THE EFFECT OF FUNGAL GROWTH ON THE PROTEIN CONTENT OF SOME SELECTED GRAIN LEGUMES

***[1]ODUWOBI, O.O. and [2]NOAH, A. A.**

[1]The Department of Science Laboratory Technology, [2]The Department of Food Technology,

[1,2]The Federal Polytechnic, Ilaro,

Ogun State.

*E-mail: dwheazy2012@gmail.com; 08057954550

ABSTRACT

Common beans; *Phaseotus vulgaris*, is a member of that unique family of plants, the leguminous (Fabaceae) which comprises about 600 genera and about 13,000 species. Legumes are grown agriculturally, primarily for their grain seed called 'Pulse', for livestock forage and soilage and as soil enhancing green manure. Moulds are one type of microorganisms and they grow on food materials like bread, fruits, vegetables, jam/jelly etc. This research work is based on the ability to reduce the protein content of some selected grain legumes. Three different fungal strains belonging to three genera were repeatedly isolated. The fungi isolated are: *Phytophthora infestans*, *Aspergillus niger* and *Fusarium moniliforme*. The grain legumes (healthy and diseased) tested for protein all showed a positive indication for the presence of protein. The spectrophotometric readings of the healthy and diseased beans' range were from 4.32 to 4.75mg/ml and 2.45 to 2.98mg/ml respectively. The spectrophotometric readings of the healthy and diseased soya beans were from 4.79 to 4.96mg/ml and 3.01 to 3.56mg/ml respectively. By this investigation, it can be deduced that the diseased grain legumes contained a lesser concentration of protein as compared with the healthy ones. Fungal infestation should be prevented or avoided by the application of appropriate and approved fungicides on the grain legume crops while still in the fields.

Keywords: Pulse, Moulds, Spectrophotometric readings, Fungicides, Fields

INTRODUCTION

Common beans; *Phaseotus vulgaris,* is a member of that unique family of plants, the leguminous (Fabaceae) which comprises about 600 genera and about 13,000 species. The genus *Phaseolus* includes 150-200 species of plants, many which are cultivated as food or garden ornamentals. The specific name *Phaseolus vulgaris* refers to hundreds of varieties and cultivars of common beans which have been in cultivation for thousands of years (Ramirez and Vohra, 2014).

Legumes are grown agriculturally, primarily for their grain seed called 'Pulse', for livestock forage and soilage and as soil enhancing green manure. A legume fruit is a simple dry fruit that develops from a simple carpel and usually dehisces (opens along a seam) on two sides. Legumes are notable in that most of them have symbiotic nitrogen fixing bacteria in structures called root nodules. For that reason, they play a key role in crop rotation (Weichselbaum, 2012). Right behind cereals, legumes are the second most important source of human food and animal storage. It is through nitrogen fixation that legumes provide plant tissue that is high in protein. In agricultural practice, legumes are used as organic manure to amend and reduce crop nutrient deficiency. Mankind has known for millenniums of the importance of legumes environmentally.

Legumes are able to provide the benefits of nitrogen fixation thanks to a strain of soil bacteria. Rhizobia bacteria penetrate legumes' roots, creating pink nodules that bind nitrogen gas found naturally in the atmosphere. The fixation provides a useable source of nitrogen to the legumes in return for carbohydrates needed by the bacteria. This mutual interaction between the two species is known as symbiotic relationship (Staats *et al*., 2007).

Planting legumes with others forage crops helps improve nitrogen availability to companion crops. The USDA states that in legume and perennial grass mixtures, legumes not only supply their own nitrogen but also supply approximately the nitrogen needs of grass crops. They also function as a non-chemical weed control for crop fields and pastures (ICMSF, 2002). Legumes used as green crop, or cover crop, provide many benefits to the soil. According to the USDA, legumes improve soil quality by increasing soil organic matter, improving soil porosity and structure, recycling nutrients, decreasing soil pH, diversifying microorganisms and alleviating disease problems, planting legumes as a cover crop also helps reduce soil erosion.

