

**LEVELS OF DAMAGE, ASSOCIATED ORGANISMS AND TRANSMISSION OF SEED-BORNE FUNGI OF GROUNDNUT SEEDS GROWN IN SOUTHEASTERN NIGERIA.**

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**ABSTRACT**

Groundnut samples collected from commercial markets in Southeastern Nigeria were examined for seed-borne fungi organisms and their level of losses were quantified. Laboratory experiments were conducted to determine the occurrence of fungi organisms on the seeds. Identification of fungi was generally by blotter method. Results obtained showed that only about 29% of groundnut seeds were without damage. This high rate of damage is a factor in low viability of groundnut seeds in Southeastern Nigeria. Major fungal species encountered in the study were *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* Spp, *Rhizoctonia* Spp and *Alternaria* Spp. In the five states, *Aspergillus flavus* was the most predominant fungus isolated from the groundnut seeds, while *Alternaria* Spp was the least frequently isolated organism. On the transmission test, two pathogens namely *Alternaria* spp and *Fusarium* spp were responsible for transmission from seed to germinating seed and seedling.

**Keywords:** Groundnut, seed-borne organisms, losses transmission.

**INTRODUCTION**

Groundnut (*Arachis hypogaea* L.) also known as peanut, is one of the most important tropical annual grain legumes grown all over the world (Norden et al, 1982). It is native to South America, and is cultivated in areas of the world between 40°N and 40°S (Salunke and Desai, 1986; Oluma and Nwankiti, 2007). It is produced mainly in Asia, Africa North and Central America. Nigeria, an important groundnut producing country supplies 3% of the total world output (Ekefan and Adie, 2003).

It is an important cash and food crop in many parts of the tropic (Oluma and Nwankiti, 2007). They are second largest source of vegetable oil, the largest being soybean (Kochhar, 1981). Nutritionally, groundnuts contain about 26% protein, 2.7% minerals, 43% oil and 24% carbohydrates. It is rich in vitamin E, calcium, phosphorus and iron (Young and Ahmed, 1982). It is usually eaten directly or processed into products

like cheese, butter, creams, curds and yoghurt. The groundnut seed also serves as planting material for the crop (Ekefan and Adie, 2003).

For effective yield, viable seeds are among the most important things in agronomic practices. Global losses in food production due to seed borne diseases are important negative factors in world agriculture; hence seeds should be examined on regular basis. Seeds have long been appreciated as the most important biological input to sustainable agricultural production and food security. It has been proved seed transmission is responsible for the perpetuation of plant diseases leading to drastic yield reduction (Oluma and Nwankiti, 2007). Mohammed and Mohammed, (2001), reported the transmission of *Cephalosporium gregata* in soybean. In Southeastern Nigeria, groundnut seeds for planting is usually obtained from open markets, farmers own reserve, seed companies or agricultural development programs.

Moreover, every year, seed-borne fungi cause a heavy yield loss on the groundnut with some fungi acting as a primary source of infection. Pathogens associated with seed deterioration in groundnut may differ with location and cultivars. Hence, there is the need to routinely assess the seed health within a region and to determine the transmission of the seed-borne fungal organisms from seed to seedling. This work was conducted to;

Identify seed borne organisms associated with groundnut seeds grown in South-eastern Nigeria, quantify the level of damage to groundnut seeds by these organisms and to evaluate the transmission of these seed borne organisms from seeds to seedlings.

**MATERIALS AND METHODS**

**The Study Area:** The study was conducted in Southeastern Nigeria. Southeastern Nigeria comprises five states – Abia State, Enugu State, Imo State, Anambra State and Ebonyi State (latitude 4°45' and 5°50'N and longitude 6°40' and 8°15'E). soil here are dominated by ultisols (Onweremadu, 2006).

The study area has humid tropical climate with total annual rainfall ranging from 1800-2500mm with a total annual temperature range of 26-30°C. It has a rain forest vegetation, comprising many forms of plants arranged in tiers. Farming, fishing, hunting, gathering from wild and cottage industries are major socio-economic activities in these areas.

Two groundnut seed cultivars –Ngwongworo (Samnut 21) and Nwaerente (Spanish 205) were used for this experiment. The experiments were conducted in Crop Science and Technology Laboratory, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria.

