Journal of Agriculture, Environmental Resource and Management
ISSN2245-1800(paper) ISSN 2245-2943(online)
5(3)701-708; Dec.2020



**MICROBIAL CONTAMINATION OF BAMBARA SEED (*Vigna subterranean)* IN THREE MARKETS IN UMUAHIA, ABIA STATE.**

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## *ABSTRACT*

*A survey was conducted in three Umuahia markets namely; Ahia Ukwu Olokoro, Ubani Modern and Ndoro markets. The major aim of this research was to determine the microbial contamination level of Vigna subterranean seeds. The study was conducted at the laboratory of the Department of Plant Health Mangement, Michael Okpara University of Agriculture, Umudike, Nigeria. The seeds were washed and sterilized with 0.5% sodium hypochlorite and rinsed three times in sterile water. The sterilized seeds were then inoculated onto nutrient agar medium inside 20ml Petri dishes and allowed for 24 - 48hours at 30$^{o}$C and 60 - 70% relative humidity. Results obtained showed that all the seeds from the three markets were contaminated ranging from 200 – 240. This experiment shows that variable white bambara seed from Ndoro Market with 60.00% microbial contamination scored the highest and so farmers and marketers should prefer the ones from Ahia Ukwu Olokoro with 53.33%*

**Keywords:** *Vigna subterranean* seeds, microbial contamination, sterilized, inoculated.

**INTRODUCTION**

*V. subterranean* groundnut is a traditional or indigenous crop, mainly grown as a subsistence crop by farmers and to some degree for income generation (Mwang'ombe *et al*., 2007; Mhango, 2013). The crop is found in the wild from Central Nigeria eastwards to Southern Sudan and cultivated throughout tropical Africa (Brink *et al*., 2006) and has the potential to seeds security in view of its ability to withstand drought especially in sub-Saharan Africa, where rainfall is low to support most leguminous crops.

Adu-Dapaah *et al*. (2004) described *V. subterranean* groundnut as the most resistant pulse crop producing under conditions of high temperature and low rainfall where other pulses fail to survive. Legumes are associated crop types usually intercropped with maize or other cereals which are typically the major crops, a cropping practice common with small-scale farmers in the tropics (Tsubo *et al*., 2001). Intercropping has many benefits to the farmer

including a reduction in farm inputs, diversification of diet, addition of cash crops, reduced labor cost, and reduced risk of crop failure (Hauggard-Nielsen *et al*., 2007).

In developing countries seeds sold by street sellers are the major source of seeds borne illness and their microbiological safety is not always certain (Kibret and Tadesse, 2013). A study in Silchar city Assam, India has reported detection of isolates of E. *coli* (37.5%), *Salmonella* (5.36%), *Shigella* (19.64%) and other microbial species from 37 street-sold seeds samples (Sharma and Mazumdar, 2014). In Orissa, India E *coli*, *Klebsiella* spp, *Salmonella paratyphi*, *Shigella dysenteriae*, and *Vibrio* spp were detected in 12 Panipuri samples (Das *et al*., 2012). Marketed seeds vending along locations linking various geographical regions/states of Nigeria is an emerging new form of seeds/nut vending resulting from high level of unemployment and failed family and community values. This situation however, has resulted that seeds sanitary measures and proper seeds handling have been transferred from individual, families to the seeds sellers who rarely enforce such practices (Musa and Akande, 2002).

The market sellers have no fixed station/location/route. They move to different routes and locations on highways via commercial motorcycles/vehicles, and their movement is dependent on newly identified failed portions on the highways, repair/construction works on the highways, security posts (check points), accident spots on the highways and possibly they move to different locations/routes to deliberately sale bad products (Gilbert *et al*., 2000). According to Mamun *et al*. (2013), seeds from street sellers are perceived to be a major public health risk due to the lack of basic infrastructure and services and also the difficulty in bringing the large numbers of street seeds sellers under effective control measures (Rane, 2011). In view of the health risk posed from street-sold seeds in densely populated cities of developing countries, study of published literature revealed that seeds sellers have inadequate knowledge and awareness regarding seeds safety issues.

This study was carried out to assess microbial contamination of *V. subterranean* from three different markets in Abia State, Nigeria. The aim was to ascertain the level of microbial contaminants in *Vigna subterranean* groundnut from the three different markets, isolate and identify the major microbial organisms present in the seeds.

