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Nutritional changes induced by fungi on cowpea (*Vigna unguiculata* L. Walp) seeds

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Abstract

Seeds are usually infected by microorganisms and pests during storage, causing deterioration and reduction in the nutritive and market value of these seeds. In this study, the proximate composition of *Vigna unguiculata* seeds inoculated with different fungal organisms was determined to ascertain the level of deterioration caused by fungi on the seeds. The fungi used in the study were *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Aspergillus niger*. There was a significant increase ($p<0.05$, 0.008) in the protein content of seeds inoculated with fungi. *Fusarium oxysporum* (29.45%) caused the highest increase in protein followed by *Aspergillus niger* (28.14%), *Botryodiplodia theobromae* (27.85%) and *Rhizopus stolonifer* (27.50%). The increase could be attributed to the proteineous content of the fungal mycelia. There was a significant increase ($p<0.05$, 0.005/0.014) in moisture and ash content of inoculated seeds respectively. *Fusarium oxysporum* caused the highest increase in ash (7.93) while *Rhizopus stolonifer* (5.4) caused the lowest increase. The increase in ash content is due to the presence of minerals like potassium and phosphorus in the mycelia of the fungi. There was a significant decrease ($p<0.05$, 0.019) in the carbohydrate, lipid, fibre and dry matter content of fungi-inoculated seeds when compared with the control. *Fusarium oxysporum* (36.6) caused the highest decrease while *Rhizopus stolonifer* (43.2) caused the lowest decrease in dry matter of inoculated seeds. Decrease in dry matter may be as a result of production of enzymes by these fungi.

Keywords: Fungi, Nutritional changes, Proximate analysis, *Vigna unguiculata*

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INTRODUCTION

V. unguiculata is an important grain legume grown in the tropics where it serves as good source of protein for millions of people (Boukar *et al*., 2017). Cowpea is mostly produced and consumed in the sub-Saharan Africa especially Central and West Africa. The main world producers are Nigeria, Brazil and Niger (Marques *et al*., 2015). Nigeria has an annual grain production of approximately 2.14 million metric tonnes (FAOStat, 2017). Burkina Faso and Niger Republic are other major producers with 0.57 and 1.59 million metric tonnes respectively per annum. Other countries that cultivate cowpea in Africa are: Senegal, Ghana, Mali, and Cameroon (Directorate Plant Protection, 2011).

Vigna unguiculata is usually better adapted to drought, high temperatures and other biotic stresses such as damage caused by nematodes, insects, weeds etc compared with other legumes (Martins *et al*., 2003). The seeds are a major source of plant protein and vitamins for man, feed for animals (Gebre *et al*., 2012) and also a source of income for farmers. The young leaves and immature pods are eaten as vegetables. The mature grain contains 20 to 25% protein (Adoo-Quate *et al*., 2011), 1.3 to 1.5% lipids and 5.1 to 5.8% crude fibre (Tshovhote *et al*., 2003). Cowpea is high in protein, resistant to drought, adapts to different soil types and intercropping systems, and has the ability to prevent erosion and improve soil fertility. This makes it an important economic crop in many developing countries (Gogile *et al*., 2013). Cowpea being an important legume has several environmental, agronomic and economic advantages, improving the diets and income of peasant farmers in Asia, Africa and South America (Hall, 2012). Some biotic stresses adversely affect the productivity of cowpea. Cowpea is affected by many fungal, viral and bacterial diseases leading to drastic reduction in yield (Boukar *et al*., 2017) and deterioration of seeds during storage. Jaradi *et al*. (2018) reported *Rhizoctonia solani Curvularia muehlenbeckiae* on *Vigna unguiculata*. Jonbozorgi *et* al. (2019) also reported *Alternari destruens, Macrophomina phaseolina, Fusarium chlamydosporum, f. nygamai, F. falciforme, F. proliferatum* and *Pythium oxalicum* on cowpea. Afolabi *et al*. (2020) reported moulds belonging to *Aspergillus, Penicillium* and *Fusarium* on difeerent cowpea varieties sold in Nigerian markets. Adebayo *et al.* (2020) also reported *Rhizopus stolonifer, Aspergillus fumigatus, A. flavus, A. niger, Mucor mucedo, Colletotrichum lindimathianum* and *C. gleosporoides* on cowpea seeds. Seeds under storage are usually attacked by microorganisms which reduce the nutritive value of the plant produce. This study was carried out to assess the nutritional changes of *Vigna unguiculata* seeds inoculated with *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Aspergillus niger*. These organisms were used for this study because they were isolated from cowpea seeds.

