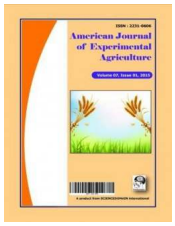

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Assessment of Post-harvest Insect and Mould Infestation of *Vigna subterranea*

P. T. Nnaji[1*] and A. A. Brooks[1]

[1]Department of Microbiology, University of Calabar, Cross River State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author AAB designed the study. Authors AAB and PTN wrote the protocol. Author PTN wrote the first draft of the manuscript, reviewed the experimental design and all drafts of the manuscript. Authors PTN and AAB managed the analyses of the study. Author PTN identified the growths. Author PTN performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was carried out with the aim of assessing the post harvest insect and moulds that infest *Vigna subterranea*.
Study Design: The randomized complete block experimental design with three replications for each variety was used to for the assessment of these varieties.
Place and Duration of Study: This research lasted for a period of four months in the University of Calabar, Cross River State Nigeria.
Methodology: Standard microbiological methods were used to obtain pure culture of fungi. Spore head and hyphae were chiefly compared with standard mycological atlas. Insects pest and infested nuts were sent to zoology department for identification of insect.
Results: Four varieties of *Vigna subterranea* namely; Cream Black Eye Variety (CBEV), Cream No Eye Variety (CNEV), Speckled Flecked Spotted Variety (SFSV) and Brown Variety (BV) were assessed for post harvest insect and mould infestation. *Aspergillus niger, Penicillium species, Mucor species, Trichophyton species and Rhizopus species* were isolated after plating in Sabouraud

**Corresponding author: E-mail: nnajipraisetochukwu@gmail.com;*

Dextrose Agar with antibiotics concentration of 50 µg/1000 ml. growth was not significant at P=0.05, but was significant at P=0.01. Cream black eye variety, brown variety and speckled flecked spotted variety were susceptibility to insect and mould infestation. Cream black eye variety had very high resistance to insect but was susceptible to mould infestation. *Callosobruchus maculatus* was identified as the insect that carried out the damage in *Vigna subterranea.*

Conclusion: Insect infestation in *V. subterranea* can result to increase in fungi growth. Cream no eye variety *V. subterranea* posses some properties that exhibits resistance to insect.

Keywords: Vigna subterranea; mould; insect; resistance; susceptible; infestation; post-harvest.

1. INTRODUCTION

The crop *V. subterranea* is a crop with many hidden potentials. Studies have shown that the crop has the capacity of contributing to food security in the world. The crop can be cultivated in regions of the world were rain is low. Besides, high disease resistance capacity has been found as one of the inherent potentials of the crop. Infact its been noted as the most resistant pulse crop with high yield in regions with low rainfall [1]. History has it that the crop originated from the north eastern states of nigeria to the rest parts of the country, excluding the riverine and swampy zones of the country.

In line with the world's population demand for nutrient rich food stuff, *V. subterranea* is nutritionally endowed to cob malnutrition since it has been proven to contain a reasonable amount of some major food classes such as protein, carbohydrate, and lipids. About 63% carbohydrate, 6.5% oil and 19% protein is present in *V. subterranea* seed. No harm is associated with consumption of nut at different stages of maturity (so far it's well processed) [2-4]. *Vigna subterranea* also has an appealing flavor which has highly influenced its demand from the local markets where producers sell them. It shares high nutritive value like other popularly consumed African legumes. *V. subterranea* is capable of growing in arid land where Ground nut *(Arachis hypogea),* maize *(Zea mays* L.) and sorghum *(Sorghum bicolar)* have failed [3]. Acccording to [4], Nigerians are the highest producers of *V. subterranea* in Africa.

However,the good qualities of this nut, does not actually mean total absence of infestation by pest, as various extreme conditions may over power the resistance of this crop. Reseachers in some publications have disclosed that pest and disease are major problems affecting the high yield of *V. subterranea* in africa [4,1,3]. Poor processing and storage system are practices that cotributes to post-harvest contamination and commercialization problems of bambara nut [3].

