Science World Journal Vol 12 (No 1) 2017
www.scienceworldjournal.org
ISSN 1597-6343

GERMINATION OF SEVERAL GROUNDNUT CULTIVARS IN RELATION TO INCIDENCE OF FUNGI

Ahmed O.[1] Olayinka B.U.,[2] Garuba, T.,[2] Ahmed J.[2] and Etejere, E.O[2]

[1]Crop protection Department, Faculty of Agriculture, University of Ilorin, Nigeria
[2]Plant Biology Department, Faculty of Life Sciences, University of Ilorin, Nigeria

Corresponding author's email: ahmelad2007@yahoo.com

ABSTRACT
This experiment is concerned with the germination of nine cultivars of groundnut grown in Nigeria in relation to incidence of fungi. The cultivars were NHK 5V8, NUTII 288, Samnut 10, 11, 21, 22, 23, 24 and MK 373. Germination potential was assessed after 10 days of planting in petri-dishes. Parameters such as seedling vigour and electrical conductivity were assessed. Potato dextrose agar (PDA) was used to isolate the fungi grown in each seed types and the fungi were identified. Four species of fungi: *Aspergillus niger*, *A. flavus, Fusarium* sp and *Penicillium* sp were isolated in all the nine cultivars at varying degree except *Aspergillus niger* that was absent in Samnut 24. *Fusarium* sp. was preponderant in NUTII288, Samnut 22, 23 and 24. Whereas *A. flavus* was highest in NHK5V8, MK 373 and Samnut 21. The species such as *A. niger* and *Penicillium* sp were respectively high in occurrence in Samnut 10 and 11. Samnut 23 showed highest percentage germination followed by Samnut 24 and 21 (100-90%). All other cultivars had percentage germination between 70-80%. The results of speed, ability and seedling vigour followed similar trend as recorded for percentage germination. Seeds with higher vigour showed lower conductivity test as compared to those with low vigour. Generally, cultivars with high germination potential showed low incidence of fungal attack.

Keywords: Germination, Seedling Vigour, Conductivity, *Aspergilus niger.* Groundnut

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the most popular and universal crops cultivated in more than 100 countries in six continents and it ranked as the 4th most important oilseed crops of the world (Nwokolo and Smartt, 1996). In Nigeria, it has good potential as a legume crop due to its ability to add nitrogen to the soil, low cost production and multiple uses. There are several factors responsible for low yield of this crop in which diseases play a prominent role.

Groundnut production is threatened by the presence of various pest among the pathogens are seed borne viruses, bacteria, nematodes and most especially seed borne fungi. Most of these pathogens have been known to affect viability of the seeds as well as seed quality (Pitt *et al*., 1991). The quality of groundnut seeds has been found to be compromised or lost gradually through contamination especially by those of the genus of *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus* (Pitt *et al*., 1991; Fernandez *et al*., 1997; Almeida *et al*., 1998). Among fungi species, the species of *Aspergillus* have frequently been reported to cause greater quantitative and qualitative losses, and produce highly toxic and carcinogenic chemical substances known as aflatoxins (Chavan and Kakde, 2008).

Aside *Aspergillus,* groundnut is attacked by a number of other pathogenic fungi of economic importance like *Alternaria dianthicola, Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum.* This pathogen causes discoloration, rotting, shrinking, seed necrosis, and loss in germination capacity and toxicity to the oilseeds (Elwakil and El-Metwally, 2001; Chavan and Kakde, 2008).

The frequency of occurrence of the foregoing seed borne fungi pathogens and their effect on the germination of several cultivars of groundnut grown in Nigeria appear scanty in literature. With this consideration in mind, this present research was performed to fill this gap.

MATERIALS AND METHODS

Test Materials

Nine groundnut cultivars were obtained from the Institute of Agricultural Research (IAR) Zaria and College of Agriculture Mokwa, Niger State. The cultivars were NHK 5V8, NUTII 288, Samnut 10, 11, 21, 22, 23, 24 and MK 373. Pods of each cultivar were manually shelled to obtain the seeds. The seeds were cleaned by removal of foreign particles before they were subjected to germination studies and isolation of seed borne fungi after one month in dry storage.