MATERIALS AND METHODS

Source of Fungi

Botryodiplodia theobromae, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Aspergillus niger* were isolated from diseased *Vigna unguiculata* seeds (Iyanyi and Ataga, 2014) using Standard Blotter Method recommended by ISTA (2010). Sterilized cowpea seeds were plated on wet Whatman's filter papers in Petri dishes using a sterile forceps and then incubated for 7days at room temperature (30^{o}C). Agar Method described by Klement and Voros (1974) was also used for the study. All fungi observed on filter paper were sub-cultured on Potato Dextrose Agar (PDA) medium under darkness at room temperature and later stored in a refrigerator at a temperature of 4^{o}C until when needed. The identification of the isolated fungi was carried out with reference to Umechuruba and Elenwo (1997) and Ataga *et al*. (2010). Pure cultures of each fungus grown on Potato Dextrose Agar were used as inocula.

Seed Inoculation with Fungi

One hundred grams of healthy-looking cowpea seeds were weighed out into 250 ml conical flasks, plugged with non-absorbent cotton wool and covered with foil and then autoclaved at 121^{o}C for 15 minutes to eliminate any seed-borne microorganisms. After autoclaving, the flasks were allowed to cool and 100 mls of sterile distilled water was added to each flask and shaken for 15 minutes to wet all the seeds. Using a sterile cork borer (1.5 cm in diameter) a disc of 7-days-old mycelia spores of each fungus obtained from the pure culture of isolated fungi was inoculated into each flask containing cowpea seeds. The flasks were shaken for about 15 minutes to obtain homogeneity or to allow the fungus to be well distributed. The control flask, received the same treatment, but there was no fungus added to it. The control flask and the conical flasks containing the inoculated seeds were placed in separate air tight plastic containers (which were previously surface sterilized) and incubated for 14 days at room temperature (30ºC).

A total of 15 conical flasks were used, 3 flasks replicate for each set of fungi-inoculated seeds and control. At the end of the incubation period, the flasks for each fungal treatment and flasks for control were harvested for biochemical analysis. The seeds in each flask were transferred into a pre-weighed watch glass, dried at 45ºC for 24 hours and the spores and mycelia of the fungi removed by sieving.

Biochemical analysis of some nutrient components of cowpea seeds in both fungi-inoculated and control flasks seeds were determined following the procedures recommended by the Association of Official Analytical Chemists (AOAC, 1995). The results obtained were analysed using Analysis of Variance (ANOVA). The analysis was done to determine the pathogenecity of the test organisms.

RESULTS AND DISCUSSION

The inoculation of cowpea seeds with the following fungi; *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Aspergillus niger* resulted in varying degrees of deterioration. The morphological characteristics of the fungal organisms used in the study are shown in Table 1. Figure 1 shows the nutritional changes in *Vigna unguiculata* seeds inoculated with the different fungi.

Table 1: Morphological characteristics of the fungal organisms used in the study

Morphological Characteristics	Fungal organism
Black colony with powdery surface	*Aspergillus niger*
Dark grey cottony colonies	*Botryodiplodia theobromae*
Copious cottony colony with black globules	*Rhizopus stolonifer*
Snow white colony with dry surface	*Fusarium oxysporum*

Source: Iyanyi and Ataga, 2014

The protein content of the seeds inoculated with fungi increased significantly ($p<0.05$, 0.008) when compared to the control (24.20%). *Fusarium oxysporum* caused the highest protein increase (29.45%) followed by *Aspergillus flavus* (28.14%), *Botryodiplodia theobromae* (27.85%) and *Rhizopus stolonifer* (27.50%). The increase may be due to the presence of proteinaceous mycelium in the fungi. Similar result was reported by Nwaukwu and Ataga (2013) in their research on the effect of fungi on *Hibiscus sabdariffa* seeds. Saxena *et al*. (2015) also reported a protein decrease of Soybean (*Glycine max* L.) seeds caused by fungi including *Aspergillus flavus* and *Fusarium oxysporum* during storage.

There was a significant decrease ($p<0.05$, 0.019) in the carbohydrate content of seeds inoculated with the test organisms when compared with control (15.6%). *Fusarium oxysporum* (10.5%) caused the highest decrease followed by *Aspergillus niger* (11.4%), *Rhizopus stolonifer* (12.3%) and *Botryodiplodia theobromae* (12%). The decrease could be as a result of utilization of storage starch and sugar as a carbon source by the fungi during respiration and also as a source of energy for microbial growth (Monday and Ataga, 2005). Khairnar (2015) obtained a similar result with wheat, maize and paddy infected with some fungal species. Nwaukwu and Ikechi-Nwogu (2012) also obtained similar result in their research on effect of fungi on *Dialum guineense*. *Aspergillus flavus* has also been known to utilize carbohydrates of seeds for its growth and aflatoxin production (Aziz and Mahrous, 2004).

