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Isolation and Identification of Seed Borne Fungi of Common Bean (*Phaseolus vulgaris L.*) from Selected Markets in Makurdi

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Abstract: Isolation and identification of seed borne fungi of common bean (*Phaseolus vulgaris*) from selected markets in Makurdi was conducted. A total of four fungi namely *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum and Botryodiplodia theobromae* were isolated from the bean samples. Percentage germination /viability of the samples ranged from 33-53%. There was no significant difference at P=0.05 in the viability of seeds from North bank and Wadata markets while seeds from Wurukum and High level markets showed significant difference ($P < 0.05$) in viability. Percentage of seeds infected with fungi ranged from 33-67% in all sample locations. There was no significant difference ($P > 0.05$) in the percentage number of seeds infected with fungi. The percentage of fungi isolated from samples ranged from 50-100% in all sampled sites. Analysis of Variance revealed no significant difference ($P > 0.05$) in the percentage of fungi on the bean samples. Frequency of occurrence of each fungi isolated ranged from 8.35-47.7%. There was no significant difference ($P > 0.05$) in the frequency of occurrence of fungi isolated from the samples. Isolation of fungal pathogens from the bean seeds indicate that they should be treated before sowing to obtain good germination and healthy crop.

Keywords: Isolation, Identification, Seed Borne Fungi, Common Bean, Makurdi

1. Introduction

Home saved bean (*Phaseolus vulgaris L.)* also known as common bean is the most important legume crop for human consumption, which provides cheap source of dietary protein for humans worldwide [1]. It is grown in over 12 million hectares and feeds more than 500 million people in Africa and Latin America alone [2].

It is commonly consumed for its delicacy, high protein content and as a source of certain antioxidants, minerals and polyphenols [3]. In addition, common beans are an excellent source of starch, dietary fibre, vitamins and minerals. The nutritional attributes of common bean makes it a potential crop for improving the nutritional security of poor communities.

The seeds of legumes are second mainly to cereals as the important source of food for humans and animals [4]. However, there are several factors which are responsible for their low production. Among them, diseases play an important role [5]. Seed-borne diseases have been found to affect the growth and productivity of the legume [6]. Diseases cause 80-100% yield loss of common bean on farms. Of all transmittable seed-borne diseases of common bean, fungi cause the most damage which includes seed rot, seed discoloration, shrinking seeds amongst others.

The present study was undertaken to find out the seed borne fungi of common bean sold in selected markets in Makurdi since the presence or absence of fungi on the seed surface is one of the most important aspects that determine seed quality.

2. Materials and Methods

2.1. Collection of Samples

Common bean seed samples were collected in polythene envelopes from four major markets namely; Wurukum, Wadata, High level and North bank in Makurdi and taken to the botany laboratory of the Benue State University for isolation of fungal pathogens.

2.2. Isolation of Seed Borne Fungi

Four replicates of 100 seeds per treatment were surfaced sterilized in 1% sodium hypo chloride for 1 minute and then rinsed in several changes of sterile distilled water. Petri dishes were lined with two layers of filter papers that were soaked in distilled water. Five of such Petri dishes were plated with 20 seeds each of common bean to represent the replicates of a treatment and these were arranged in completely randomized design. The Petri dishes were incubated for 7 days at 20-22°C. Filter papers in the Petri dishes were rehydrated at 24 hrs interval. After 7 days of incubation, fungi which grew out of the seeds were isolated on Potato Dextrose Agar (PDA) which was prepared according to manufacturers' instruction.

2.3. Identification of Fungi

Two techniques; visual observation in Petri dishes and microscopic observation were used for identification of fungi. For visual observation, growth and colony appearance were examined every day. For microscopic identification, morphology of the fungal isolates was noted using a microscope after which identification was done using recommendations given by [7] and [8].

