microscopic Examination***

For each examination, a streak of fungal mycelium was placed on a clean glass slide. One drop of cotton blue lactophenol was added and the cover slip placed. The slide was mounted on the microscope and observed at magnification of ×10, ×40 and x 100.

Morphological characteristics of fungi isolated were determined and identified using method described by Dorothea *el al.,* (1976). Lengths of the hyphae were determined with eyepiece graticule by using colonial and morphological characteristics.

***Photography***

Photographs of fungal mycelia were taken from mounted slide using camera Lucida at Biology laboratory Kano University of science and technology Wudil.

***Statistical Analysis***

The data were also analyzed statistically using One-way analysis of variance (ANOVA) and differences among the means were determined for significance at $P \leq 0.05$. This was achieved using computer program (SPSS, 16.0).

## RESULTS AND DISCUSSION

***Results***

Same colonies used in pathogenicity test were re-isolated back. A total of 375 colonies were counted and recorded during the period of investigation in both water and detergent washed tomato collected at Tarauni and Yankaba market, water washed of both the two market has the colony count of 253(67.5%) while detergent washed has the colony count of 122(32.5%). While a total of four fungal colonies were identified (Table 6) *A.niger* (Plate 1), *A.fumigatus* (Plate 2), *A.flavus* (Plate 3) and *R.stolonifer* (Plate 4). The four fungal colonies have the following composition *A.niger* 140(38.6%), *A.flavus* 124(34.1%), *A.fumigatus* 21(5.8%) and *R.stolonifer* 78(21.5%).

***Effect of Days of the Isolation on the Number of Colonies Isolated from Tarauni and Yankaba Markets***

There was a significant difference in the number total number of colonies isolated on Monday and Thursday at both Tarauni and Yankaba markets.

The results shows that more colonies were recorded on Thursday collection with a colony count of 220 (58.7%) and least colonies were recorded on Monday collection with a colony count of 155 (41.3%) throughout the study period (Table 1).

**Table 1: Total number of colonies counted from water washed and detergent washed in**

| Location | Tarauni | Yankaba | Total | Mean | %abundance |
|---|---|---|---|---|---|
| Monday | 64 | 91 | 155 | 77.5 | 41.3 |
| Thursday | 124 | 96 | 220 | 110 | 58.7 |
| TOTAL | 188 | 187 | 375 | 187 | 100 |

***Effect of Location on the Two Treatments Water and Detergent Washing of Tomato on the Number of Colonies Isolated at Tarauni and Yankaba Markets***

More colonies were isolated from water wash and detergent wash tomato collected from Tarauni188 (50.1%) than water wash and detergent wash tomato collected from Yankaba187 (49.9%). However, the difference was not statistically significant. While on the basis of treatments water washed tomato has the highest number of colonies count of 253 (67.5%) and detergent washed has the least colonies count of 122 (32.5%) throughout the period of stud (Table 2).

**Table 2: Total number of colonies counted from water washed and detergent washed tomato collected from Tarauni and Yankaba markets**

| Location | Water washed | Detergent washed | Total | Mean | % |
|---|---|---|---|---|---|
| Tarauni | 132 | 56 | 188 | 94 | 50.1 |
| Yankaba | 121 | 66 | 187 | 93.5 | 49.9 |
| TOTAL | 253(67.5%) | 122(32.5%) | 375 | 187.5 | 100 |

***Fungal Colonies Isolated from Water Wash Tomato in the Two Locations***

A total of four colonies were isolated and identified as fungal pathogens associated with post-harvest losses of water wash tomato in the two locations. The fungal pathogens has the following compositions *A.niger* has the highest frequency of occurrence with 103 (41%) followed by *A.flavus* with 89 (35.5%) and *R.stolonifer* with 43 (17.1%) while *A.fumigatus* has the least frequency of occurrence (Table 3) with 16 (6.4%).

**Table 3: Total number of fungal colonies identified from water wash tomato collected from Tarauni and Yankaba markets**

| Colonies | water washed Tarauni | water washed Yankaba | Total | Mean | %abundance |
|---|---|---|---|---|---|
| *A. niger* | 67 | 36 | 103 | 51.5 | 41.0 |
| *A. flavus* | 36 | 53 | 89 | 44.5 | 35.5 |
| *A. fumigatus* | 6 | 10 | 16 | 8 | 6.4 |
| *R. stolonifer* | 23 | 20 | 43 | 21.5 | 17.5 |
| TOTAL | 132 | 119 | 251 | 125.5 | 100 |

