African Journal of Agriculture and Food Science
ISSN: 2689-5331
Volume 3, Issue 3, 2020 (pp. 12-20)
www.abjournals.org

MICRO-ORGANISMS ASSOCIATED WITH THE SPOILAGE OF GARDEN EGGS SOLD WITHIN SOKOTO METROPOLIS

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ABSTRACT: *Solanum species (egg plants) belong to the family of Solanaceae and the plant genus Solanum with over 1,000 species worldwide. It's represented in Nigeria by about 25 species including those domesticated; with their leaves, fruits or both eaten as vegetables or used in traditional medicine. This study was aimed to isolate and identify microorganisms associated with the spoilage of garden eggs. Samples were collected from the Market (Kasuwar Daji) and processed according to standard techniques. The isolates obtained were examined and identified using microscopic examination, colony morphology and biochemical characteristics. Six bacterial and fungal species were isolated and identified. The bacterial isolates include Staphylococcus aureus (50%), Bacillus spp (25%), Staphylococcus epidermis (12.5%), Listeria monocytogens (6.3%) and Closteridium botulinum (6.3%). The fungal isolates were Aspergillus niger (42.6%) Aspergillus flavus (21.4%), and Rhizopus stoloniferMucro racemosus and Microsporum audoinii with 7.1% respectively. Among the bacterial and fungal species isolated in this study, Aspergillus niger and Staphylococcus aureus showed the highest percentage frequency of occurrence, and these organisms are capable of causing food spoilage and cause harm to consumers, so measures such as proper handling and storage should be taken to control the contamination of garden eggs fruits.*

KEYWORDS: Solanum Species, Bacterial Analysis, Fungal Analysis, Bacterial and Fungal Load, Frequency Occurrence.

INTRODUCTION

Solanum species (eggplants) belong to the family of *Solanaceae* and the plant genus *Solanum* with over 1,000 species worldwide. It is represented in Nigeria by about 25 species including those domesticated; with their leaves, fruits or both eaten as vegetables or used in traditional medicine (Bonsu *et al*., 2008: Manoko and van der Weerden, 2004). They are known as garden eggs in Nigeria and called gauta in hausa, afufa or anara in igbo or igba in yoruba. They are highly valued constituents of the Nigerian foods and indigenous medicines that are either eaten raw or cooked, very popular in mixed and rich dishes such as stews and soups (Edem *et al*., 2009), especially in the southern and western parts of Nigeria, although, they are highly cultivated in the north (Chinedu *et al*., 2011). Eggplants come in different species and varieties. They also vary in fruit color, shape, and size (Akanitapichat *et al*., 2010; Chinedu *et al*., 2011). The crops are mostly produced under rain-fed conditions during the rainy season, hence are usually at their best from August through October when they come to the market in droves. Minor season production under Irrigation also takes place. They come either in cream-color flesh or green and have pleasantly bitter taste (due to the presence of

small amount of nicotinoid alkaloids) and spongy consistency (Osei *et al*., 2010; Chinedu *et al*., 2011). According to Akinlosotu (1979) the crop can produce fruit yields of about 8 tonnes/ha to 22 tonnes/ha depending on the cultivar.

In developing countries like Nigeria, spoilage of fruits can occur directly or indirectly via animals, insects, soil and human activities such as improper preservation which causes damage in fruits and lead to difficulties in flies' control and its pathogenic organism to human. Pathogens that can be found in fruits and vegetables such as Garden egg includes the followings: *Esterobacter spp, Salmonella spp, Bacillus spp, E. coli, Listeria monocytogenes, Penicilium* spp, *Aspergillus* spp and etc (Schwartz and Gent (2007). Microbial growth as well as spoilage is controlled by environmental conditions, such conditions can greatly influence bacterial populations; the presence of free moisture on leaves from precipitation, dew, or irrigation may promote survival and growth of bacterial populations (Andrews, 1992; Beattie and Lindow, 1995).

In developing countries like Nigeria, spoilage of fruits can occur directly or indirectly via animals, insects, soil and human activities such as improper harvesting, transportation, handling, preservation which causes damage in fruits and lead to difficulties in flies' control and its pathogenic organism to human. Little or no information exists on the microbial pathogens associated with the spoilage of garden eggs in the study area.

The result of this research work will help in determining the types of microorganisms spoiling garden eggs with the view to set up prevention and control strategies in the study area. This study will also enhance human health and secure food safety as well as public enlightenment to food borne illness; there is a need to evaluate the microbial load of this garden egg available for human consumption in markets within Sokoto metropolis.The aim of this study was to isolate and identify the microorganisms associated with the spoilage of garden eggs (*Solanum specie)* within Sokoto metropolis.