The idea of storing grains and seeds for food purposes has made insect pest economically important to mankind [5]. The ubiquity of insect pest in the tropics and subtropics is a great challenge to the storage of food stuffs. Loss of weight (due to qualitative and quantitative depletion), increase in mould growth, elaboration of toxins and loss of organoleptic qualities are some aftermath effects of insect pest activities on our food and feeds [6]. Insect and mould infestations on food products are great threat to food security in Nigeria and beyond. Food products especially nuts and grains are damaged both qualitatively and quantitatively. Post-harvest mal-practices are the major reasons for insect and mould infestation of our stored food product. Farmers, traders and consumers store food products in conditions that support the growth of microorganisms and conducive for insects and rodents to feed and carry out their activities [7].

Global warming has also contributed to the increase in insect pest activity. Although increase in CO_2 due to this warming of the globe may result to vigorous growth of crops, the disaster associated with floods and droughts consumes all the benefits associated with growth. Therefore, seed production and storage drops drastically. Temperature increase will also encourage insect pest activities. More so, as the lower slopes of mountain peaks get warmer, plants, animals, and pest populations have started to migrate upward [8].

Loss of nutritional value of our food is an alarming effect of food contamination all over the globe. This low nutritional value of food results to malnutrition which is the greatest threat to the public health. Many developing countries where their climatic condition favors the proliferation of these moulds tend to suffer more of these dieting problems [9].

The tones of food lost all over the world due to contamination of food stuffs is unbearable. About one billion metric tons of food stuff was estimated lost by the United Nations (UN) and Food and Agricultural Organization (FAO) per year. FAO has disclosed that 25% of the world's grains are contaminated with mycotoxins [9].

Toxigenic moulds are one of the greatest threats associated with the contamination of food stuff by microorganisms. They produce secondary metabolites known as mycotoxins, which may be carcinogenic, tetarogenic, genotoxic and hepatoxic [10]. Besides mycotoxin production, the ubiquity of toxigenic moulds is a challenge to control of mould contamination. They thrive in humid, warm and even damp environment. According to Anyawu [11] over one thousand varieties of these moulds exist. Common occurring species of toxigenic moulds are those of *Aspergillus, Penicillium and Fusarum.* They contaminate food substrate of varying nutritional constituents; ranging from major food nutrients to minor ones. Foods rich in carbohydrates (e.g maize, rice, cassava by products and millet), Proteins (e.g Beans, wheat, fishes, etc) and other nutrients are not exceptional [12]. The fact that very little reseach effort has been directed towards the improvement of this crop is also a limiting factor [4].

2. MATERIALS AND METHODS

2.1 Study site and Sampling

Major Food markets in the South eastern Nigeria were randomly sampled. These markets include: New-market and Afor Mbu-Akpoti market in Enugu north and Isi- Uzo local government areas of Enugu state, Ose main market in Onitsha north local government area, Anambara state, Meat market in Abakaliki local government area, Ebonyi State and. Ahia Ohuru market in Aba south local government area, Abia state Nigeria.

2.2 Sample Collection

Four varieties of *Vigna suterranea* that were displayed for sell in sack bags were thoroughly mixed and randomly collected with the aid of a grain collector into a clean container and stored in a cool and dried environment for few days. Afterward, samples were transported in a well sealed clean bag to the laboratory in UNICAL for analysis.

2.3 Culture and Identification

2.3.1 Media preparation

Sabouraud Dextrose Agar (SDA) at a ratio of 65 g in 1000 ml of distilled water was prepared and sterilized in an autoclave at 121℃ for 15 min afterward the medium was supplemented with amoxicillin at a concentration of 50 µg/1000 ml pouring into plates.

2.3.2 Plating procedure

This was done according the method of Nwankwo et al. [7] with slight modifications. Each of the grains were picked up with the aid of a sterile pair of forceps and placed at the centre of the Petridishes at an average ambient temperature of 28℃ - 32℃ for 5 -7 days.

2.3.3 Purification of Isolates

Discrete fungal colonies were picked at random and sub-cultured repeatedly in sabouraud dextrose agar until an absolute pure culture was obtained. This purified isolates were stocked in Sabouraud dextrose agar slant for further studies.