**Table 4: Total number of colonies identified from detergent washed tomato collected from the two locations**

| Colonies | DW.Tarauni | DW.Yankaba | Total | Mean | %Abundance |
|---|---|---|---|---|---|
| *A. niger* | 23 | 14 | 37 | 18.5 | 33.0 |
| *A. flavus* | 23 | 12 | 35 | 17.5 | 31.2 |
| *A. fumigatus* | 2 | 3 | 5 | 2.5 | 4.5 |
| *R. stolonifer* | 7 | 28 | 35 | 17.5 | 31.2 |
| TOTAL | 55 | 57 | 112 | 56 | 100 |

*DW-Detergent wash*

***Fungal Colonies Isolated from Detergent Wash Tomato in the Two Locations***

A total of four colonies were identified from detergent washed tomato collected from Tarauni and Yankaba market, the fungal colonies identified has the following compositions; *A.niger* has the highest frequency of occurrence with 37 (33%) followed by *A.flavus* with 35 (31.2%) and *R.stolonifer* with 35 (31.2%) while *A.fumigatus* has the least frequency of occurrence (Table 4) with 5 (4.5%).

***Comparison of Total Number of Colonies Isolated from the two Treatments in the Two Locations***

Results from the study showed that at the end of the study the most occurring fungal colony from the two treatments in the two location was *A.niger* with 140 (38.6%) followed by *A.flavus* with 124 (34.1%), *R.stolonifer* with 78 (21.5%) and *A.fumigatus* has the lowest frequency of occurrence in the two locations with 21 (5.8%) Table 5.

**Table 5: Comparison of the total number of colonies identified from the two treatments in the two locations**

| Colonies | WW.TRN | DW.TRN | WW.YKB | DW.YKB | Total | Mean | % |
|---|---|---|---|---|---|---|---|
| *A.niger* | 67 | 23 | 36 | 14 | 140 | 35 | 38.6 |
| *A.flavus* | 36 | 23 | 53 | 12 | 124 | 31 | 34.1 |
| *A.fumigatus* | 6 | 2 | 10 | 3 | 21 | 5.25 | 5.8 |
| *R.stolonifer* | 23 | 7 | 20 | 28 | 78 | 19.5 | 21.5 |
| Total | 132 | 55 | 119 | 57 | 363 | 90.75 | 100 |

*Key- WW-water wash, DW-Detergent wash, YKB-Yankaba, TRN-Tarauni*

**Table 6: Shows colonial and morphological characteristic of identified fungal spp from water washed and Detergent washed tomato collected from Tarauni and Yankaba markets**

| s/n | Appearance on PDA | Morphological characteristic | Organism identified |
|---|---|---|---|
| 1. | Colony rapidly growing black in colour reverse colony is colourless or yellow later black | Numerous mycelia, conidia and conidiophores are black, spherical to oval the conidia head is globose splitting into columns | *Aspergillus niger* |
| 2. | Colony rapidly growing white cottony at first, turning brownish black at maturity | Stolons creeping, recurving to the substrate. In the form of hyphae, which are strongly raised and distance from the substrate and implemented at each node by rhizoids. The hyphae are more or less branched. Sporangiophores are unequal, irregular round or oval streak. | *Rhizopus stolonifer* |
| 3. | Colony rapidly growing velvet in texture, white at first becoming blue green to dark green. Reverse is colorless or yellow | Conidiophores smooth shoot of ten greenish upward to form apical flask shaped vesicle. Conidia green inn mass, globose, rough, mostly 2.5-3m diameter | *Aspergillus fumigatus* |
| 4. | colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green | Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 μm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened. Often more noticeable near the vesicle. Conidial head are globose. | *Aspergillus flavus* |

