Journal of Agriculture, Environmental Resources and Management
ISSN2245-1800(paper) ISSN 2245-2943(online)
5(5)650-1220; Jan.2023; pp910-921

www.saerem.com

Effect of Fungi Contamination on the Weight of Brown Cowpea Sold in Port Harcourt Metropolis

Nneji, Worukwo Emma Joy*, Nnom, F.W., Robinson V.K.
Department of Plant Science and Biotechnology, Faculty of Science, Rivers State University, Port Harcourt, Rivers State, Nigeria.

ABSTRACT
Fungi contamination on Brown Cowpea sold in major markets in Port Harcourt was investigated in the Department of Plant Science and Biotechnology, Rivers State University with the aim of evaluating the effect of fungi contamination on the weight of brown cowpea sold in Port Harcourt metropolis. Brown cowpea seeds were randomly purchased from vendors in four major markets within Port Harcourt metropolis. The cowpea seeds were transferred in clean containers to the Department of Plant Science and Biotechnology, Rivers State University for identification. Fungal isolates were inoculated by transferring 10g of cowpea seeds into 90mL sterile saline and ten-fold serial dilution was carried out and aliquots from 10^{-2} dilution was inoculated by spreading on freshly prepared Sabouraud Dextrose Agar plates in triplicates. The plates were incubated at 22℃ for 5 days. Fungal isolates were identified based on their morphology on plates and microscopy. Weight of the cowpea was used to evaluate the pathogenicity of the isolates on the cowpea and also to confirm the presence of test isolates. Results showed that fungal isolates: Aspergillus flavus, A. niger, Rhizopus and Mucor were isolated from the cowpea seeds. The percentage occurrence of the fungal isolates was A. niger (29.4%), A. niger (17.6%), Mucor sp (29.4%) and Rhizopus sp (23.5%). The weight of the brown cowpea deviated from the original weight of 0.423±0.10g after being exposed to fungal isolates and the weight loss recorded as a result of pathogenicity of A. flavus, A. niger, Rhizopus sp and Mucor sp on the brown cowpea seeds was 0.12±0.36, 0.22±0.26, 0.07±0.07 and 0.10±0.13g, respectively. In conclusion, the fungal isolates: A. flavus, A. niger, Rhizopus and Mucor sp negatively impacted on the cowpea thereby causing weight loss. Thus, contamination of these isolates on cowpea seeds could lead to the loss in quality. More so, some of these fungal isolates are known toxin producers, they could be of economic importance in eliciting allergens to immunocompromised individuals who come in contact with the products. In respect to the findings, we recommend the following; Proper practices targeted at reduction of microbial contamination during harvest, storage and distribution of cowpea should be encouraged, Proper cooking of cowpea should be adhered as microbial isolates identified in this study could cause illness if food is not properly prepared.

Keys: fungal contamination, brown cowpea

INTRODUCTION

Cowpea (*Vignaunguiculata*(L.) Walp.) is a member of the Phaseoleae tribe of the Leguminosae family. Members of the Phaseoleae include many of the economically important warm season grain and oilseed legumes, such as soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vignaradiata*). The name cowpea probably originated from the fact that the plant was an important source of hay for cows in the southeastern United States and in other parts of the world (Timko, Jeff and Philip 2007). It was been estimated that about 3.3 million tonnes of cowpea dry grains were produced worldwide in year 2000. Nigeria produced 2.1 million tonnes of this, making it the world's largest producer, followed by Niger (650,000 tonnes) and Mali (110,000 tonnes) (IITA, 2004). Cowpea is widely cultivated in the humid tropics of South-western Nigeria; however, its cultivation is faced with several setbacks such as pests and diseases. The effect of field diseases on cowpea has led to significant reduction in yield of cowpea in the humid forest of Nigeria (Adegbite and Amusa, 2008). The nutritional content of cowpea grain is important because it is eaten in quantity by millions of people who otherwise have diets lacking in protein, minerals, and vitamins. The nutritional profile of cowpea grain is similar to that of other pulses, with a relatively low-fat content and a total protein content that is two to four times greater than cereal and tuber crops (Timko *et al.*, 2007). Like other pulses, the protein in cowpea grain is rich in the amino acids lysine and tryptophan, compared to cereal grains. However, it is deficient in methionine and cystine when compared to animal proteins. In a study of 100 cowpea breeding lines in the IITA collection, seed protein content ranged from 23 to 32% of seed weight (Nielson S. S., Brandt W. E. & Singh B. B. 1993). Similarly, protein content of 12 West African and US cultivars ranged from 22 to 29%, with most accessions having protein content values between 22 and 24% (Hall, Cisse, Thiaw, Elawad, Ehlers, Ismail, Fery, Roberts, Kitch, Murdock, Boukar, Phillips, and McWatters, 2003). Cowpea

grain is also a rich source of minerals and vitamins and it has one of the highest levels of any food of folic acid, a crucial B vitamin that helps prevent spinal tube defects in unborn children (Hall *et al*., 2003). FAOUN, (1988) reported appreciable amounts of carbohydrate, fibre, protein, calcium, iron, magnesium, phosphorus, potassium, sodium, zinc and some relevant vitamin in the raw seeds of cowpea. The major economic diseases of cowpea in the humid agroecologies of South-western Nigeria include brown blotch, anthracnose, cercospora leaf spot, choaniphora pod rot, false smut, web blight and sclerotium stem blight (Ajibade and Amusa 2001). However, in Nigeria cowpea is majorly produced in the North in the savannah belt. Its yield in the South is affected by some environmental factors including rainfall hence it is seasonal. The high demand for this leguminous multipurpose crop plant is not met in the Southern part of Nigeria. The production of cowpea all year round basis in all parts of Nigeria is expected to boost production, thereby improving nutrition and contribute to food security as well as increase revenue of the producers (Agbogidi, Egho, 2012). The economic importance of fungal diseases on agricultural products is well documented. This study therefore aims to evaluate the effect of fungal isolates on the viability of the brown cowpea sold in some part of Nigeria.

MATERIALS AND METHOD

Study Area

The study was conducted in four major markets located in two local governments, Port Harcourt City Local Government Area and Obio-Akpor Local Government Area. These markets are known for high influx of traders who come from different localities to display and sell their produce. Thus, the study employed a complete randomized design aimed at determining by size and specie which variety is more prone to fungal infestation and to determine the pathogenicity of isolated fungi on healthy seeds. The map of the area under study is illustrated in Fig 1.

Sample Collection

Bean samples were randomly bought from vendors in four major markets in Port Harcourt metropolis; Mile III, Mile I, New market (Borikiri) and Rumuokoro market. The beans were taken to the Department of Plant Science and Biotechnology, Rivers State University for identification. In the laboratory, the samples were sorted according to size, and wholesomeness for further analysis.