2.3.4 Slide culture

Slide cultures were prepared from 5-7 days fungal pure cultures by the following procedures:

i. A sheet of sterile filter paper was placed in a Petri dish with the aid of a sterile forceps.
ii. U-shaped glass rod was placed on the filter paper each dish after sterilization.
iii. Sterile distilled water was poured to moisten the filter paper.
iv. With the aid of sterile forceps, glass slides were placed on the U-shaped glass rod.
v. A sterilized scalpel blade was used to cut 5-6 mm square block of solidified sabouraud dextrose agar (SDA).
vi. Agar block was aseptically transferred to the centre of slide on the U-shaped glass rod.
vii. The four sides of the agar were inoculated with spores or mycelia of fungus with the aid of a sterile wire loop.
viii. A sterile cover slip was aseptically placed on the inoculated agar block.
ix. Petri dish was carefully covered and incubated at an average ambient temperature of 28^{o}c for 48 hour and more hours for those with no sufficient growth.

x. All agar blocks were discarded after confirmation of growth on cover slips. Afterwards, an aliquot of lacto phenol cotton blue was dropped on a sterile glass slide before the cover slip with growth was slightly flamed and placed on it.

2.3.5 Identification and characterization of isolates

Both macroscopic and microscopic morphology as well as physiological characteristics were studied for proper fungi identification. Proper observation of the rate of growth, presence or absence of spores, colouration of culture and other morphological characteristics were carefully studied. The stained slides from the slide culture were mounted and viewed using x40 objective lens on a microscope linked to the computer for appropriate morphological characteristics. Standard mycological atlas was used to identify the moulds. The resulting spore heads and mycelia were compared with existing pictures in the atlas.

2.4 Percentage Frequency of Fungal Occurrence in *Vigna subterranea*

The percentage frequency of fungal occurrence in *V. subterranea* was determined by the formula stated below.

$$\frac{X}{Y} \times \frac{100}{1} = \% \text{ frequency}$$

Where:

X = Total number of each organism in a variety.
Y = Total number of all identified organism in a variety.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Methods of storage of *V. subterraanea*

V. subterrenea (Bambara nut) in sack bags and kept in ware houses were the most prevalent method of grain storage among traders in South Eastern Nigeria as shown in Plate 1. However, grains were stored by local farmers in plastic gallons to prevent insect attack as shown in Plate 2.

Plate 1. Bambara nut stored in sack bags in new market ware house Enugu, Enugu state

Plate 2. *Vigna subterranea* store in plastic gallons by local farmers of Mbu-Akpoti, Enugu State, to prevent insect mould attack

3.1.2 Distribution of fungal isolates in varieties of *V. subterranea*

Out of the seven known varieties of *V. subterranea*, only four namely; Cream Black Eye Variety (CBEV), Cream No Eye Variety (CNEV), Speckled Flecked Spotted Variety (SFSV) and Brown Variety (BV) were sampled in South Eastern Nigeria for this study.

Table 1 illustrates the distribution of fungal isolates across the four varieties of *V. subterranea* from some major markets in South Eastern Nigeria.

Table 1. Distribution of different fungal species in *V. subterranean*

Fungal species	CNEV	BV	SFSV	CBEV
Aspergillus niger	+	+	+	+
Trichophyton spp	+	-	-	-
Penicillium spp	+	-	+	+
Rhizopus nigricans	+	+	+	+
Mucor spp	+	+	+	+

3.1.3 Percentage growth of fungi in *Vigna subterranea*

The percentage frequency of the total fungal growth (Table 2) was calculated in order to determine the rate of dominance of the five species that were identified in across the *V. subterranea* varieties.

3.1.4 Rate of insect/mould infestation on varieties of *Vigna subterranean*

After sample collection and initial assessment of various varieties for mould contamination, a pest which was not introduced or inoculated during study was observed to attack the nuts. This pest was identified by the entomology arm of the department of Zoology in the University of Calabar, Cross River state Nigeria as *Callosobruchus maculatus.* The plate (Plate 3) below illustrates the extent of deterioration by the insect and mould in the most susceptible and resistant variety after four months of sampling.

Fig. 1 illustrates the percentage deterioration and resistivity of *V. subterranea to* insect and mould.Within the space of four months, *Callosobruchus maculatus* successfully attacked three varieties (CBEV, SFSV and BV) out of the four varieties. The highest damage occurred in CBEV (Cream Black Eyed Variety), followed by BV (Brown Variety), thirdly by SFSV (Speckled Flecked Spotted Variety) and CNEV (Cream No Eye Variety) had no attack as represented in Fig. 1.