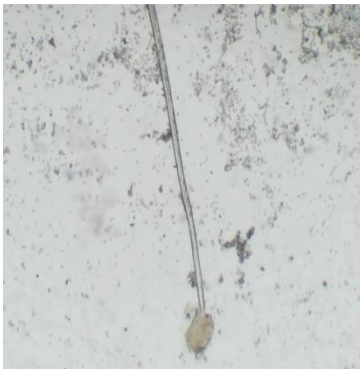

**Plate 1: A Microscopic view of *A. niger* under cotton blue lactophenol x 100**

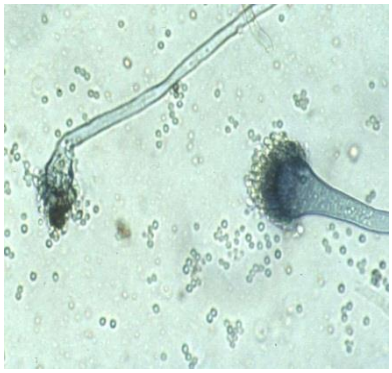

**Plate 2: A Microscopic view of *A. fumigates* under cotton blue lactophenol x100**

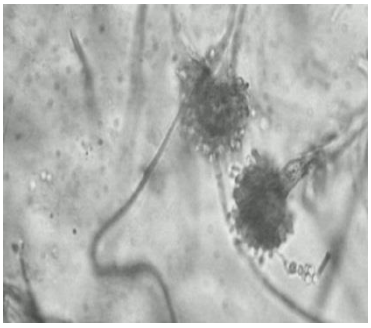

**Plate 3: A Microscopic view of *A.flavuss* under cotton blue lactophenol x100**

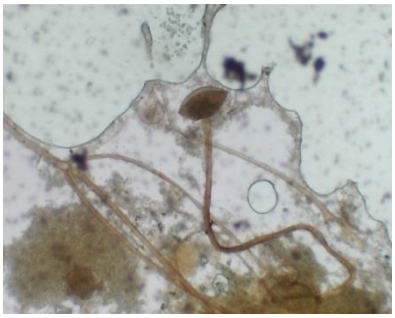

**Plate 4: A microscopic view of *R. stolonifer* under cotton blue lactophenol x 100**

***Discussion***

Four fungal colonies comprising *A.niger, A.fumigatus, A.flavus* and *R.stolonifer* were isolated from both water washed and detergent washed tomato in the two locations. High number of colonies was recorded from the water wash tomato than detergent wash tomato in both the two locations. Also more colonies were recorded from samples collected from Tarauni market than samples collected from Yankaba market.

In the present study a total number of 375 fungal colonies were counted and recorded during the period of investigation in both water and detergent washed tomato collected from Tarauni and Yankaba market, water washed of both the two market has higher colony count of 253(67.5%) while detergent washed has the low colony count of 122 (32.5%) (Table 2). Water washed tomato collected from Tarauni has the high colony count of 132 (36.3%) than water washed tomato collected from Yankaba market with the colony count of 119 (32.9%).

In the study a total of 57 (15.7%) colonies were isolated from detergent washed tomato collected from Yankaba market. While55 (15.1%) colonies were isolated from detergent washed tomato collected from Tarauni market (Table 2 and 3).Similar fungal species were identified from the water washed tomato collected from the two locations which consist of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Rhizopusstolonifer.* Out of the colonies *A.niger* has the highest frequency of occurrence with 103 (41.0%) followed by *A.flavus* with 89 (35.5%) and *R.stolonifer* with 43 (17.1%) while *A.fumigatus* has the least frequency of occurrence with 16(6.4%) (Table 3). While colonies isolated from the detergent wash tomato collected from the two markets were also identical to the colonies isolated from water wash tomato out of the colonies *A.niger* has the highest frequency of occurrence with 37 (33%) followed by *A.flavus* with 35 (31.2%) and *R.stolonifer* with 35 (31.2%) while *A.fumigatus* has the least frequency of occurrence with 5 (4.5%) (Table 4).The finding of this research could be related to the result of Sani and Alao (2006) who assessed fungal deterioration of tomato fruits in Kano state Nigeria and found that 70% losses in tomato was attributed to the activities of fungal pathogens *A.niger,*

*A.fumigatus* and *R.stolonifer* which account for 20% and 10% respectively. The result also agrees with the work of Hayatu (2000) who isolated *A.niger, A.flavus, R.stolonifer* and *mucor species* from sample of tomato grown at irrigation sites of Nassarawa local government area of Kano state.

In a related experiment carried out at Keffi, Nassarawa State, Mahovic *et al.,* (2004) reported a wide range of fungi which was responsible for the storage decay of tomato fruits.The result of the present study also agrees with the result of Chuku *et al.*, (2008) and Ogaraku *et al.,* (2010). Their result showed that out of the 48 samples of tomatoes collected 34 tomatoes had fungal infection, while 14 samples were without fungal isolates.

The species of fungi isolated and identified from the infected tomatoes were *Aspergillusniger, Aspergillus flavus, Alternaria alternate, Alternaria solani, Fusarium oxysporium* with the frequencies of occurrence of 47.06%, 17.65% 14.71%, 11.76% and 8.82% respectively.

The result of the study indicated that colonies counted showed that water washed tomato Tarauni has the highest colony count of 132 followed by water washed tomato from Yankaba with 199 colonies count. While 57 colonies were counted from detergent washed tomato collected from Yankaba, but 55 colonies was counted from the detergent washed tomato collected from Tarauni. Therefore, from the result of the study it can be concluded that higher numbers of colonies were isolated from water wash tomato. Therefore the less colonies isolated from detergent wash tomato is an indication that washing with detergent is more effective in reducing the fungal pathogens from the surface of the tomato thereby reducing post-harvest losses. It is therefore, recommended that washing of tomato with detergent after harvest should be encourage.

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