According to Yu and Sutton (1997), a staple food or simply a staple is a food that is eaten routinely in such quantities that it constitutes a dominant portion of a standard diet for a given people. Most staple foods are derived either from vegetables and animals products including cereals (such as rice, wheat, maize, millet or sorghum) starchy tubers or root vegetables (such as potatoes, cassava, yams or taro), meat, fish, eggs, milk and cheese. Other staple foods include pulses (dried legumes).

Moulds are organisms that reproduce by releasing spores that create mould colonies when the spores settle on damp surfaces, including food grains, over a period of time, as they provide a suitable environment for their growth. The increased number of mould colonies can destroy the surfaces they feed off and digest (Alexopolous, 2004). Moulds are one type of microorganisms and they grow on food materials like bread, fruits, vegetables, jam/jelly etc. Grains legumes provide fungi with essential amino acids needed for their survival, hence, their susceptibility to fungal attack or rot.

Moulds are not only unsightly and smelly; they can also lead to huge economic losses. Common grain legumes are important legumes with high nutritional value. Fungal deterioration not only reduces their economic value by reducing their protein content, their infestation on them can also lead to fungal food poisoning or intoxication if consumed.

MATERIALS AND METHODS

SAMPLE COLLECTION

This investigation was carried out in the Microbiology laboratory at The Federal Polytechnic, Ilaro, Ogun State, Nigeria. Healthy Soya beans and beans grains were purchased at Sayedero market in Ilaro town, Ogun State, for this research and the experiment was conducted at the microbiology laboratory of The Federal Polytechnic, Ilaro. Half proportion of the soya beans and beans samples were left to naturally rot to obtain the diseased samples by sprinkling water on the seeds intermittently.

MEDIUM PREPARATION

Potato dextrose agar was the medium used for the growth and maintenance of the fungal isolates. The PDA was prepared according to the manufacturer's instruction, which is to dissolve 39g of the dehydrated PDA in 1 Litre of distilled water (Cheesbrough, 2000). 250ml of PDA was prepared by homogenizing and then autoclaving at 121^0C for 15 minutes. As a general rule 0.5g antibiotic per liter of agar will reduce bacterial contamination significantly. Therefore, about 0.125g of Chloramphenicol was sterilized by first dissolving it in 5ml of sterile water and then passed through a commercial membrane filter (Whatman filter paper) of 0.45ml pore size. The medium was allowed to cool to about 35^0C and the antibiotic was incorporated to suppress

bacterial contamination. Approximately 12ml of the molten PDA was aseptically dispensed into the sterile disposable 90mm Petri plates and then allowed to gel and then inverted (Okigbo *et al*., 2009).

ISOLATION

Sterile cotton buds were moistened by dipping into distilled water and then gently used to randomly swipe the two types of diseased grain legumes to collect fungal strains. The cotton buds were aseptically used to streak the already prepared Potato Dextrose Agar (PDA). The plates were then incubated for 72hours (3 days) at room temperature of 28 + 2^0C. Sub-culturing was carried out to obtain pure culture of the isolates. The pure isolates were prepared on agar slants. Agar slants for the fungal isolates were preserved in McCartney bottles and stored at 28^0C + 2^0C until the appearance of distinct and appreciable fungal colonies before they were then stored in a refrigerator. The pure cultures were kept refrigerated as stock cultures. The experiment was conducted in duplicate. Labeling of the sample containers was done accordingly.

CULTURAL IDENTIFICATION

The isolates were identified on the basis of their morphological and cellular characteristics, as described by Barnet and Hunter (1999). The isolates were identified by first mounting each fungal mycelium on a glass slide then stained with Lactophenol-in-cotton blue dye, covered with a cover slip and then mounted on a compound microscope. The slides were viewed under x40 objective lens. The physical characteristics were compared to a Fungi Atlas for identification.

TEST FOR PROTEIN

The Biuret's test was qualitatively used to test for protein in the food samples (healthy and diseased). The test is a chemical test used for detecting the presence of peptide bonds which are the basis protein formation. An aqueous sample of the food was prepared by crushing the grain legumes in a mortar using a pestle. A few drops of distilled water were added and then the liquid was decanted. About $2cm^3$ of the aqueous sample was added to $2cm^3$ of the Biuret's reagent in a test tube and viewed against a white background. In other words, the aqueous sample of the food was treated with an equal volume of 1% of a strong base (NaOH) and then mixed carefully, followed by a few drops of 1% aqueous Copper (II) sulphate, without shaking. If the solution turns purple from blue, it is an indication for the presence of protein (Ramirez, 2014).