2.4. Determination of Seeds Germination/Viability

Germ inability of the seeds was determined by visual observation. Seed germination was assessed by counting the seeds with seed leaf and dividing by the total number of seeds in the Petri plates expressed as a percentage. Thus;

$$\text{Percentage germination} = \frac{Number\ of\ seeds\ with\ seed\ leaf}{Total\ number\ of\ seeds\ per\ plate} \times 100$$

2.5. Determination of Seeds Infected with Fungi

Seeds with fungal infection were determined by counting the infected seeds and dividing by the total number of seeds expressed as a percentage. Thus;

$$\text{Number of seeds infected} = \frac{Number\ of\ seeds\ infected\ with\ fungi}{Total\ number\ of\ seeds\ per\ plate} \times 100$$

2.6. Percentage Occurrence of Fungi

Occurrence of fungi was determined by counting the number of times each individual fungus occurred divided by the total number of fungi and expressed as a percentage. Thus;

$$\text{Percentage occurrence of fungi} = \frac{Number\ of\ times\ each\ fungi\ occurred}{Total\ number\ of\ fungi\ per\ plate} \times 100$$

2.7. Data Analysis

Data obtained from this study was analyzed using Analysis of Variance (ANOVA) and the Fishers Least Significant Difference was used to separate the means at 5% level of significance.

3. Results

3.1. Fungi Isolated from Samples

A total of four fungi were isolated from the bean samples. They are *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Botryodiplodia theobromae* as shown in table 1.

Table 1. *Characterization and Identification of fungal isolates from bean samples.*

Micro characteristics	Macro characteristics	Probable organism
Conidia are radiate, Phailides are present. Conidiophores are smooth walled and brownish.	Colony is dark brown in colour and fast growing.	*Aspergillus niger*
Conidia heads are spherical, splitting into poorly defined columns. Conidiophores are hyaline and heavy walled.	Colony is usually spreading and colour is light green to deep green.	*Aspergillus flavus*
Abundant micro conidia are formed, usually hyaline, one celled but occasionally two celled, oval - club shaped.	Colony grows rapidly with white aerial mycelium often becoming tan to orange	*Fusarium oxysporum*
Pycnidium is brownish and has a septum	Colony colour is white then become greyish black	*Botryodiplodia theobromae*

3.2. Determination of Seed Germination/Viability

Table 2. *Percentage germination / viability of seeds.*

Replicates/Location	North bank	Wurukum	High level	Wadata
R_1	40	53	40	33
R_2	33	40	47	33
R_3	40	47	53	33
R_4	40	47	53	40

Key: R_1-R_4– Replicate 1 - 4

Table 3. *Analysis of Variance in the germination / viability of seeds.*

Location	Germination of seeds/Plate
N/Bank	38.25 ± 1.75^{a}
Wurukum	46.75 ± 5.31^{bd}
H/Level	48.25 ± 3.09^{cd}
Wadata	34.75 ± 3.50^{a}
LSD (0.05)	**7.35**

Means tagged with different alphabets are significant at P=0.05, otherwise, they are the same.

The percentage of seed germination of the bean samples ranged from 33-53% in all the locations sampled as shown in

table 2. There was no significant difference in the viability of seeds from North bank and Wadata while seeds from Wurukum and High level showed significant difference in viability as shown in table 3.

3.3. Determination of Bean Seeds Infected with Fungi

Percentage of seed infection determined by visual observation and fungal isolation ranged from 33-67% in all the sample locations as shown in table 4. There was no significant difference in the percentage number of seeds showing fungi in all locations as shown in table 5.

Table 4. *Percentage number of seeds infected with fungi.*

Replicates/Location	North bank	Wurukum	High level	Wadata
R_1	33	40	53	60
R_2	47	53	40	47
R_3	53	40	40	67
R_4	60	40	40	53

Key: R_1-R_4– Replicate 1 - 4

Table 5. *Analysis of Variance in the percentage of seeds infected with fungi.*

Location	Fungi on seed/Plate
N/Bank	48.25±5.73[a]
Wurukum	43.25±3.25[a]
H/Level	43.25±3.25[a]
Wadata	56.75±4.32[a]
P=0.137 (P>0.05)	NS

Means followed by same alphabets are the same at P=0.05
Key: NS -No significant difference

3.4. Percentage of Fungi on Bean Samples

The percentage of fungi on samples ranged from 50-100% in all sampled sites as shown in table 6. Analysis of Variance showed that there was no significant difference in the percentage of fungi on the samples as shown in table 7.