**MATERIALS AND METHOD**

*V. subterranean* was collected from three markets in Abia State. The markets under study were Ubani Modern Market, Ndoro Market, and Ahia Ukwu Olokoro. One hundred grams (100g) weight of *V. subterranean* seeds was sampled from each of the three different markets giving a total of 300g. The samples were collected in sterile polyethylene bags, and separately labelled according to their locations and taken to the laboratory for microbial isolation and identification.

## Pathogenicity Test

The sterilized seeds were then inoculated onto nutrient agar medium inside 20ml Petri dishes and allowed for 24 – 48hours at 30$^{o}$C and 60 – 70% relative humidity. The experiment was

allowed for seven (7) days and colony number incidence observed.

## Preparation of Culture Media

Nutrient agar culture media was used for bacteria. A sensitive balance was used to measure 28 grams of nutrient agar powder and was then dissolved in 1000ml of distilled water in a conical flask and shake vigorously. Thereafter, the mixture was put into an autoclave, sterilized at 121°C/15psi for 30minutes and was allowed to cool to 50°C. 15ml aliquot of the media was dispensed into 9cm diameter Petri dishes and left to solidify at ambient temp.

## Bacteria Isolation and Identification

One gram of *Vigna subterranean* sample showing visible signs of spoilage by moulds was inoculated onto Sabouraud Dextrose Agar in Petri dishes onto which ampicillin was added to hinder the growth of bacteria. The inoculation was done in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25°C ± 3°C (Samson *et al.*, 1981). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates was obtained after a series of isolations.

The bacteria spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The bacteria were identified based on their spore and colonial morphology, mycelia structure and other associated structures (Samson *et al.*, 1981).

## Experimental Design/Statistical Analysis

The design of experiments used was in C.R.D. (Complete Randomized Design). All the data obtained were subjected to statistical analysis of variance and means were separated using Least significant difference LSD at 5% level of probability.

**RESULTS AND DISCUSSIONS**

## Isolated Bacteria

Three different bacteria colonies were isolated from the various diseased *V. subterranean* seeds. The bacteria include; *Erwinia* which were abundant in the variety (brown, white and black) with
223, 240, and 243 occurrences respectively, for each varieties according to their position in the table BS545, BS599 and BS520.

## Pathogenicity Test

Each of the three variety of the *V. subterranean* reisolated gave a pure colonies and were tested for 24 - 48 hours according to Koch's postulate. Result indicates that the bacteria isolates under investigation were able to contribute bacteria common diseases of *V. subterranean*. The test showed that *Erwinia* was the pathogenic organism that can cause contamination when the *V. subterranean* seeds is consumed. *Erwinia* has been reported to be associated with common bacteria disease of *V. subterranean* (Nghomoud *et al*., 2005).

## Table 4.1: Effect of Pathogen on Three Varieties of *V. subterranean* Seeds from Week 1

| Variable | Colony Number | Contamination Frequency |
|---|---|---|
| White Seeds | 60.00 | 200.00 |
| Brown Seeds | 53.33 | 240.00 |
| Black Seeds | 53.33 | 205.33 |
| LSD(0.05) | 24.918 | 26.01 |

## Table 4.2: Effect of Pathogen on Three Varieties of *V. subterranean* Seeds from Week 2

| Variable | Colony Numbers of Bacteria | Contamination Frequency of Bacteria |
|---|---|---|
| White Seeds | 66.67 | 210.00 |
| Brown Seeds | 46.67 | 243.33 |
| Black Seeds | 46.67 | 223.33 |
| LSD(0.05) | 16.31 | 35.86 |

For the colony numbers, the result from table 4.1 showed that there is no significant difference between the varieties (brown, white and black) of *V. subterranean* seeds at (P>0.05) while for contamination frequency, the result showed that variety BS520 has the highest number of colonies (240.00) while the BS599 has (205.33) and the BS545 (200.00) has the least colony numbers and was significantly different at (P>0.05).

For colony numbers, the result in table 4.2 showed that variety BS545 had the highest number of colonies (66.667) while the BS599 and BS520 recorded least colony numbers (46.667) showing that there is significant difference between the various varieties of *V. subterranean* seeds sampled. Whereas result of contamination frequency, showed that there was no significant difference between various varieties of *V. subterranean* seeds sampled (P>0.05).