QUANTITATIVE DETERMINATION OF PROTEIN

Absorption spectroscopy using a spectrophotometer set at a wavelength of 540nm was used to quantify the amount of proteins in mg/ml for each sample (healthy and diseased) for comparative analysis. This was preceded by the Biuret's test. After the Biuret's test, each sample was transferred into a cuvette for spectroscopy and the readings were taken twice in absorbance. The mea value of each reading was determined. The test result was compared to a standard using distilled water for a negative protein test while the main standard was commercial albumin powder which was employed as a positive protein test. Different concentrations of the standard albumin powder were prepared and their spectrophotometric readings were taken (Harris, 2003). A standard curve was obtained from the known concentrations of the albumin powder, after the spectrophotometric analysis, which was the reference protein. The quantity of protein for each sample was then extrapolated from the standard curve and expressed in mg/ml.

RESULTS AND DISCUSSION

Three different fungal strains belonging to three genera were repeatedly isolated. The fungi isolated are: *Phytophthora infestans*, *Aspergillus niger* and *Fusarium moniliforme*.

From the result obtained, it is obvious that the grain legumes can act as an organic source which could easily be utilized by the fungi as a natural carbon source, under favourable conditions such as the presence of moisture and warmth.

The grain legumes (healthy and diseased) tested for protein all showed a positive indication for the presence of protein by the change in colour from blue to purple for the Biuret's test.

Table 1: Fungi isolated from the Soya beans and Beans

Isolates	Colonial appearance	Microscopy	Organisms
SB-2	Black mould	Colonies spots jet-black spherical conidia, profuse conidia, biseriate.	*Aspergillus niger*
B-1	Brown mould	Lumpy appearance, aplerotic.	*Phytophthora infestans*
SB-1	Green mould	Macroconidia with septation.	*Fusarium moniliforme*
B-2	Green mould	Macroconidia with septation.	*Fusarium moniliforme*

Keys: SB = Soya beans, B = Beans

Fig. 1 shows the values of the spectrophotometric analysis conducted on the albumin standard, which is depicted as a linear graph. The graph was then used to extrapolate the values in mg/ml of the beans and soya beans (healthy and diseased) after obtaining their spectrophotometric readings in absorbance. Table 2 shows the spectrophotometric readings of the healthy and diseased beans and the range was from 4.32 to 4.75mg/ml for the healthy beans and 2.45 to

2.98mg/ml for the diseased beans. Table 3 shows the spectrophotometric readings of the healthy and diseased soya beans and the range was from 4.79 to 4.96mg/ml for the healthy soya beans and 3.01 to 3.56mg/ml for the diseased soya beans. It was observed that the healthy beans and soya beans both had higher protein contents as compared with the protein contents of the diseased beans and soya beans respectively.