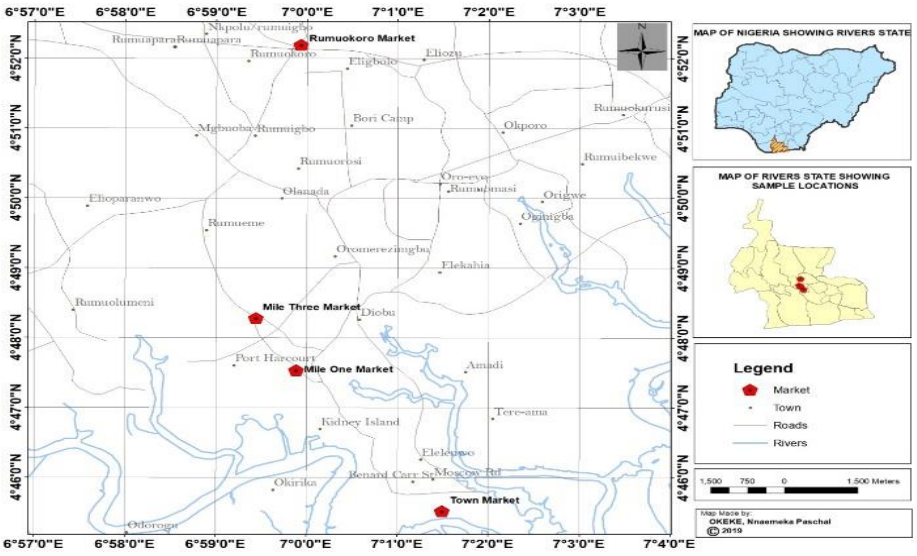

Fig. 1. Map of the study locations

Microbiological Analysis

Isolation of Fungi

The spread plate method was used in the isolation of fungal isolates associated with cowpea seeds (Amadi, Kiin-Kabari, Kpormon, Robinson, 2014). In this method, 10g of the cowpea seeds were immersed in conical flasks containing 90 mL of sterile normal saline. After serially diluting the samples to dilution of 10^{-6}, an aliquot (0.1mL) of the 10^{-1} and 10^{-3} in duplicates were carefully transferred into well-labeled freshly prepared Sabouraud Dextrose agar plates with the aid of a sterile 1mL pipette. The inoculated plates were carefully spread using a flame-sterilized bent glass rod and were finally incubated at 22°C for 2-5 days (Douglas and Robinson, 2018; 2019). The fungal isolates were identified using their morphological features such as colony color, shape, texture, and size of the colony followed by microscopic examination (conidial shape, arrangement of hyphae, and type of spore) of their wet mounts prepared with lactophenol cotton blue (Robinson et al., 2020) and reference made to fungal identification manual (Sarah, Catriona, Helen and David, 2016)

Pathogenicity Test

Pathogenicity test as described in Koch's postulates was carried out. Fungal isolates from the cowpea seeds were inoculated onto healthy seeds. The inoculation was carried out by transferring the fungal spores into 20 mL of sterile distilled water. The turbidity matched the 0.5 McFarland. The water which contained the spore was sprayed directly

on healthy cowpea seeds which were kept in sterile Petri dishes. Before inoculation, the weight of the healthy seeds was measured using an electronic weighing balance. The weight of the sprayed seeds was observed by weighing them on an electronic weighing balance after seven days and observed for any negative impact on the seeds. The infected seeds were analyzed using microbiological techniques to determine the presence of the inoculated organisms. This was done to ensure that the infections on the seeds were as a result of the organisms inoculated.

Statistical Analysis

Complete Randomized Design (CRD) was used for relevant parts of the data. 3 treatments comprising the various varieties were analyzed and ANOVA was used to check for a significant differences. Scheffe's Post Hoc test was used for mean separation in areas showing significant differences.

RESULTS

Microbial Characterization

Results of the fungi characteristics and genera is presented in Table 1. The morphological and microscopic characteristics of the fungal isolates showed that they were *Rhizopus*sp, *Mucor*sp, *Aspergillus niger*, and *Aspergillus flavus.*

Table 1. Cultural Characteristics of the Fungal Isolates

Isolates	Macroscopy	Microscopy	Probable Identity
A	Dark yellow-green colonies, brown reverse	Septate hyphae with globose conidia. Hyaline stipes of the conidiophore	*Aspergillus flavus*
B	Fluffy white cottony, white reverse	Aeseptate h sporangiospores	
C	Fluffy white to grey cottony, yellow reverse	Aeseptate h sporangiospores	
D	White periphery, dense black spores, brown reverse	Septate hyphae wi	

Pathogenicity Test

The pathogenicity test showing the effect of *Aspergillus flavus, A. niger* and *Rhizopus* sp on the weight of the big brown beans, and small brown beans in season one is presented in fig 1, 2, 3, 4, 5 and 6, respectively. Results showed that the fungal isolates affected the weight of the different beans by causing loss of weight. Despite the loss in weight of the bean varieties, there was no significant difference between the weight before inoculation, the weight of the control (uninoculated bean), and the weight of the inoculated beans.

In season two, the weight of the bean varieties before and after treatment with fungal isolates such as *A. niger*, *Mucor* sp and *Rhizopus* sp is illustrated in figs 7, 8, 9, 10, 11 and 12, respectively. The results showed that all the fungal isolates impacted negatively on the various bean seeds thereby causing a loss in the weight of the bean. Irrespective of the loosed weight of the various bean seeds after treatment with the respective fungal isolates, there were no significant differences between the weight of the beans before inoculation, the control, and the weight after inoculation of the fungal isolates.