### Physical Examination of Seeds

Four hundred groundnut seeds from each of the two cultivars of groundnut were examined physically and categorized into various damaged categories in accordance with International Seed Testing Association Specification (ISTA, 1976). Each cultivar constituted a treatment and each was replicated 10 times (40 seeds per replicate) and the experiment was repeated three times. The cumulative mean of damaged seed categories were subjected to Analysis of variance (ANOVA) and mean were separated using Least Significant Difference (LSD).

### Examination of seed after incubation

This was to determine the occurrence of fungal organisms on groundnut seeds collected from five South-Eastern states of Nigeria. The states constituted the treatments and the experiment was arranged in completely randomized design and replicated 10 times. Each treatment, 400 seeds were plated on moist filter paper (10 seeds per plate) using the blotter method, after they had been surface-sterilized for 1 minute in 0.05% sodium hypochlorite and rinsed with sterile distilled water. The plates were incubated for 7 days at ambient conditions of light and temperature.

The seeds were then examined under dissecting microscope for fungal growth. Each fungal organism observed was purified by repeated sub-culturing using growth medium that permitted good growth-Potato-Dextrose, Agar (PDA). Slides were examined under stereomicroscope for fungal identification using recommended keys and texts (Barnett and Barry, 1987) and percentage occurrence of each fungus identified was determined.

### Transmission of certain pathogenic fungi from seed to seedling

The test tube seedling symptom test developed by Basak and Lee (2002) was used for this study. Test tube slants prepared by pouring 6ml of 2 % water Agar and sterilized in autoclave for 10 minutes and 15 lb pressures at 121°C. The groundnut sample having highest percentage of seed infection was employed in this experiment. Fifty seeds were used at the rates of one seed per test tubes. Then, the test tubes with the seeds were incubated on the laboratory desk at room temperature. The mouths of the test tubes were properly plugged with cotton and test tubes were placed on the wooden test incubation.

The germinated seeds and seedlings in the test tube were examined for the presence of visible symptoms (seed rot, germination failure, infection or death of emerged seedlings caused by the pathogens present in the seed).

The symptoms produced on the germinating seeds and seedlings by the associated pathogens were confirmed by examining the seeds under stereomicroscope.

The seeds and seedlings were examined under dissecting microscope for fungal growth. Each fungal organism observed, was purified by repeated sub-culturing using Potato-Dextrose Agar (PDA) media and incubated for 7 days. Slides were examined under stereomicroscope for fungal identification using recommended keys and texts (Barnett and Barry, 1987).

## RESULTS AND DISCUSSION

### Physical Examination of Seeds

The result of the physical examination of seed without incubation was shown in Table 1. Three various damaged categories of seeds identified were cracked/broken seeds, shriveled/reduced and discoloured seeds (Plate 1 ). Analysis of variance showed that there was significant difference in the frequency of occurrence of the various damaged categories in the two groundnut cultivars examined.

There were significant differences in frequency of occurrence of broken and discoloured seeds and there were significant difference in the frequency of occurrence of shriveled/reduced seeds and the discoloured seeds. Between the two cultivars examined, shriveled seeds had the highest cumulative frequency of occurrence (10.50) while broken/cracked seeds had the lowest cumulative frequency score of about 8.35. Between the two cultivars, Spanish 205 (Nwaerente) had the highest percentage of seed damage 74.50 %. It also recorded the highest frequency of cracked, shriveled and discoloured seeds (24.00 %, 31.00 % and 95.50 %, respectively).

Plate 1: Damaged categories of groundnut seeds.

Scale: X0.01

Values presented here are mean percentages of 10 replicates

**Table 1: Cultivar differences on damaged categories of groundnut seed**

Cultivars	Damaged categories			
	Cracked/broken	Shriveled/Reduced	Discoloured	Good quality seed
Nwaerente	24.00	31.00	19.50	25.00
Ngwongoworo	17.50	21.25	27.50	33.00
Mean	20.88	26.25	23.50	29.00
LSD <sub>0.05</sub>	1.60	2.49	1.70	2.1



#### Discoloured seeds

##### Examination of seed after Incubation

Table 2 showed the percentage occurrence of fungal organisms identified on the groundnuts (*Arachis hypogea*) seeds gotten from the five Southeastern states of Nigeria. The various organisms that were consistently isolated were as follows; *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* spp, *Rhizotonia* spp and *Altermaria* spp.