**Table 4.3: Effect of Pathogen on Three Varieties of *V. subterranean* Seeds from Week 3**

| Variable | Colony Number of Bacteria | Contamination Frequency of Bacteria |
|---|---|---|
| White Seeds | 70.00 | 216.67 |
| Brown Seeds | 60.00 | 216.67 |
| Black Seeds | 60.00 | 240.00 |
| LSD(P=0.05) | 36.48 | 29.78 |

For colony numbers and contamination frequency, the result in table 4.3 showed that there is no significant difference between the various varieties of *V. subterranean* seeds sampled (P>0.05).

## Table 4.4: Effect of Pathogen on Three Varieties of *V. subterranean* Seeds from Week 4

| Variable | Colony Number of Bacteria | Contamination Frequency of Bacteria |
|---|---|---|
| White Seeds | 60.00 | 200.00 |
| Brown Seeds | 53.33 | 240.00 |
| Black Seeds | 53.33 | 208.33 |
| LSD | 24.92 | 26.01 |

For colony numbers, the result in table 4.4 shows that there is no significant difference between the various varieties of *V. subterranean* seeds sampled at (P>0.05) whereas contamination frequency, the result showed that variety BS520 has the highest number of colonies (240.00) while the BS599 has (208.33) and the Ubani Modern (200.00) has the least colony numbers, these in turn shows that there is significant difference between the various varieties of *V. subterranean* seeds sampled.

## Discussion

This experiment shows that all the test aqueous extracts gave various zones of inhibition against the bacterial soft rot pathogen (*Erwinia*

*carotovora*) in culture. However, high incidence of *Erwinia* was more susceptible in Ndoro market than other markets and such seeds when planted can reduce farmers yield in the field (Agrios, 2006). This Ready-to-eat seeds/nuts have been reported to be easily available, affordable, provide diverse/variable food source, employment and with a potential for improving seeds security and nutritional status and general social security (Draper, 1996) however, it is a veritable source of food borne pathogen (Mensah *et al*., 2002).

The bacterial disease that is caused by *Xanthomona scampestris* pv. *phaseoli* and *X. campestris* pv. *Phaseoli* var. *fuscans* affects almost all legumes. It's destructive during high rainfall, humidity and temperature (25-35˚C) with maximum development occurring at 28˚C and results in yield and quality losses (Akhavan *et al*., 2013). It affects all legumes and yield losses have been reported to vary between 22% and 45% (Yoshii, 1980). This disease also affected the seeds resulting in high seed contamination. When infection occurs early in the growth cycle of susceptible cultivars, yield loss of up to 100% can occur (Fern´andez *et al*., 2000). The characteristic symptoms include pod lesions that are sunken encircled by a slightly raised black ring surrounded by a reddish border, small circular water soaked spots or streaks which develop a reddish discoloration can be seen on pods. Light cream or silver colored bacterial ooze associated with the spots can be observed which cause discoloration, shriveling and bacterial contamination of seeds, that is a major source of infection (Hall, 1994). This can be the systemic cause of yellowing and death of new foliage thus, control is by the use of resistant cultivars and clean seeds (Nkalubo *et al*., 2007) during propagation. Serious losses have been documented in Lesotho, Rwanda and Zimbabwe (Allen *et al*., 1998), also yield losses of 43% have been reported in experimental conditions (Fourier, 2002).

Recommended control measures of halo blight includes cultural practices like deep ploughing, crop rotation, use of clean seeds, use of resistant varieties (such as GLP 92) and use of fungicides. Field sanitation, crop rotation, destruction of infected crop debris, and avoiding collateral hosts near the crop may help in reducing the incidence (Pande *et al*., 2007).

**CONCLUSION AND RECOMMENDATIONS**

Generally, seeds from street sellers are perceived to be a major public health risk due to lack of basic infrastructure, services and the difficulty of bringing large number of street seed sellers under effective control measures. In view of the health risk posed from street-sold seeds in densely populated cities of a developing country such as Nigeria, studies have revealed that seeds sellers have inadequate knowledge and awareness regarding seeds safety issues.

The results obtained from this study reveals that seeds of *Vigna subterranean* can be contaminated from farms to market and finally to consumers due to several factors such as unhygienic handling by marketers and transporting means. Also, *Vigna subterranean* groundnut seed collected from Ahia Ukwu Olokoro market showed minimum contamination on the three varieties studied. Hence farmers and sellers in Abia State should get their seeds from Ahia Ukwu Olokoro market, which indicated the least microbial contamination of seeds, hence reduced disease incidence and crop loss.

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