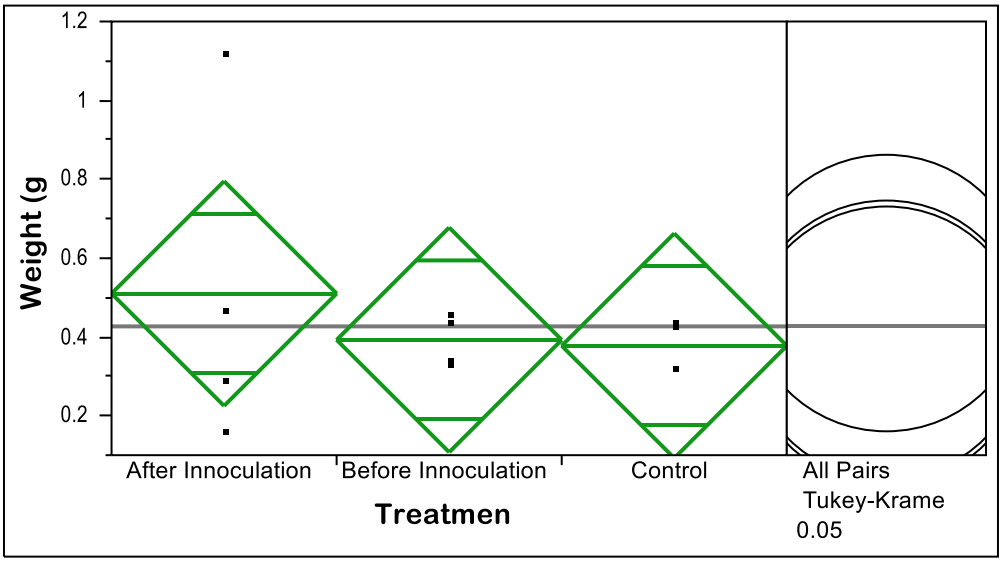

Fig. 1. Pathogenicity test of *Aspergillus flavus* on Big brown cowpea seed in season one.

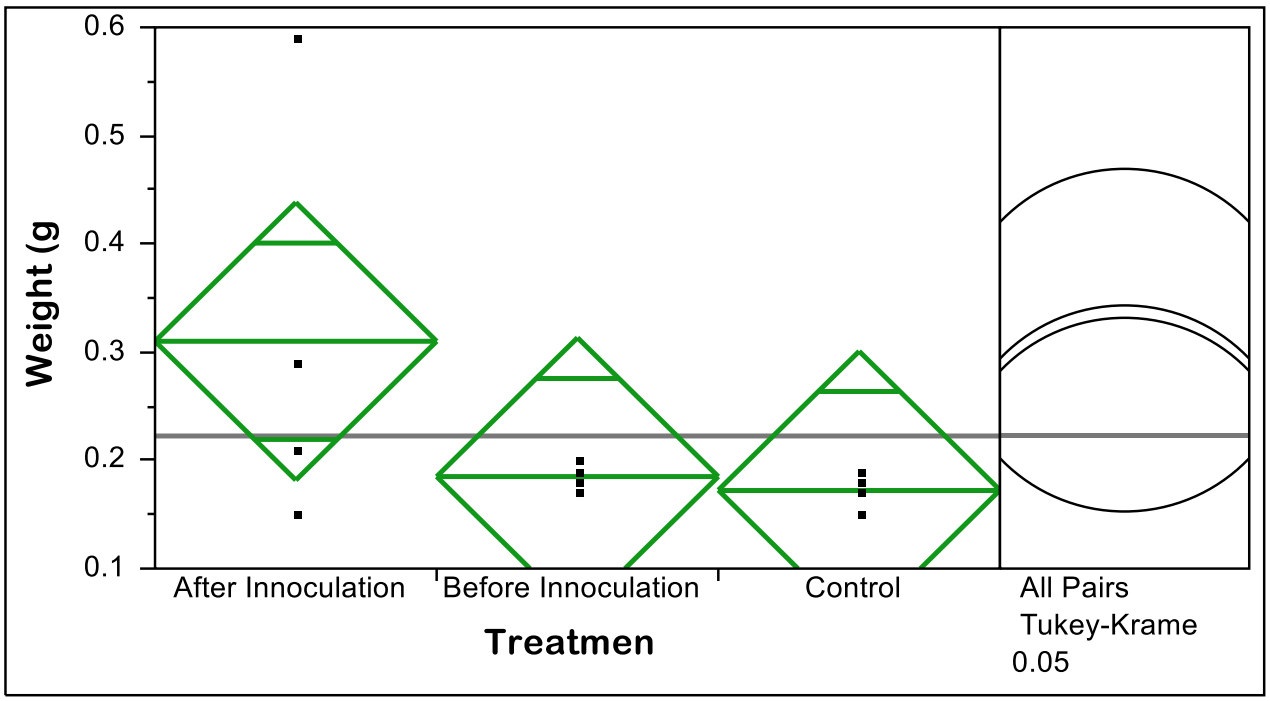

Fig. 2. Pathogenicity test of *Aspergillus flavus* on small brown cowpea seed in season one

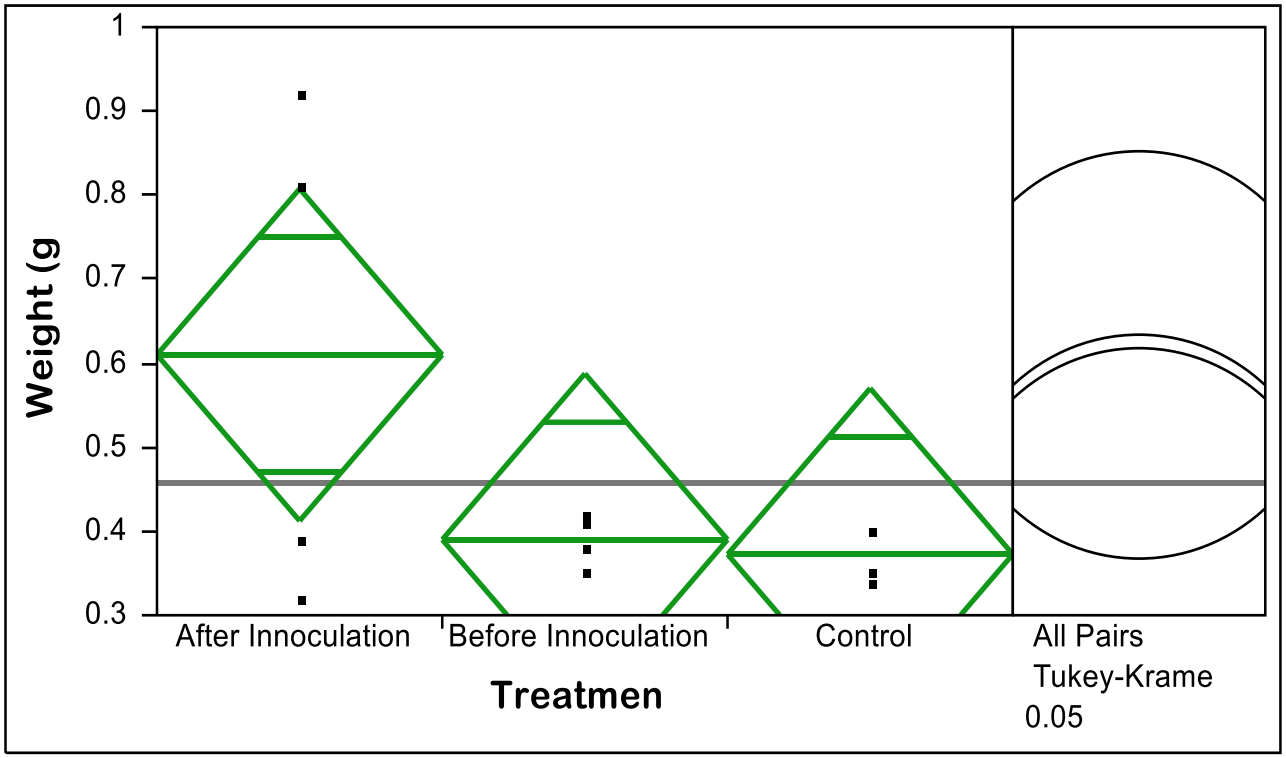

Fig. 3. Pathogenicity test of *Aspergillus niger* on Big brown cowpea seed in season one.