Percentage of occurrence of *Aspergillus Flavus*, was significantly ( $p < 0.05$ ) highest for Anambra and Abia states than for Imo, Enugu and Ebonyi States. Percentage of occurrence of



#### Shriveled seeds

*Aspergillus niger* was significantly ( $P < 0.05$ ) highest for Imo State and Anambra State than for Abia, Enugu and Ebonyi states. Percentage of occurrence of *Fusarium* spp was significantly ( $P < 0.05$ ) higher for Ebonyi, compared with Anambra State, and there were also significant differences ( $P < 0.05$ ) among in the percentage of occurrence of *Rhizotonia* spp in Ebonyi and Abia States. There was significant difference ( $P < 0.05$ ) among the states in the occurrence of *Alternaria* spp.

**Table 2: Percentage occurrence of fungal contaminants recorded in seed lots of groundnut collected from five states in Southeastern Nigeria.**

State	<i>Fusarium</i> spp	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Rhizoctoria</i> spp.	<i>Alternaria</i> spp.
Imo	16.4	24.0	36.0	13.0	10.0
Enugu	22.0	12.0	43.0	18.0	8.0
Anambra	3.0	20.0	61.0	7.5	12.0

Abia	9.5	9.7	54.0	16.0	5.7
Ebonyi	28.0	6.0	38.0	23.0	8.3
LSD (p=0.05)	1.0	1.1	2.9	1.0	1.2

**Fungus transmission from seed to seedling**

Results of transmission of two seed borne fungi of groundnut seed to germinating seeds and seedlings (as determined by test tube seedling symptom test ) are presented in Table 3 (Plate 2). In all, two kernel rot pathogens namely *Alternaria* spp and *Fusarium* spp, found to transmit from seed to the germinating seeds causing rot were higher than that of transmission in seedling infection or seedling mortality.

*Alternaria* spp first produced brownish black fluffy on the seeds and around the base of seedlings. The fungus infected the cotyledons, as a result, the cotyledonary leaves could not open.

Some infected seedlings collapsed and finally died (Plate 2). On the total seed borne infection of 10 % caused by *Alternaria* spp, 4.0 % was seedling infection, 2.0 % was death of emerged seedlings .On the other hand, 4.0 % seeds could not germinate at all. In field, this pathogen produces *Alternaria* blight disease.

The *Fusarium* spp produced whitish fluffy colony on the seeds and around the base of seedlings .In total seed borne infection of 74.00 %, on an average, 28 % of seed could not germinate, 18 % of seedling infection and 28 % post emergence death (Table 3).

**Table 3: Transmission of seedborne fungi of groundnut from seeds to germinating seeds and seedlings as determined by test tube seedling symptom test**

Seedborne Fungi	Percentage of seedborne fungi (%)				
	Seed rot	Pre-emergence death	Post-emergence death	Total disease development	
<i>Alternaria</i> spp.	4.0	4.0	2.0	10.0	
<i>Fusarium</i> spp	28.0	18.0	28.0	74.0	

**Plate 2: Transmission of seed-borne fungi from seeds to seedling as determined by the test tube seedling symptom test**

Scale: X0.01



## DISCUSSION

The result of the physical examination of seeds without incubation revealed that only about 29 % of groundnut seeds sold in our markets in South Eastern Nigeria were of good quality. The remaining 71 % of the seeds were damaged and therefore of low quality. Ekefan and Adie, (2003), reported that seed of high quality is recognized as an important factor for optimum agricultural production. This level of damage on our groundnut seeds is alarming and unacceptable when compared with international standards as reported by Okoro and Nwankiti (2003). It was reported by Smith, (1966), that the official grain standard of the United States for groundnut has only three grades. The lowest which is grade 3 has percentage of damaged kernels of 45 %, anything above that is unacceptable. The high percentage of damage to our groundnut seeds is dangerous to both man and animals and to the growers/producers. The damages are mostly caused by microorganisms, as reported by Shetty, (1988). Some of these micro organisms produce mycotoxins that can cause health problems to man and animals consuming them (Yousef and Naresh, 2010). These organisms also cause some biochemical reactions on the seeds which may change their taste and the economic value of the seeds are also affected negatively (Okoro and Nwankiti, 2003). This calls for an immediate action by all institutions and researchers involved in groundnut research to reverse the trend. The Nigerian government should urgently set up an official grain standard for the nation, not only for groundnut but for other grains grown in Nigeria as the Nigerian National seed Decree NO. 72 of 1992 was silent on the regulation of the quality of imported or unnotified seeds being traded in the market (Usman, 1996). The high rate of seed damage is also responsible for low viability of groundnut seeds in South-Eastern Nigeria. Okoro and Nwankiti (2003), stated that any seed with germination percent below 70 % and a high incidence of seed borne organism is regarded as having low seed quality. Low quality seeds, if not treated properly, can cause groundnut disease epiphytotic in the nearest future, since most illiterate farmers use uncertified seeds for planting. Results from the study showed that Ngwonworo cultivar of groundnut had higher seed quality. (Table 2) This cultivar should be improved upon.