There was also a significant decrease ($p<0.05$, 0.027) in the lipid content of inoculated seeds when compared to the control (1.44%). *Fusarium oxysporum* (0.74%) caused the highest decrease when compared to the other fungi. This was followed by *Aspergillus niger* (0.93%), *Botryodiplodia theobromae* (1.02%) and *Rhizopus stolonifer* (1.03%). Similar report was reported by Srivastava *et al*. (2013) on *Jatropha curcas* seeds infested with *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium chlamydosporum* and *Penicillium* glabrum. Results by Tripathi and Kumar (2020) also showed that there was a significant decrease in oil content of *Salvadora oleoides* and *Salvadora persica* seeds infected with *Aspergillus candidus, A. flavus, A. niger, Fusarium oxysporum, Penicillium chrysogenum, Rhizoctonia solani, Rhizopus nigricans* and *Sclerotium rolfsii*.

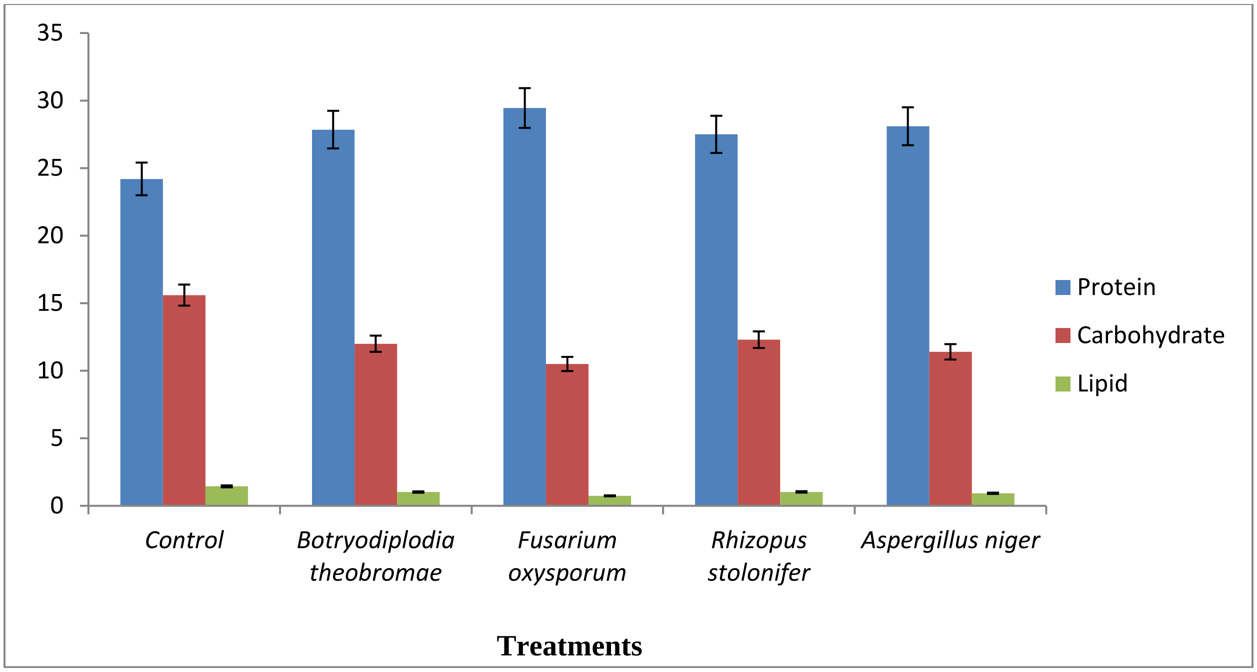

Figure 1: Changes in protein, carbohydrate and lipid contents of *Vigna unguiculata* seeds inoculated with fungi at 30^{o}C

Figure 2 shows the changes in moisture and dry matter in *Vigna unguiculata* seeds inoculated with the different fungi. The moisture content of the fungi inoculated seeds increased significantly ($p<0.05$) when compared with the control (52%). *Fusarium oxysporum* (63.4%) caused the highest increase in moisture followed by *Aspergillus flavus* (59.4%), *Botryodiplodia theobromae* (58.2%) and *Rhizopus stolonifer* (56.8%). Seeds, being concentrated packages of high nutritive materials like starch, protein and lipid are attractive food supplies for a number of organisms. The increase caused by the fungi is due to their utilization of components of the seeds as food nutrient thereby producing water in the process. Similar results were recorded by Embaby *et al*. (2013) with *Phaseolus vulgaris*, *Pisum sativum* (peas) and *Glycine max* (soyabeans) infected with *Aspergillus parasiticus*.