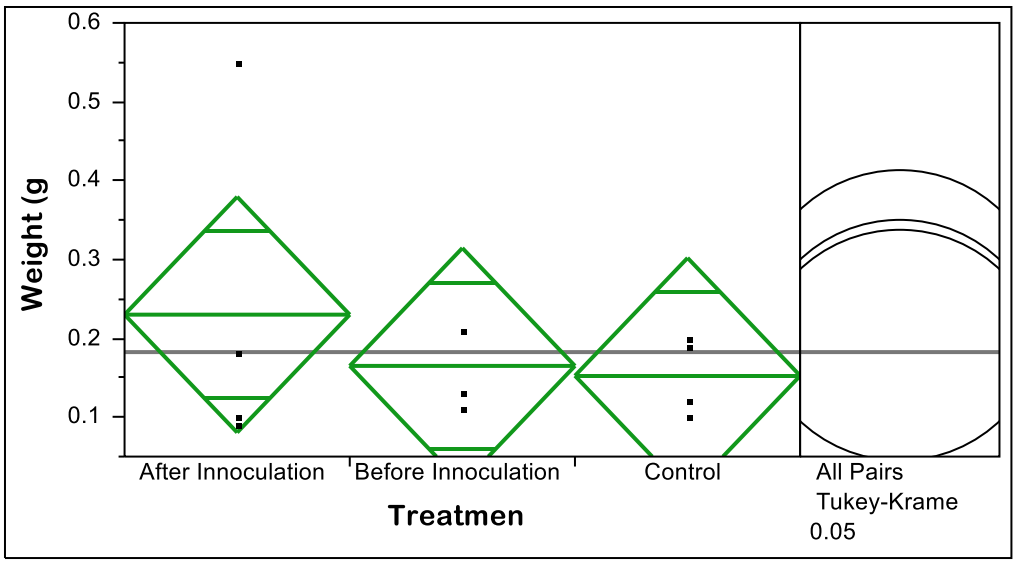

Fig. 4. Pathogenicity test of *Aspergillus niger* on small brown cowpea seed in season one

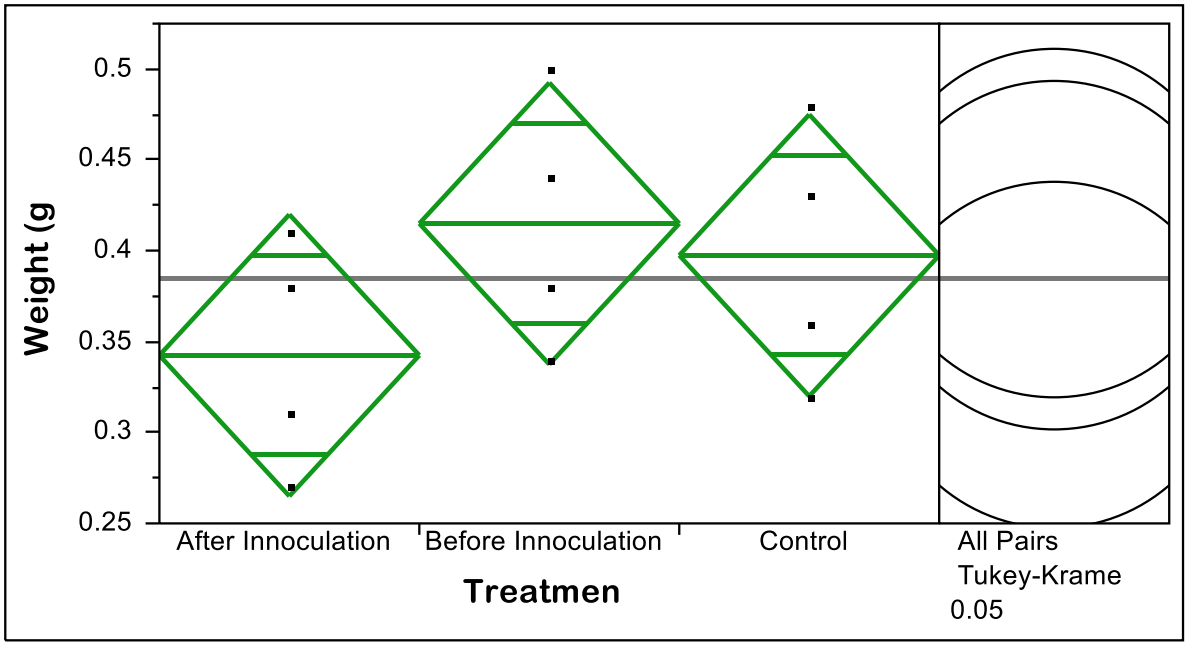

Fig. 5. Pathogenicity test of *Rhizopus* on Big brown cowpea seed in season one.