3.1.5 Statistical analysis

Analysis of variance was used to statistically determine the point at which the mould growth was significant among the set of means. At P=0.05, there was no significant growth of moulds from *V. subterranea* after six days of growth in ambient temperature and relative humidity. However, at P=0.01, growth was significant.

Table 2. Percentage growth of fungi in *Vigna subterranean*

Name of organism	% in CNEV	% in SFSV	% in BV	% in CBEV
Aspergillus niger	10.00	10.00	16.00	24.00
Trichophyton Spp	5.00	7.00	4.00	7.00
Penicillium spp	10.00	18.00	50.00	21.00
Rhizopus nigricans	35.00	30.00	20.00	24.00
Mucor spp	40.00	35.00	10.00	24.00

Plate 3. Rate of resistance and susceptibility of *V. subterranea* to insect/mould infestation. (A) Whole grain of CBEV of *V. subterranea* before insect attack (B) Deteriorated grain of CBEV of *V. subterranean* after four months (C) Whole grain of CNEV of *V. subterranea* after sample collection (D) Whole grain of CBEV of *V. subterranea* after four months

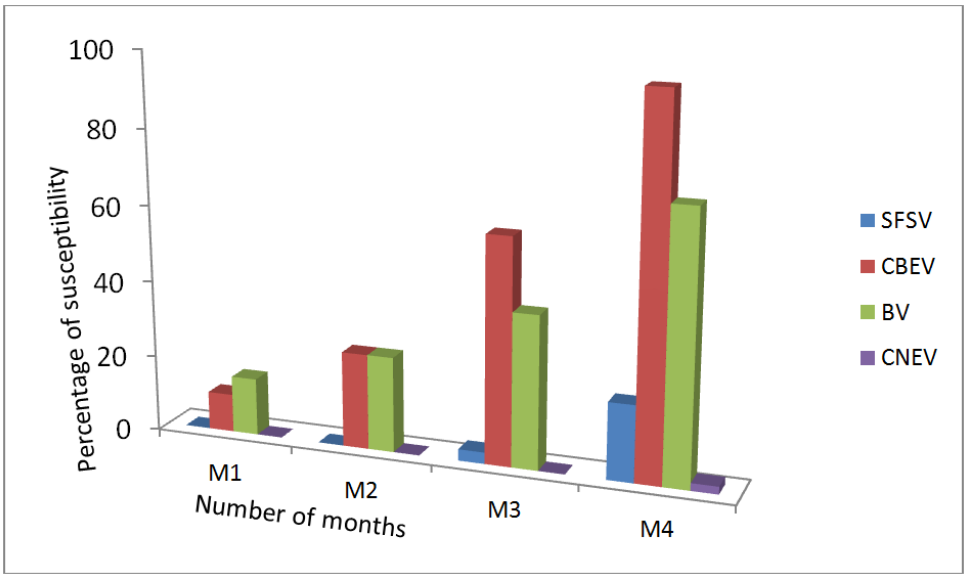

Fig. 1. Rate of insect infestation (%) in four varieties of *Vigna subterranea* within the space of four months

KEY: M1-First month; M2- Second month; M3-Third month; M4- fourth month

Table 3. Anova of for viable counts of moulds isolated from varieties of *V. subterranea* after six days

SOV	Degree of Freedom (DF)	Sum of Square (SS)	Mean Square (MS)	F-Value	F-Table (5%)	F-Table (1%)
Total	15	3184.94				
Block	3	650.69	216.89	2.27		
Treatment	3	1674.69	558.23	5.85	3.86	6.99
Error	9	859.56	95.50			

3.2 Discussion

A total of five species of fungi were isolated from the four varieties of *Vigna subterranea,* which are known as the varieties that are popularly consumed as food in South Eastern Nigeria. *Aspergillus* and *Penicillium* species which were referred to as the most common storage fungi according [13,14], were also major contaminants. Although the percentage range of occurrence varied slightly in the later.

The paucity of good reliable storage practices is a contributory factor to the recurrent contamination of *Vigna subterranea* by these moulds that posse's toxin producing potentials. Poor method of storage, low hygiene of storage environment, management of warehouses, other food grains on the floor, presence of insects in other food seed in the same warehouse and some crushed grains of *V. subterranea* could attract rodents and fungal contamination. It is believed that this pest that attacked the nuts were those that infested them right from the warehouses where the nuts were stored in the markets, since they were not by any means inoculated in the samples in the laboratory.