The fungi isolated in this work have been associated with groundnut seeds in Southeastern Nigeria, other states in Nigeria and other parts of the world. In this investigation several fungi including *Aspergillus flavus*, *Aspergillus niger*, *Rhizoctonia* spp and *Alternaria* spp and *Fusarium* spp were identified on groundnut seeds (Table 3). The presence of these organisms indicates that groundnut seed is vulnerable to many infections,

which reduce the seed quality. In a study in Egypt, Mohammed and Mohammed (2001), reported the occurrence of all the organisms found in this investigation on groundnut seed. They also found other organisms such as *Fusarium oxysporium*, *Fusarium solani* and *Sclerotium rolfsii*, which were not detected in this study. In a study in Imo state, Ihejirika et al (2005), reported the occurrence of all the organisms found in this investigation on groundnut seed except *Aspergillus niger* and *Alternaria* spp which were not detected. This is also similar to the report of Oluma and Nwankiti (2003), in their investigation of seed-storage mycoflora of peanut cultivars grown in Nigerian savannah, they found other organisms such as *Rhizopus stolonifer*, *Penicillium* spp, *Trichoderma* spp which were also not detected in this investigation. These indicate that the distribution of fungal organisms on groundnut seeds may differ from one location to another. The disparity in the distribution of fungal organisms among seeds from different states may be attributable to differences in humidity of the states. For example, Onitsha main market in Anambra State which was one of the locations from where the seeds were collected from, is located by the riverside and might likely have higher humidity. Hence, the groundnut seeds had higher percentage of *Aspergillus flavus*. Ntare et al. (2008) reported that several species of *Penicillium* and *Aspergillus* invaded seed that were stored at moisture conditions above 14 %.

The consistent isolation and presence of *Aspergillus* spp. from groundnut seeds from all the locations confirmed the earlier report of Ihejirika et al, (2005), Oluma and Nwankiti, (2003), Ekefan and Adie, (2003) Mohammed and Mohammed, (2001), that *Aspergillus* spp were the prominent fungi isolated from stored groundnut seeds.

One of the objectives of this study was to determine the ability of some important seed-borne fungi of groundnut to be transmitted from seed to seedling. The fungi which have been detected during test tube seedling symptom test that were transmitted from seed to germinating seeds and seedling were *Fusarium* spp and *Alternaria* spp. They caused pre- and post emergence rot resulting in root rot and damping off of seedlings. Similar observations were made by Basak and Lee, (2002), who reported that *Alternaria* spp and *Fusarium* spp were found responsible for transmission from seed to germinating seeds and seedlings in maize plant. Mohammed and Mohammed, (2001), similarly reported that *Fusarium* spp was transmitted from seed to germinating seeds and seedlings and other parts of matured groundnut plant. They further reported the transmission of *Fusarium moniliforme* and *Fusarium oxysporium* in *Capsicum* spp and *Fusarium solani* in coriander

plant from seed to matured plant. These fungi under favourable environmental conditions can infect the growing plants and can also serve as a source of inoculum for field crop.

#### Conclusion and Recommendation

The various damages categories of seeds identified were cracked/broken seeds, shriveled seed and discoloured Seeds. The organisms identified in this study were *Aspergillus flavus*, *Aspergillus niger*, *Rhizoctoria* spp, *Fusarium* spp and *Alternaria* spp. On the transmission test, *Fusarium* spp and *Alternaria* spp were found to be transmitted from seed to germinating seeds and seedling causing seed rot and seedling infection.

The Nigerian Government should set up an official grain standard for the nation, not only for groundnut, but for other grains grown in Nigeria, as the Nigerian National seed decree, No. 72 of 1992, was silent on the regulation of imported and unnotified seeds sold in our markets.

Seeds for planting should be obtained from certified seed sources as much as possible. Seeds that have been stored for more than one year should not be used during planting. Infected groundnut seeds should not be preserved for planting.

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