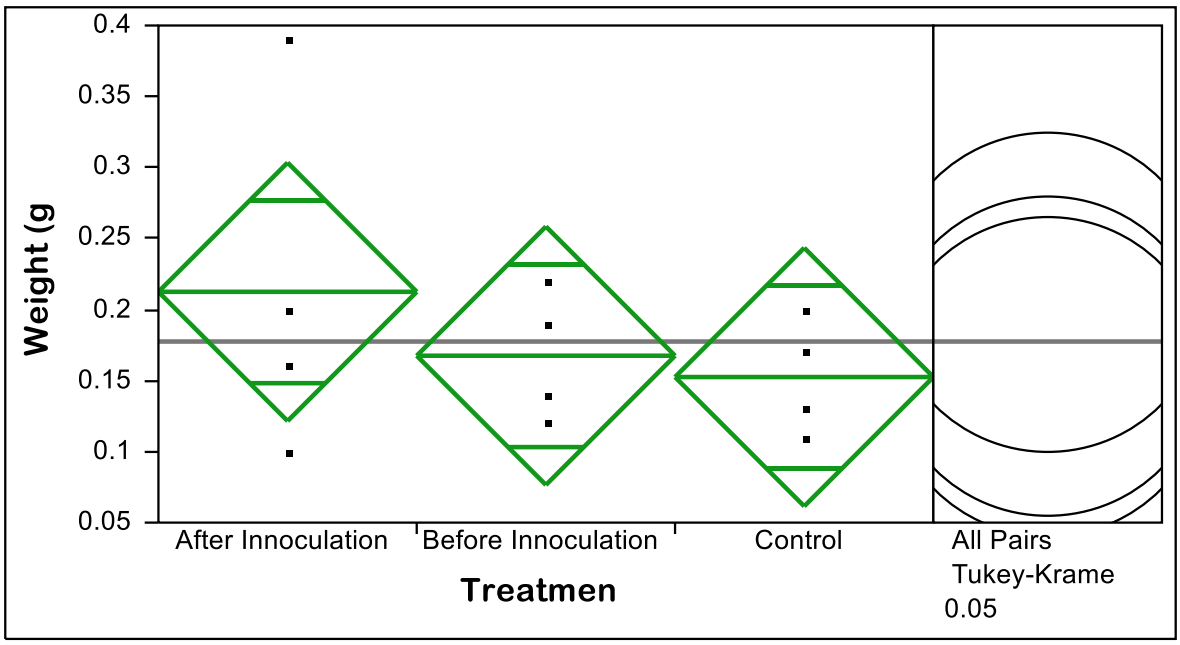

Fig. 6. Pathogenicity test of *Rhizopus* on small brown cowpea seed in season one

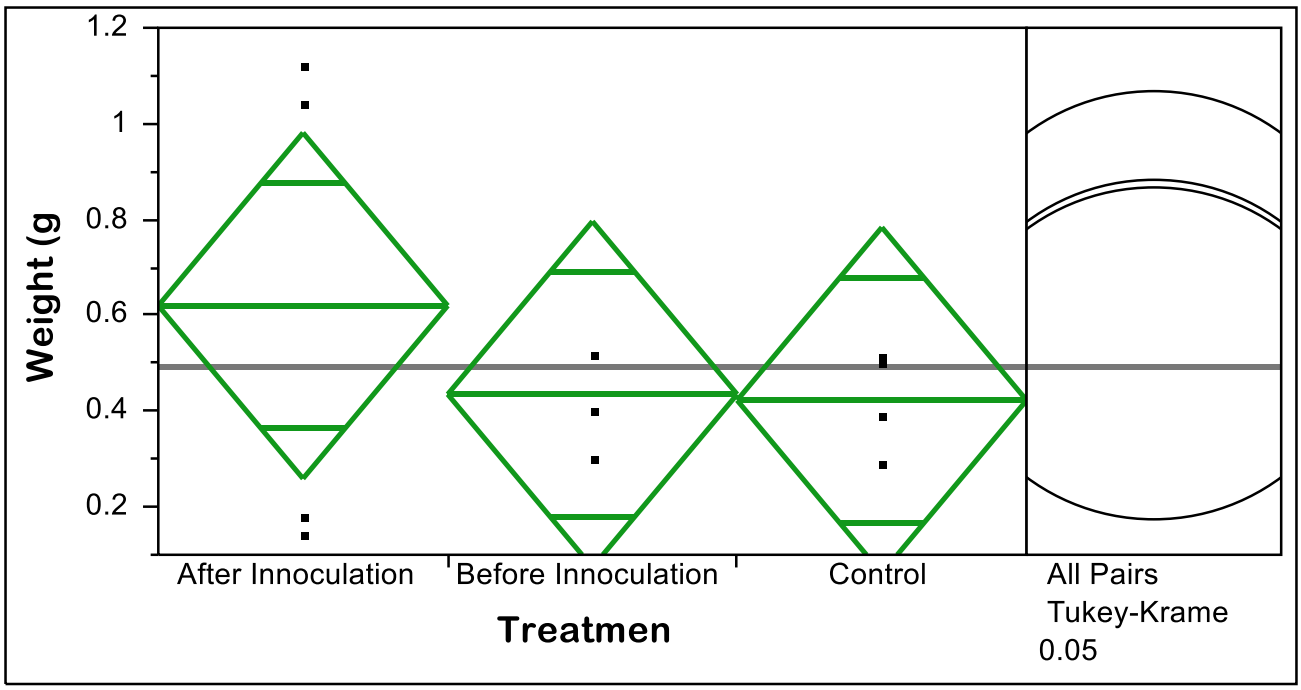

Fig. 7. Pathogenicity test of *Aspergillus niger* on Big brown cowpea seed in season two.