There was a significant decrease ($p<0.05$, 0.005) in the dry matter content of the seeds inoculated with the individual fungi when compared with the control (48%). *Fusarium oxysporum* (36.6%) caused the highest decrease followed *Aspergillus niger* (40.5%), *Botryodiplodia theobromae* (41.8%) and *Rhizopus stolonifer* (43.2%). This fungus produces extracellular cellulolytic and pectic enzymes; and secondary metabolites which may be responsible for the drastic depletion of dry matter (Okonkwo *et al*., 1990). The result is in line with the report of Nwaukwu and Ataga (2012) on the biochemical changes in *Hibiscus sabdariffa* seeds caused by five pathogenic fungi, and Khairnar (2015) on seed-borne fungi, bio deterioration of seeds and control.

Figure 3 shows the changes in ash and fibre contents in *Vigna unguiculata* seeds inoculated with the different fungi. There was a significant increase ($p<0.05$, 0.014) in ash content of fungi-inoculated seeds when compared to the control (3.73%). *Fusarium oxysporum* (7.93%) caused the highest increase followed by *Aspergillus niger* (5.93%), *Botryodiplodia theobromae* (5.56%) and *Rhizopus stolonifer* (5.4%). Khairnar (2015) also reported an increase in ash content of some cereals associated with *Aspergillus flavus* and *Curvularia pallescens*. Ataga and Akueshi (1997) resolved that the increase could be attributed to the presence of minerals like potassium and phosphorus in the fungal mycelia.

There was a significant decrease ($p<0.05$, 0.006) in the fibre content of fungi inoculated seeds when compared with the control (7.3%). *Fusarium oxysporum* (1.01%) caused the highest decrease followed by *Aspergillus niger* (1.34%), *Botryodiplodia theobromae* (2.07%) and *Rhizopus stolonifer* (2.21%). Kakde and Chavan (2011) obtained similar results with sesame and groundnut seeds infected with *Fusarium oxysporum Fusarium equiseti*, *Penicillium digitatum* and *Penicillium chrysogenum*.