Table 6. *Percentage of fungi on Bean samples.*

Replicates/Location	North bank	Wurukum	High level	Wadata
R_1	75	50	50	75
R_2	50	75	50	50
R_3	75	75	75	100
R_4	75	50	50	75

Key: R_1–R_4.Replicate 1 - 4

Table 7. *Analysis of Variance in the percentage fungi on bean samples.*

Location	Percentage of fungi isolated
N/Bank	68.75±6.25[a]
Wurukum	62.50±7.21[a]
H/Level	56.25±6.25[a]
Wadata	75.00±10.20[a]
P=0.383 (P>0.05)	NS

Means having same alphabets are the same at P=0.05
Key: NS - No significant difference

3.5. Frequency of Occurrence of Fungi

Frequency of occurrence of fungi ranged from 8.35-41.7% as shown in figure 1. There was no significant difference in the frequency of occurrence of fungi isolated from the various sampling locations as shown in table 8.

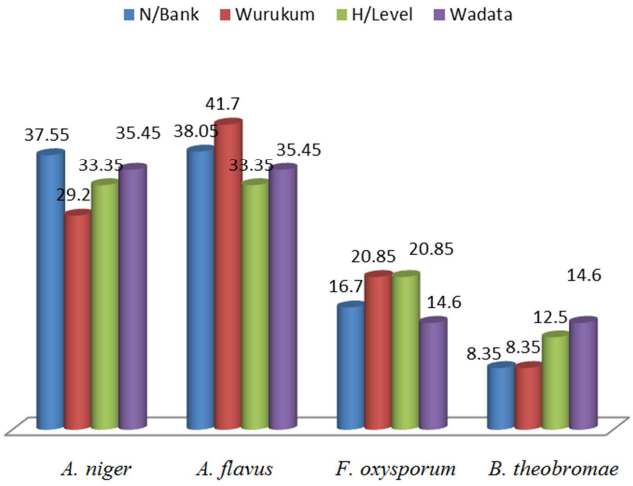

Figure 1. *Frequency of occurrence of fungi isolated.*

Table 8. *Analysis of Variance in the frequency of occurrence of fungi.*

Location / Fungi	*A. niger*	*A. flavus*	*F. oxysporum*	*B. theobromae*
N/Bank	37.58± 4.15[a]	38.05± 4.01[a]	16.70± 9.64[a]	8.35± 0.83[a]
Wurukum	29.20± 10.49[a]	41.70± 4.79[a]	20.85± 12.50[a]	8.35± 0.84[a]
H/Level	33.35± 11.78[a]	33.25± 11.78[a]	20.85± 12.50[a]	12.50± 0.13[a]
Wadata	35.45± 5.23[a]	35.45± 5.23[a]	14.60± 8.60[a]	14.60± 0.86[a]
(P>0.05)	NS	NS	NS	NS

Means followed by same alphabets are the same at P=0.05
Key: NS - No significant difference

4. Discussion

In this study, *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Botryodiplodia theobromae* were isolated from the common bean samples. This is similar to that of [9], who reported same fungi on some Legumes. Seed borne pathogens reduce crop yield in the range of 20-92% if infected seeds are grown in the field.

The viability of the seed samples ranged from 33-53% which indicates low seed germination. This may be due to the seed borne pathogen present externally, internally, or associated with the seed as a contaminant which causes reduction, elimination of germination, seedling damage, seed rot and seed necrosis [10].

Seeds infected with fungi ranged from 33-67%. This indicates that the bean seeds were strongly infected with different fungi located at the surface and internal of seeds especially the cotyledon. This is in agreement with [11] who reported several fungi from the testa, tegmen and embryo of sponge gourd seeds.

The percentage of fungi from samples ranged from 50-100% in all the sample sites and frequency of occurrence of fungi ranged from 8.35-41.7% for all fungal isolates. This indicates that the occurrence of fungi on the bean seeds differed greatly. Maximum fungal association was *Aspergillus flavus* and the least was *Botryodiplodia theobromae*. Many important diseases

of plants caused by fungi are reported to be seed borne [12]. Analysis of seed infection level is a valid investigation tool to foresee the disease development transmitted by seeds because a healthy seed is a foundation for a healthy plant and a necessary condition for good yield.

5. Conclusion

Among various factors which affect seed health, the most important are seed borne fungi which cause reduction in seed germination. Isolation of these fungal pathogens indicates that seeds should be treated before sowing to obtain good germination and healthy crop.

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