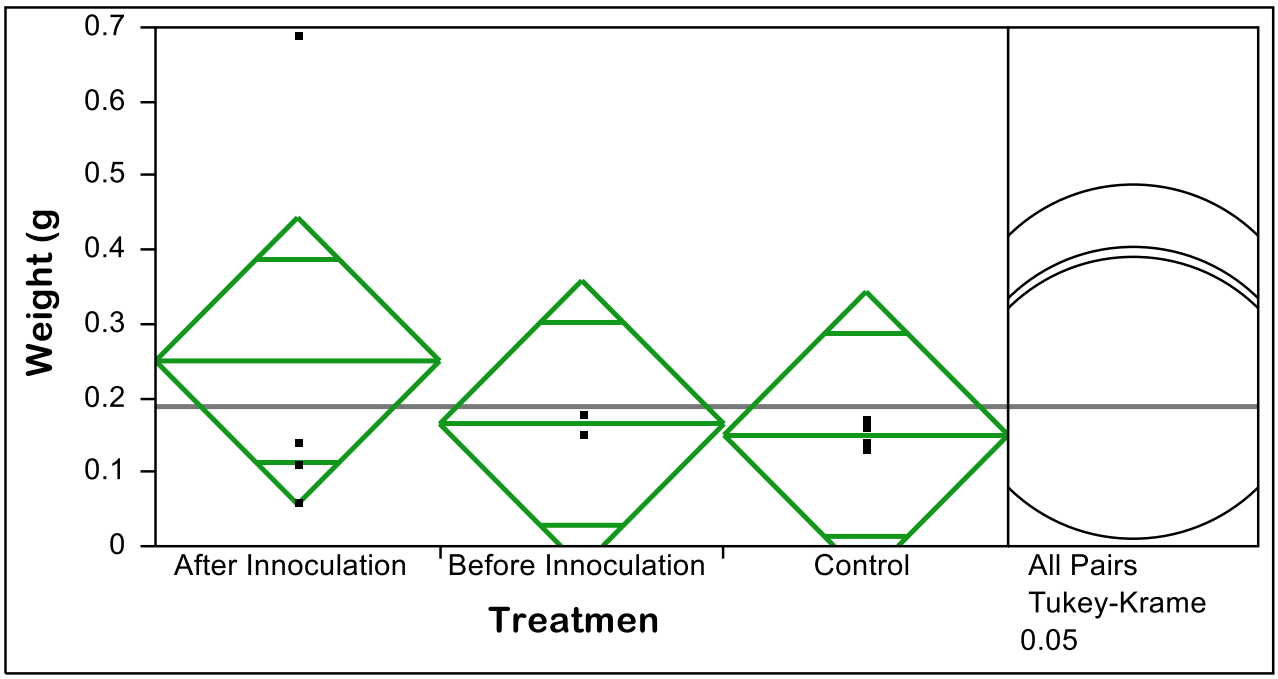

Fig. 8. Pathogenicity test of *Aspergillus niger* on small brown cowpea seed in season two