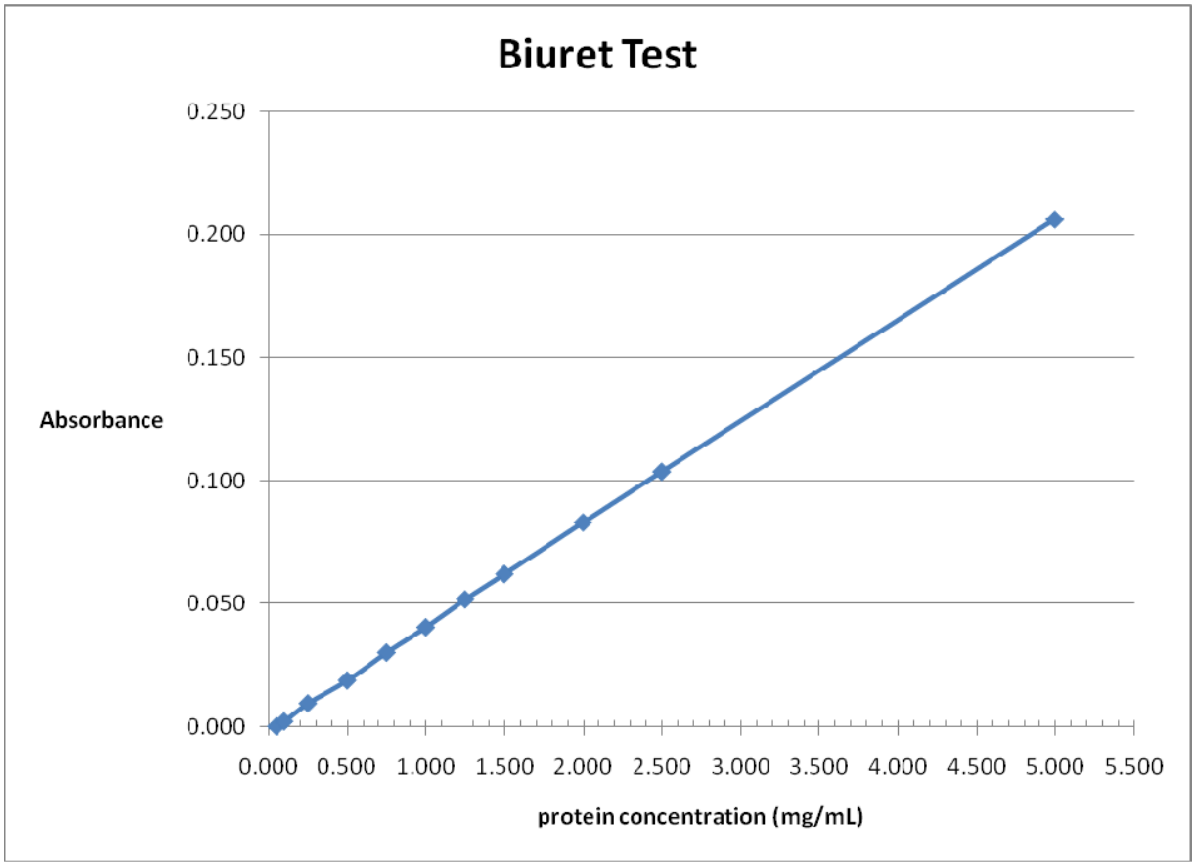

Fig 1: Spectrophotometric Chart of the Albumin Standard

Table 2: Spectrophotometric Readings of the Healthy and Diseased Beans

Absorbance (abs)	Healthy beans (mg/ml)	Absorbance (abs)	Diseased beans (mg/ml)
0.1800	4.75	0.1100	2.78
0.1250	4.32	0.1080	2.68
0.1570	4.68	0.1001	2.45
0.1478	4.59	0.1360	2.98

Table 3: Spectrophotometric Readings of the Healthy and Diseased Soya Beans

Absorbance (abs)	Healthy beans (mg/ml)	Absorbance (abs)	Diseased beans (mg/ml)
0.1945	4.96	0.1401	3.01
0.1780	4.88	0.1469	3.78
0.1845	4.79	0.1430	3.45
0.1902	4.82	0.1457	3.56

This study assessed the quantitative effect of fungal rot on the protein content of some diseased beans and soya beans. Fungi are ubiquitous organisms that make up approximately 25% of the earth's biomass. Fungal health effects are dependent on the species present, the metabolic products, the concentration and exposure duration (Young *et al*., 2005). Fungal infestation on food crops is of particular interest due to the potentiality of an epidemic occurrence of fungal food-borne diseases and economic losses if the situation is not curbed.

The quantization of protein content is important and has many applications in clinical laboratory practices and in research especially in the field of biochemistry. The accurate quantization of protein content is a critical step in protein analysis. Spectrophotometric methods which utilize visible radiation are called colorimetric analyses (Harris, 2003). The absorbance of the analyte versus its concentration is plotted in a calibration curve.

For routine use, the Biuret procedure is simple to perform, producing a stable color that obeys Beer's Law. UV-Vis Spectroscopy is primarily used for quantitative analysis in chemistry and one of its many applications is in protein assays. One commonly used method for determining the total protein in a sample is the Biuret method. The Biuret method is based on the complexation of Cu^{2+} to functional groups in the protein's peptide bonds. The formation of a Cu^{2+}protein complex requires two peptide bonds and produces a violet-colored chelate product which is measured by absorption spectroscopy at 540nm (Kingsley, 2009). Over a given concentration range, the measured absorption at 540nm is linear with respect to the concentration of total protein. The intensity of the color and hence the absorption at 540nm, is directly proportional to the protein concentration, according to the Beer Lamber's law. The amount of color produced is proportional to the amount of peptide bonds such as size, amount of protein/peptide (Robinson & Hogden, 1999).