MATERIALS AND METHODS

Sample Collection

A total of nine (9) samples of three different varieties of (three each) garden egg was collected from Kasuwar Daji of Sokoto Metropolis. They were aseptically collected in sterilized samples bags and then transported to Microbiology laboratory of the Faculty of Science, Usmanu Danfodiyo University Sokoto for Microbiological analysis.

Sample Processing

Rotten parts of garden eggs were surfaced sterilized with absolute alcohol after which they were cut through using of a sterile scapel. Cutting was done beginning from the healthy portion (so as to get area of rot). For each sample 1g was taken aseptically using sterile spatula and transferred into first test tube and then mix. 1 ml from the first test tube was also taken and transferred to the second test tube and mix. The procedure was repeated up to the last test tube (Prescott *et al.*,2008).

Bacteriological Analysis of the Samples

For each sample after serial dilution, 0.1ml from 10^{-5} to 10^{-6} dilutions was taken using a sterile syringe and transferred onto the centre of prepared nutrient agar. It was spread all over the agar surface with sterile L-bend glass using spread method technique. The inoculated plates were labelled properly for easy identification and then incubated at 37ºC for 24 hours (Ogofure *et al.,* 2015). The colonies on each plate were counted; this was done to determine the bacterial concentration in a given sample from each area and to compare the amount of growth of bacteria under various condition (Onyeagba, 2004).

Distinct colonies were sub-cultured, which was aseptically transferred into newly prepared Nutrient agar using sterile wire loop by streaking the culture on Nutrient agar plate and incubated at 37ºC for 24 hours. (John, 2003; Fox, 2011).

Identification / Characterization of Bacterial Isolates

The bacterial isolates were characterized and identified by gram staining method, microscopic examination and biochemical test. These tests include Gram staining, Methyl red- Voges-proskaeur test, Citrate utilization test, Catalase test, H_2S gas production, Mannitol salt, Triple sugar iron test (TSI) and Coagulase test as described by (Oyeleke and Manga, 2008; Cheeesbrough (2000).

Fungal Analysis of the Samples

The method of Samson *et al.* (1995) was employed in the mycological analysis of the garden eggs sample.

One ml of each sample solution in a test tube containing $X10^{-3}$ was inoculated into PDA plates differently and then incubated for 72 hours at room temperature that is 25^0C. After 72 hours, the plates were observed. Those PDA plates that show mixed growth were sub-cultured to obtain pure culture. A small portion of each different fungal colony was singly placed in the centre of potato dextrose agar (PDA) plate and incubated at room temperature (25^0C) for 5- 7 days. The developing fungal colonies was sub-cultured repeatedly using sterile cork-borers to cut out 2mm disc from advancing region of the cultured colonies on fresh PDA plates until pure cultures of isolates were obtained (Chiejina, 2006).

The pure culture of the isolate obtained was subjected to microscopic examination with the view to identify the organism present in the garden egg samples. Clean glass slide was used for the identification. A drop of water is place in the center of the slide; a small portion of the fungal culture was cut out with a sterile inoculating needle. The piece was put directly in the water and a smear is formed; a cover slip was used to cover the teased portion. The slides were mounted on the microscope stage, damped with chips, using lower and higher magnification (X10 and X40) objectives. The isolates were identified by comparing characteristics under microscope with diagrams in text book of mycological atlas (Samson *et al.,* 1995)

The identification was based on colonial appearance, pigment production and micro morphology of the spore produced in accordance with Bamett and Hunter, (1999) and Alexopoulous *et al.* (2002).

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RESULTS

From the results obtained in this research, Table 1 shows the viable count of bacteria isolates associated with the spoilage of garden eggs samples, which gives the average mean count ranging from 37.5× 10^7 to 72.7× 10^7cfu/ml. sample C has the highest mean count (72.7 ×10^7cfu/ml), followed by B (48.3 10^7cfu/ml) and C (37.5× 10^7 cfu/ml).

Table 2 represents the biochemical characterization of the bacterial isolates and organism identified. The identified bacterial organisms include *Staphylococcus aureus*, *Bacillu*s spp, *S. epidermis*, *Listeria monocytogens* and *Clostridium botulinum*

Table 3 represents the frequency of occurrence of the isolated bacteria which shows that *Staphylococcus aureus* (50%) has the highest percentage frequency followed by *Bacillus* spp (18.8), *Bacillus subtilis*, *Listeria monocytogens* and *Clostridium botulinum* has the least percentage frequency (6.3%).