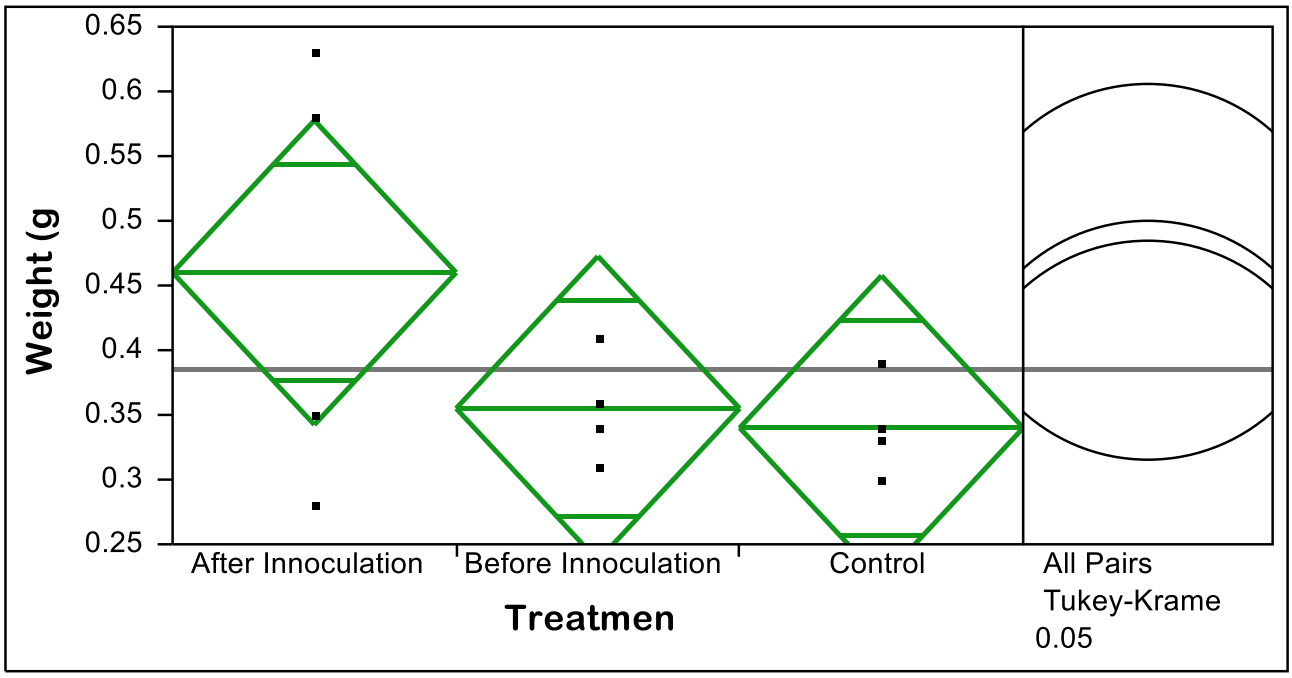

Fig. 9. Pathogenicity test of *Mucor* on Big brown cowpea seed in season two

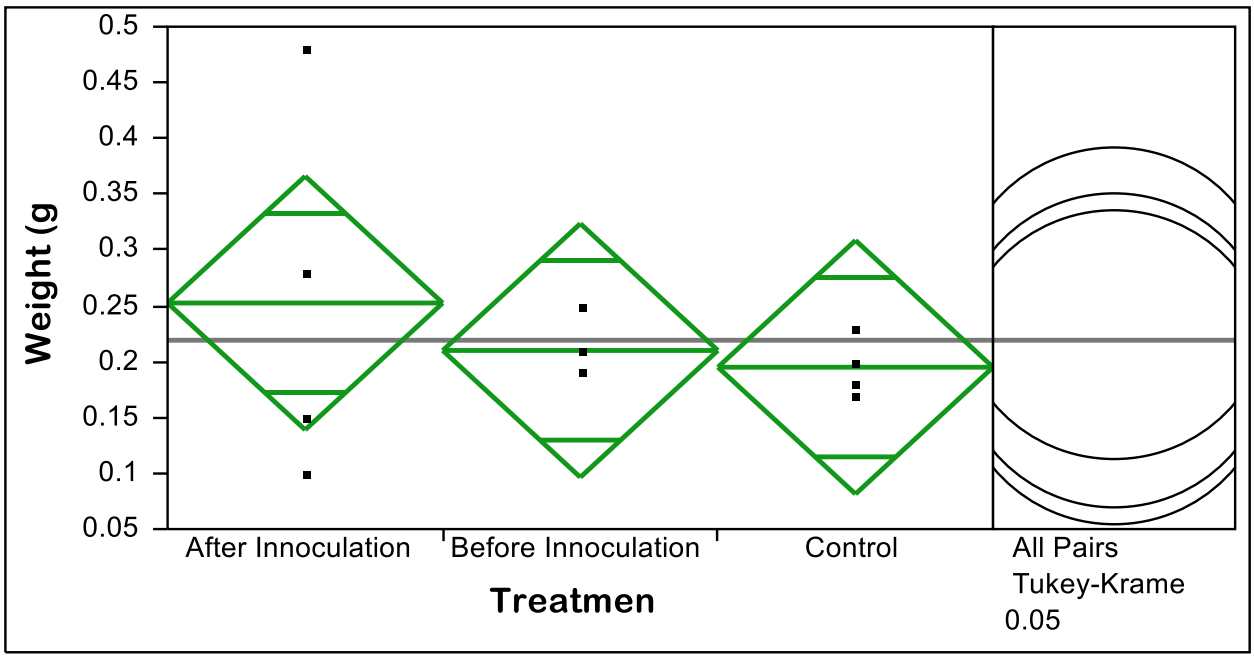

Fig. 10. Pathogenicity test of *mucor* on small brown cowpea seed in season two

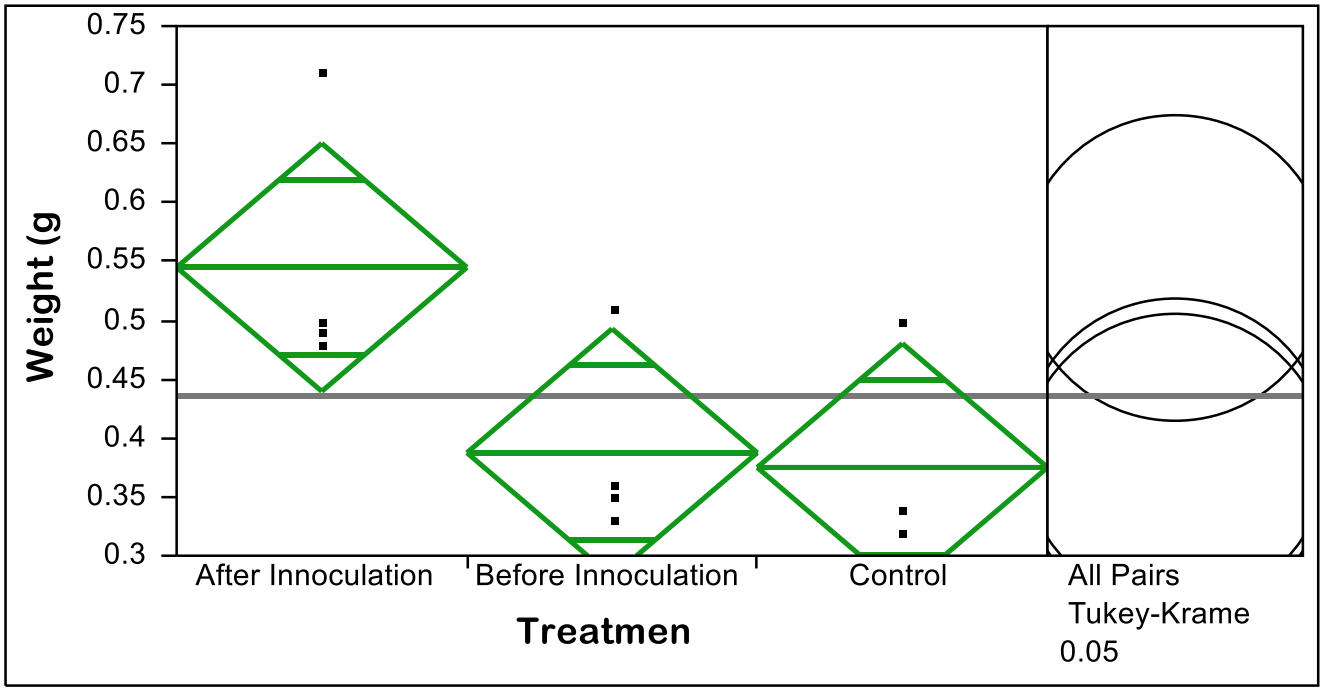

Fig. 11. Pathogenicity test of *Rhizopus* on Big brown cowpea seed in season two

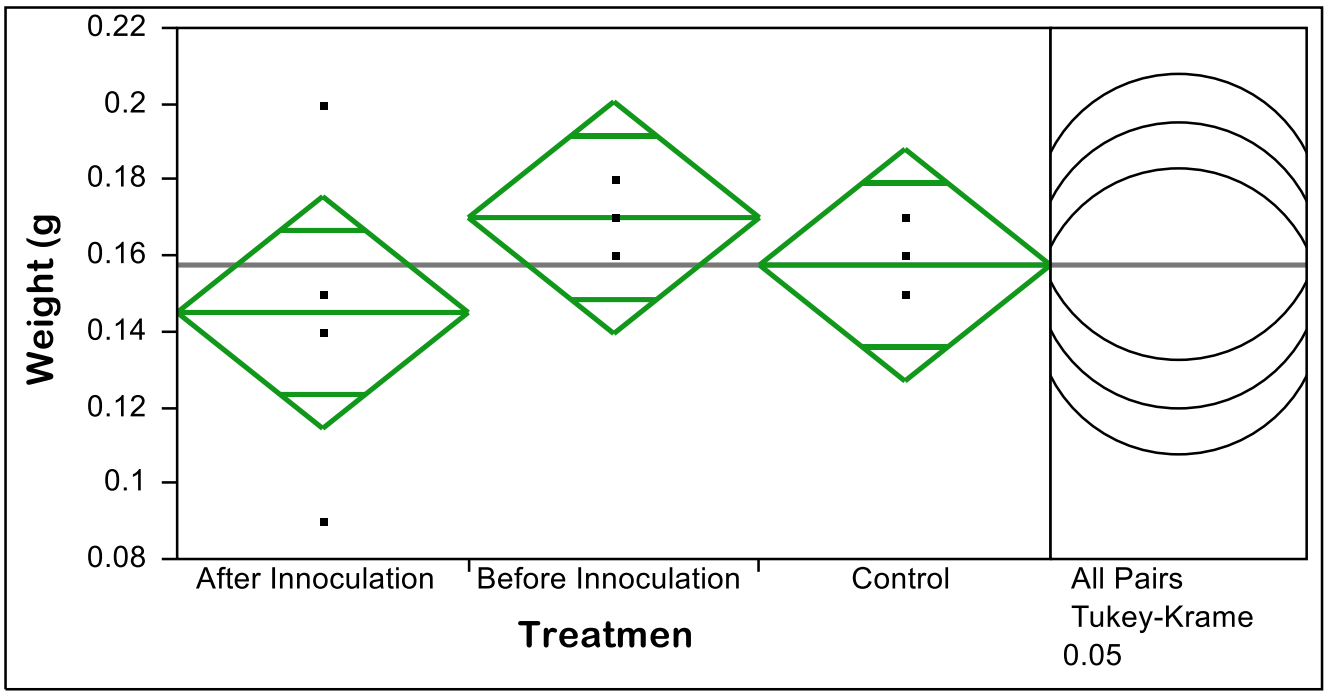

Fig. 12. Pathogenicity test of *Rhizopus* on small brown cowpea seed in season two

DISCUSSION

Microbial Types

The quality of two varieties of raw (uncooked) cowpea sold in major markets in Port Harcourt, Rivers State were evaluated. Although microorganisms play key roles in fermentation and other industrial processes, they are also responsible for the spoilage of foods or food materials which leads to the deterioration of the food. The fungi identified in this study are amongst the fungal isolates reported by (Gabriel and Ruth 2012) on jack beans. Also, early work done on cowpea by previous authors has reported the presence of *A. niger*, *A. flavus*, *Mucor*, and *Rhizopus* sp (Wakessa, 2010; Bishaw, Sahilu, and Simane, 2008). The microbial isolates identified in this study on the cowpea varieties could be normal flora of the cowpea or they could be a contaminant that settled on the bean surfaces. This is because fungi are widely distributed in nature (Prescott, Harley, and Klein 2011) and their mode of distribution such as wind and contact with humans is readily available.