It is important to state that during the laboratory study of these varieties, there was no observable significant difference in the rate of susceptibility or resistivity to insect and moulds infestation owing to the methods of storage (either in sack bags or plastic containers). However, rural farmers attested that continuous storage in plastics containers has often resulted to little or no exposure to insect pest. This is because the thick plastic containers served as protection against insect and rodents which may want to feed on the nuts. This sole reason is responsible for such practices of storage in plastic containers by rural famers in South Eastern Nigeria over decades.

The mould infestation associated with all the varieties despite the high level of resistivity to insect by CNEV suggests that the difference in the rate of susceptibility and resistivity to insect may be due to some variation in some bioactive compounds or genetic makeup across the four varieties. The high increase in mould growth that was observed in susceptible varieties as a result of the severe insect infestation is in agreement with [15]. Quality loss and weight loss and were synergistic aftermath of fungal/insect degradation in *V. subterranea*. This was barefaced by the conspicuous putrid odor of fatty acid degradation by microbes as the nuts were no longer fit for propagation and even consumption purposes.

4. CONCLUSION

This study has obviously unraveled some inherent potential of some varieties of *V. subterranea* as CNEV possessed high level of resistance to insect infestation. Cream No Eye Variety of *V. subterranea* posses some inherent properties that exhibits resistance to insect. Information about the inherent properties of this variety that is responsible for such resistance may be helpful in the war against insect pest. Insect infestation in *V. subterranea* can result to increase in fungi growth.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Akpalu MM, Atubilla IA, Sekyere DO. Assessing the level of cultivation and utilization of bambara groundnut (*Vigna subterrenea (L)verdo*) in the sumbrungun community of Bolgatanga, upper east region, Ghana. International Journal of Plant, Animal and Environmental Sciences. 2013;27:28-32.
2. Okonkwo SI, Opara MF. The analysis of bambara nut *(Vandezia subterrenea* (L) thouars) for sustainability in Africa. Research Journal of Applied Science. 2010;5:394-396.
3. Hillocks RJ, Bennett C, Mponda, OM. Bambara nut: A review of utilization, market potential and crop improvement. African Crop Science Journal. 2012;20:1-16.
4. Aviara NA, Lawal AA, Atiku AA, Hague MA. Bamabara groundnut processing, storage andutilization in North Eastern Nigeria. Continiental Journal of Engeneering Sciences. 2013;8:28-36.
5. Harold HS. Insect infest stored grain and seed. Reprinted June 1947 Bulletin 340.
6. William RJ, McDonald D. Grain moulds in the tropics: Problems and importance. Ann. Rev. Phytopathol. 1983;21:153-178.
7. Nwankwo JI, Ogunbodede TT, Ukpai EG. Mycogenera of stored cereal grains in Ogbete main market, Enugu State, South East Nigeria. International Journal of Current Microbiology and Applied Science. 2015;4:875-883.
8. Available:www.williamairdner.com/formal/2007/7/4/global warming -in-a-nutshell.html
9. Kouadio IA, Koffi LB, Dosso MB. Prevention of crops contamination by fungi and mycotoxins using natural substances derived from *Lycopersicon esculentum* mill leaves. Journal of Food Security. 2013; 1:16-26.
10. Mohamed EZ. Impact of mycotoxin on humans and animals. Journal of Saudi-Chemical Society. 2011;15:129-144.
11. Anyawu EC. The validity of the environmental neurotoxic effects of toxigenic moulds and mycotoxin. The Internet Journal of Toxicology. 2007;5:2.
12. Saravanakumar K, Kumaresan G, Sivakumar K. Incidence and characterization of ochratoxigenic moulds in curd (Dahi). Research Journal of Agriculture and Biological Sciences. 2007;6:818- 820.
13. Amadi JE, Adeneyi DO. Mycotoxin production by fungi isolated from stored grains. African Journal of Biotechnology. 2009;8:1219-1221.
14. Francisco FG, Uberti R. Seed health of common been stored at constant moisture

and temperature. Science Agriculture (Piracicaba, Braz.). 2008;65:613-619.
15. Gabriel OA, Puleng L. Strategies for the prevention and reduction of mycotoxins in developing Countries. In: Hussaini AM, editor. Mycotoxin and Food Safety in Developing Countries; 2013.

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