Table 4 showed the fungal colony count isolated from garden eggs samples, the mean fungal count ranged between 2.7 ×10^3 to 3.3 ×10^3 cfu/ml. Sample B has the highest mean colony count of 3.3 ×10^3cfu/ml while Sample A and C has the least mean colony count (2.7 ×10^3 cfu/ml)

Tables 5 represent the macroscopic and microscopic characteristics/features of fungi isolated from spoiled garden eggs samples which includes *Aspergillus niger, Aspergillus flavus, Rhizopus stolonife*r, *Rhizopus oryzae, Mucro racemosus*and *Microsporum audoinii*

Tables 6 represent the percentage frequency of occurrence of fungi isolated from spoiled garden eggs by which *Aspergillus niger* occurred frequently (42.6), *Aspergillus flavu*s (21.4%), *Rhizopus stolonife*r (14.3%) and *Rhizopus oryzae, Mucro racemes* and *Microsporum audoinii* with 7.1%.

Table 1:Bacterial Colony Count (cfu/ml) in Garden Egg Samples

Sample	Bacterial Mean count (Cfu/ml)
A	37.5×10^7
B	48.3×10^3
C	72.7×10^3

KEY: Cfu/ml = Colony forming unit per mil

Table 2: Biochemical Identification of Bacteria Isolated from Garden Eggs

Isolates	G. rxn	Glucose	Lactose	Sucrose	Gas	H_2S	Mort	MSA	Cat	Coa	Citr	MR	VP	Org. Identified
1	+ve cocci	-	-	-	-	-	-	+	+	+	NA	NA	-	*Staphylococcus aureus*
2	+ve cocci	-	-	-	-	-	-	+	+	-	NA	NA	-	*Staphylococcus epidermis*
3	+ve rods	+	+	-	-	-	-	NA	NA	NA	+	+	-	*Listeria monocytogens*

4	+ve rods	-	+	-	-	-	+	NA	NA	NA	+	-	+	*Bacillus species*
5	+ve rods	-	-	-	-	-	+	NA	NA	NA	-	+	-	*Closteridium botulinum*

Key: H_2S= Hydrogen Sulphide, G. rxn = Gram reaction, MR= Methyl red, VP= Voge's Proskauer, MSA = Mannitol salt agar,
NA= Not Applicable

Table 3: Frequency of Occurrence of Bacteria Isolated from Garden Eggs

Isolates	Occurrence	% Frequency of Occurrence
Bacillus species	4	25%
Staphylococcus aureus	8	50%
Staphylococcus epidermis	2	12.5%
Listeria monocytogens	1	6.3%
Closteridium botulinum	1	6.3%
Total	**16**	**100%**

Table 4: Fungal Colony Count (cfu/ml) in Garden Eggs Samples

Sample	Fungal Mean count (Cfu/ml)
A	2.7×10^3
B	3.3×10^3
C	2.7×10^3

KEY: Cfu/ml = Colony forming unit per mil

Table 5: Macroscopic and Microscopic Characteristics of Identified Fungal Isolates

Isolates	Macroscopic Characteristics	Microscopic Characteristics
Aspergillus niger	Pin like or Black powdery myceliated, spreading and zonated colonies	Non-branched conidiophores with bulb end carries conidia like sun rays.
Aspergillus flavus	Appeared rough, woolly and yellowish green at first, but later turned dark green with age.	Non-Branched conidiophore with bulb end carries conidia.
Rhizopus stolonifer	Colonies light grey, growing extremely rapidly and filling the petridishes with dense cottony mycelia producing mass of sporangia.	Sporangia contain spores, have rhizoids
Rhizopus oryzae	it has a rapid growth rate and a wooly texture; the colonies are typically grayish to brownish.	Sporangia are ovoid, have rhizoids, hyphae broad, not or scarcely septate.

Mucor racemosus	A very white cottony like growth became brownish to grey with age observed.	Tall (up to 2cm) needle like sporangiosphore and large sporangium
Microsporum audoinii	The colonies grew with a moderately rapid pace. Colonies were flat and spreading with a radiating margin. The appeared to have a gray-white to tan beige coloration	Hyphae are septate and often show pectinate (comb-like) and racquest cells

Table 6: Percentage Frequency of occurrence of Identified Fungi associated with Garden Eggs

Identified Fungi	Total number of Occurrence	% Frequency of Occurrence
Aspergillus niger	6	42.6 %
Aspergillus flavus	3	21. 4%
Rhizopus stolonifer	2	14.3%
Rhizophus orezea	1	7.1%
Mucor racemosus	1	7.1%
Microsporum audoinii	1	7.1%
Total	**14**	**100%**

DISCUSSION

Microorganisms associated with the spoilage of garden eggs were isolated and identified using standard techniques. The mean colony count ranged from 37.5 $\times 10^7$ to 72.7 $\times 10^7$ cfu/ml. Sample C has the highest mean count (72.7 $\times 10^7$), followed by sample B (48.3 $\times 10^7$) and A had the lowest average mean count (37.5 $\times 10^7$) cfu/ml. The high colony recorded was due to the spoilage of the fruit. This is not in agreement with the findings of Yaji *et al*. (2016) and Agurou *et al*. (2015) who studied the microorganisms associated with fresh garden eggs. They recorded bacterial colony count that ranged between 1.9 $\times 10^5$ to 1.1 $\times 10^5$ cfu/ml and 5.0 $\times 10^7$ to 6.4 $\times 10^7$ cfu/ml. These may be associated with the different samples (fresh and spoiled fruit) used, during the study.