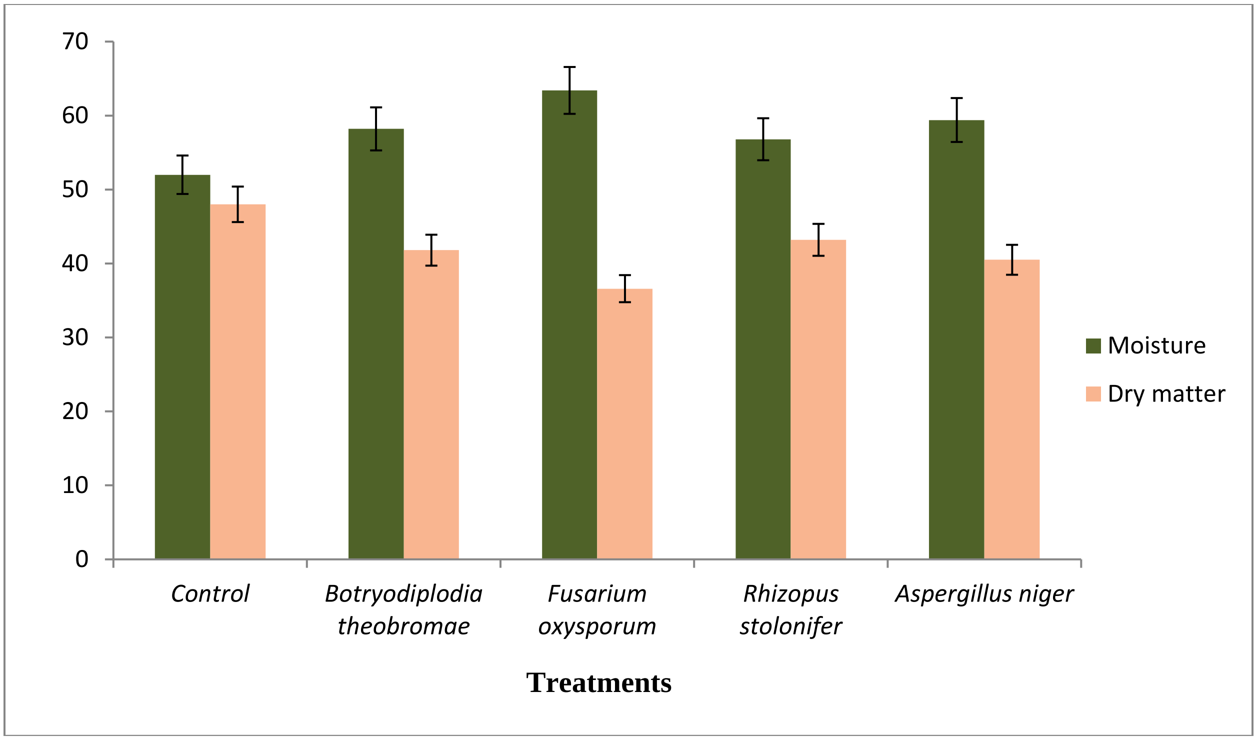

Figure 2: Changes in moisture and dry matter contents of *Vigna unguiculata* seeds inoculated with fungi at 30^{o}C

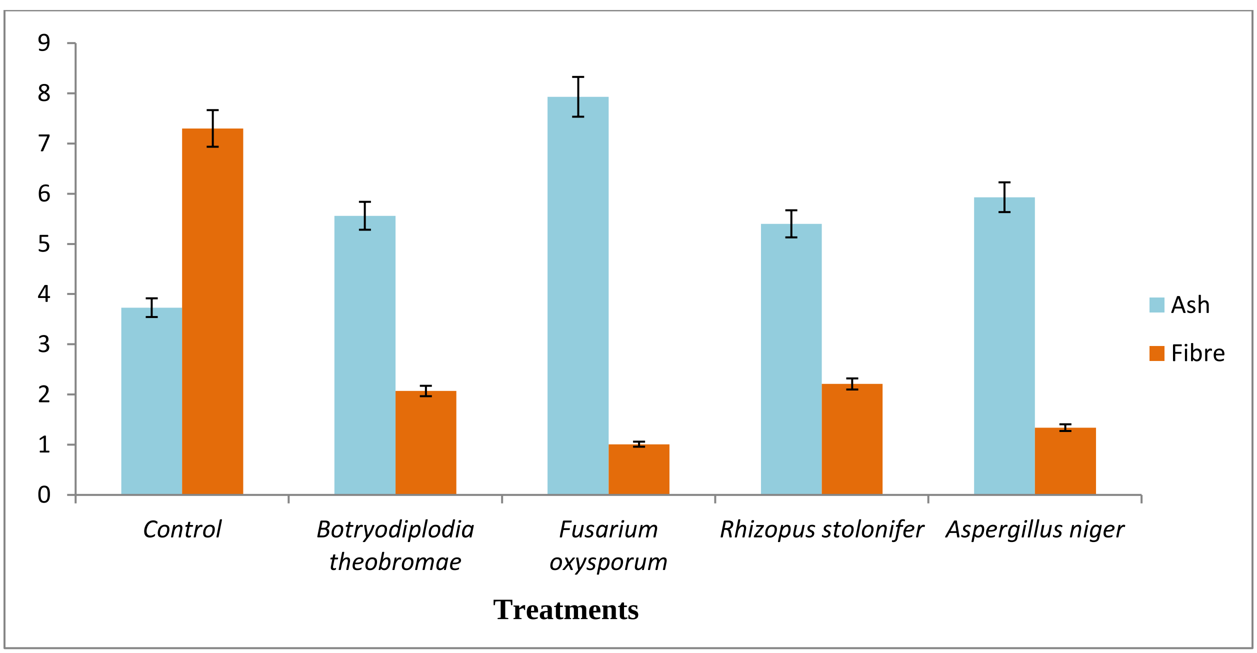

Figure 3: Changes in ash and fibre contents of *Vigna unguiculata* seeds inoculated with fungi at 30^{o}C

CONCLUSION

The results obtained from this study revealed that the inoculation of fungi in stored cowpea resulted in significant changes in the nutritional and proximate compositions of *Vigna unguiculata*. The various fungi resulted in different degrees of alterations in the chemical components of the seeds and consequently reduced its nutritive value. Improving the methods of preservation and storage of these seeds is key in preventing or reducing the incidence of fungi on seeds as most of these pathogenic fungi are seed borne, attacking plant produces especially during storage. There is need to ensure the continuous and sustainable production of cowpea for the teeming population of Africa.

Conflict of Interest

Authors have no conflict of interest to declare.

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