Pathogenicity

All the microbial isolates affected the weights of the cowpea varieties thereby causing a loss in weight as compared to the original weight. Though the controls which were not inoculated or treated with any microbial inoculant had slight weight loss even though the observed weight loss was not as those observed with the microbial-treated seeds. The reduced weight observed in this study after treatment with microbial inoculants could be attributed to the activities of the microorganisms which have caused the cowpea seeds to deteriorate. Deterioration of food materials as a result of microorganisms could lead to loss of the physical properties of the food material. This agreed with (Pitt and Hocking 2006) who stated that biodeterioration of food materials leads to the loss in the food's physical and chemical properties. Conclusively, the fungal isolates: *A. flavus*, *A. niger*, *Rhizopus* and *Mucor* sp negatively impacted on the cowpea thereby causing weight loss. Thus, contamination of these isolates on cowpea seeds could lead to the loss in quality. More so, some of these fungal isolates are known toxin producers, they could be of economic importance in eliciting allergens to immunocompromised individuals who come in contact with the products. In respect to the findings, we recommend that proper practices targeted at reduction of microbial contamination during harvest, storage and distribution of cowpea should be encouraged, and cowpea should be properly cooked to reduce microbial load which could cause illness if food is not properly prepared.

REFERENCES

Adegbite A. A. and Amusa N. A. (2008). The major economic field diseases of cowpea in the humid agro-ecologies of South-western Nigeria. African Journal of Biotechnology, 7 (25): 4706-4712.

Agbogidi, O. M. &Egho, E.O. (2012). Evaluation of eight varieties of cowpea (*Vignaunguiculata*(L.) Walp) in Asaba agro-ecological environment, Delta State, Nigeria. *European Journal of Sustainable Development*, 1(2): 303-314.

Ajibade S. R. &Amusa N. A. (2001). Effects of Fungal diseases on some cowpea lines in the humid environment of South-western Niger. J. Sust. Agric. Environ. 3: 246-253.

Amadi E.N, Kiin-Kabari D.B, Kpormon, L.B, Robinson V.K.K. (2014). Microbial flora and nutritional composition of adult Palm - Wine Beetle (Rhynchophorus phoenicus). International journal

of Current Microbiology and Applied Science, 3(11), 189-192

Bishaw, Z., Sahilu, Y. and Simane, B. (2008). The status of the Ethiopian seed industry: In: M. H. Thijssen, ZewdieBishaw, AbdurahmanBeshir, and W. S. de Boef, Farmers seed and varieties: Supporting informal seed supply in Ethiopia (eds.). 23 - 32. Wageningen, the Netherlands, Wageningen International 2008.

Douglas, S. I. & Robinson, V. K. (2018). Fungal Pollution of Indoor Air of Some Health Facilities in Rivers State. *International Journal of TROPICAL DISEASE & Health*; 32(2): 1-7.

Douglas, S. I. & Robinson, V. K. (2019). Indoor Microbiological Air Quality in Some Wards of a Tertiary Health Institution in Port Harcourt, Nigeria. *Journal of Pharmacy and Biological Sciences*; 14: 44-50.

Robinson, V. K., Samuel-Penu, B., & Minimah, S. O. (2021). Susceptibility of Medical Fungal Isolates to Commonly used Antifungal Agents. International Journal of Pathogen Research, 8(1), 37-42.

Ajibade S. R. &Amusa N. A. (2001). Effects of Fungal diseases on some cowpea lines in the humid environment of South-western Niger. *J. Sust. Agric. Environ.* 3: 246-253.

Amadi J. E. (1995). Chemical control of Cercospora leaf spot disease of cowpea (*Vignaunguiculata* [L.] Walp.). *Agrosearch.* 1: 101-107.

Bishaw, Z., Sahilu, Y. and Simane, B. (2008). The status of the Ethiopian seed industry: In: M. H. Thijssen, ZewdieBishaw, AbdurahmanBeshir, and W. S. de Boef, Farmers seed and varieties: Supporting informal seed supply in Ethiopia (eds.). 23 - 32. Wageningen, the Netherlands, Wageningen International 2008.

Food and Agriculture Organization of the United Nations (FAOUN) (1988). Traditional Food Plants. FAO, Food and Nutrition, Paper 42

Gabriel, A. and Ruth A. (2012). Biodeterioration of Thermally Treated Jack Beans (Canavaliaensiformis, L) Cotyledons. *Journal of Scientific Research;* 4 (3), 689-700.

Gabriel, A. R. (2012). Biodeterioration of Thermally Treated Jack Beans (Canavaliaensiformis, L) Cotyledons. *Journal of Scientific Research;* 4 (3): 689-700.

Hall A. E., Cisse N., Thiaw S., Elawad H. O. A., Ehlers J. D., Ismail A., Fery R., Roberts P., Kitch L. W., Murdock L. L., Boukar O., Phillips R. D. &McWatters K. H. (2003). Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. *Field Crops Res* 82:103–134.

Pitt II, Hocking AD. Fungi and food spoilage. 3rd ed. New York: Springer; 2006.

International Institute of Tropical Agriculture (IITA) (2004). IITA Crops and Farming Systems. www.ita.org/crop/cowpea.htm

Nielson S. S., Brandt W. E. & Singh B. B. (1993). Genetic variability for nutritional composition and cooking time of improved cowpea lines. *Crop Sci* 33:469–472.

Prescott, L. M., Harley, J. P. and Klein, D. A. (2011). Microbiology. 8th ed. McGraw Hill, London

Sarah, K., Catriona, H., Helen, A. And David, E. (2016). Descriptions of Medical Fungi (3rd edn). 113-155.

Timko M. P., Jeff D. E. & Philip A. R. (2007). Cowpea (*Vignaunguiculata*). *Genome Mapping and Molecular Breeding in Plants*, 3: 49- 67.

Wakessa, T. (2010). Assessment of common bean (phaseolus vulgaris l.) seed quality of different seed sources in central rift valley, Ethiopia, MSc Thesis, Haramaya University 2010; 84.