Five bacterial isolates were identified from spoiled garden eggs samples which includes; *Staphylococcus aureus*, *Bacillus* spp, *Staphyloccus epidermis*, *Listeriamonocytogens* and *Closteridium botulinum.* These organisms are easily found in contaminated air, water and soil. The bacterial species isolated from garden eggs fruit is due to the possession of high level of nutrients and water, suitable for the growth of pathogens by garden eggs and certain environmental influence. The result of this study is in agreement with the report of Aguoru *et al*. (2015)) who identified bacterial species such as *Bacillus* spp and *Staphylococcus aureus* as the bacterial organisms responsible for spoilage of garden eggs.

The frequency of occurrence of the isolated bacteria (Table 3) showed that *Staphylococcus aureus* occurred the most having a percentage frequency of 50%, *followed* by *Bacillus spp* (25) and *Staphylococcus epidermis* with percentage frequency of 12.5% while *Listeria monocytogens* and *Clostridium botulinum* has the least percentage frequency of occurrence of 6.3% each. This is in consonant with the findings of Yaji *et al*. (2016) who reported a frequency of occurrence for *Staphylococcus aureus* and *Bacillus* spp of 36.7% and 10% respectively. The highest frequency of *Staphylococcus aureus* might be due to unhygienic handling of fruits, storage and certain environmental factors. *Staphylococcus aureus* which has the highest frequency is a normal flora of man which can be found in the nose, respiratory tract, and on the skin, it might be commonest in fruits in large number being its normal flora and people touch fruits without washing their hands. *Clostridium botulinum* which occurred in small number comes in contact with the fruit when its falls to the ground which lead to the spoilage of fruits. This agreed with the findings of Anna (2002) who indicated that Gram positive organisms may be responsible for initiating and causing spoilage in cucumber, garden egg and pawpaw fruit.

The fungal count isolated from garden eggs samples ranged between 2.7×10^3 to 3.3×10^3cfu/ml. This is attributed to the spoilage of fruits which may be due to the change in physiological state of the fruits as a result of either post harvest handling, transportation which may result in production of microorganisms. And also the rate of contamination might be as a result of improper handling during production, packaging and storage of fruits.

Different species of fungi were identified from garden eggs based on their macroscopic and microscopic characteristics namely *Aspergilus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Rhizopus oryzae*, *Mucor racemosus* and *Microsporum audoinii* as described by Barnet and Hunter, (1999) and Alexopoulous *et al.* (2002). These organisms are commonly found in soil, water, air and can cause food spoilage when it's come in contact with the food, the higher moisture content of fruits make it susceptible to attack by pathogenic microorganisms, which can cause rots and produce mycotoxins. The result of this present work is in agreement with the findings of Gambari *et al*., (2013) who identified *Aspergiluus niger*, *Aspergillus flavus* and *Mucor racemosus* as potential contaminant of garden eggs spoilage.

The percentage frequency of the fungi isolated from spoiled garden eggs (Table 6) showed that *Aspergillus niger* occurred most frequently (42.6%), followed by *Aspergillus flavus* (21.4%) and *Rhizopus stolonifer* (14.3%). The least fungal species encountered were isolates of *Rhizopus oryzae, Mucor racemosus and Microsporum audoinii* with 7.1% each respectively. The highest frequency of occurrence recorded by *Aspergillus niger* could be related to its high speculating capacity and production of toxins which inhibit the growth of other fungal pathogens. These organisms might have gained entry through stomatal openings, growth cracks or surface injuries. The results of this research is in agreement with the work of Gambari *et al*., (2013), in which *A. niger* has the highest frequency of occurrence (40.3%) and *Muco*r spp has the least frequency (26.82%).

CONCLUSION

The study on microorganisms associated with the spoilage of garden eggs has revealed that garden eggs are sensitive to physical damage as well as can serve as good medium for the

growth of pathogenic microorganisms due its nutrients and moisture contents, high colony count were recorded in all the samples processed. Five bacterial species and six fungal species were isolated and identified that are commonly found in air, soil and water, which are potential causal organisms responsible for the spoilage of *Solanum melongena* (Garden egg). This indicates that spoiled garden eggs collected within Sokoto Metropolis, harbor pathogenic microorganisms which can lead to deleterious health implications.

RECOMMENDATIONS

Consumers should avoid consumption of spoilt fruits (Garden eggs) to avoid health implications that may lead to death.

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