A standard curve is a type of graph used as a quantitative research technique. Standard curve for protein concentration is often created using known concentrations of bovine serum albumin (BSA). In protein quantization assays, BSA serves as a reference protein that is used to construct protein standard curves. Other proteins can be used depending on the physical/chemical properties of the protein of interest (Weichselbaum, 2012).

CONCLUSION

By this investigation, it can be deduced that the diseased grain legumes contained a lesser concentration of protein as compared with the healthy ones. This is an indication that the isolated fungi must have utilized some of the available protein in the diseased grain legumes for their growth and survival as they simultaneously caused rot in the grain legumes.

RECOMMENDATIONS

Fungi, particularly the food-borne ones, are undoubtedly, some of the micro-organisms of medical importance, hence:

- Vendors of grain legumes should meticulously pay extra attention and care to their produce by ensuring that they are free from moisture while also maintaining their sanitary integrity.
- Fungal infestation should be prevented or avoided by the application of fungicides on the grain legume crops while still in the fields.
- Farmers should be educated and introduced to fungi-resistant seed varieties through Agricultural extension agents.
- The populace should be better educated on the profound health implications of the consumption of grain legumes with fungal infection which could range from food poisoning or intoxication to consumption of such legumes with inconsequential protein yield, through the various mass media platforms.

REFERENCES

Alexopolous, C. J., Charles W. & Blackwell, M. (2004). Introductory Mycology, (4th Ed.) John Wiley and Sons, Hoboken, 67-126.

Barnett, H. L. & Hunter, B. B. (1999). Imperfecti Fungi. 4th ed. London: macmillian publisher; Pp.126-130.

Cheesbrough, M. (2000). Microbiology tests. District laboratory practices in tropical countries. Volume 2. Cambridge University Press; Pp. 35-70.

Harris, R. J. (2003). Epidemiological determinants of spread of causal agents of diarrhoeal disease. *Lancet*. 361:1761-1765.

ICMSF (International Commission on Microbiological Specifications for Foods). 2002 Microorganisms in Foods. Microbiological testing in food safety management New York USA, Kluwer Academic / Plenum Publishers.

Kingsley, G. (2009). The Determination of Serum Total Protein, Albumin, and Globulin by the Biuret Reaction. *J. Biol. Chem.*, 131, 197-200.

Okigbo, R.N., Ramesh, P. & Achusi, C.T. (2009). Post harvest deterioration of tomato and its control using essential oil. New York. *Journal of Chemistry* 6 (4), pp 1274-1280.

Ramirez, D. B. and Vohra, P. (2014). Nutritional improvement of tannin containing sorghums (*Sorghum bicolor*) by sodium bicarbonate. *Cereal Chem.* 67: 533-537.

Robinson, H, & Hogden, C. (1999). The Biuret Reaction in the Determination of Serum Proteins. I. A Study of the Conditions Necessary for the Production of a Stable Color which bears a Quantitative Relationship to the Protein. *J. Biol. Chem.*,135. 707-725.

Staats, M., Van-Baarlen, P., Schouten, A., Van-Kan, A. L. & Bakker, F. T. (2007). Positive selection in phytotoxic protein-encoding genes of Botrytis species. *Fungal Genetics and Biology*. 44: 52–63.

Weichselbaum, T.E. (2012). An accurate and rapid method for the determination of proteins in small amounts of blood, serum and plasma. *Am, J. Clin. Path., Tech. Suppl.,* 10, 40-49.

Young, N. P., Turner, G. & Bennett, J. W. (2005). "Fungal secondary metabolism - from biochemistry to genomics". *Nature Reviews. Microbiology*. 3 (12): 937–47.

Yu, H. & Sutton, J. C. (1997). Morphological development and interactions of *Gliocladium roseum* and *Botrytis cinerea* in raspberry. *Canadian Journal of Plant Pathology*. 19 (3), 237–246.