Preparation of Growth Medium

The method described by Adamu *et al.* (2009) was adopted for preparation of the growth medium. Potato Dextrose Agar (PDA) was used as culture medium. For the preparation, 19.5 g of PDA was weighed and transferred aseptically using a sterile piece of aluminum foil into a sterile 500 ml conical flask. It was then made up to the 500 ml graduation mark with sterile distilled water. The resulting solution was boiled to remove precipitates and shook intermittently. It was then autoclaved at 121^{o}C for 15 minutes and chloramphenicol (30mg/L) was added to inhibit bacterial growth.

Isolation and Identification of Seed Borne Fungi

The seeds of each cultivars were surface sterilized with 70% ethanol using swab method, then briefly rinsed in a sterilized distilled water. These seeds were dried on sterile filter papers under filtered air in a laminar flow. The seeds were aseptically transferred to petri dishes containing solidified amended PDA. The plates were incubated in inverted position and incubated at 25 ± 2^{o}C for 5 days. Sub-culturing was carried out to obtain pure

culture of each fungal isolates. The procedure of Amadi *et al*. (2014) was used to identify the isolates.

Germination Tests

Seeds of each cultivar were surface sterilized with 0.5% sodium hypochlorite solution for 30 seconds and rinsed in several changes of sterile distilled water, using the method of Dezfuli *et al.* (2008). Ten seeds each were then plated on double-layered filter paper contained in 9 cm Petri dishes. The filter papers were moistened with 10 ml of sterile distilled water. The Petri dishes were incubated under 12 hours of light and darkness. Germination counts were estimated by a clear emergence of the radicle made after 10 days. The experiment was a simple randomized design with five replications for each variety. Five seedlings per replicate were randomly selected and their lengths measured using a meter ruler. The average lengths of these selected seedlings were multiplied by percentage germination to record seed vigour index.

The germination index (GI) was calculated as number of seedling emerging in each day divided by the day after planting (G.I = n/d). Ability of seeds to germinate, expressed as 'activation value' was calculated for each variety using the formula:

Activation Value (AV) $=$

$\frac{1}{T50}$ x % germination (Masselink, 1980)

Percentage germination $= \frac{N}{A} \times 100$ (Labouriau, 1983)

Where N = number of germinated seeds and A = total number of seeds tested.

The speed of germination termed as coefficient of velocity was also calculated according to the formula of Kotowski (1926).

Speed of germination = $\frac{A1+A2+ \ldots\ldots\ldots\ldots Ax}{A1T1+A2T2 \ldots\ldots\ldots\ldots AxTx}$ x 100

Conductivity Test

The seeds of different cultivars were sterilized using 0.5% sodium hypochlorite solution. Sterilized seeds about 5 g from each cultivar were immersed in 100 ml of water at room temperature for 10-12 hours. The seeds were removed with sterilized forceps, the steep water left was termed as leachate was poured into conical flask and the conductivity of each leachate was measured using a conductivity meter (Perry, 1984b). The conductivity meter was warmed for 30 minutes before testing, first the conductance of a distilled was measured and recorded, the electrode was then cleaned with tissue paper and the conductance of the leachates was read cleaning the electrode with tissue paper after reading the conductance of each leachate. The electrical conductivity (EC) of the leachate recorded from each cultivar was calculated as shown below:

EC ($\mu S\ cm^{-1}\ g^{-1}$) = EC of each leachate – EC of distilled water

Note that the lower the EC, the higher the seed vigour.

Data Analysis

Data were subjected to analysis of variance using SPSS statistical package version 16, the mean were separated using the least significant difference p = 0.05.

RESULTS

Prevalence of Seed Borne Fungi

Fungi associated with different cultivars of groundnut seeds are presented in Table 1. *Aspergillus niger*, *A. niger, Fusarium* sp. and *Penicillium* sp. were isolated from all the cultivars with varying degree of occurrence except *A. niger* that was absent in Samnut 24. The occurrence of *A. flavus* was highest in NHK5V8, MK 373 and Samnut 21 with percentage incidence of 51.85%, 42.86%and 69.44% respectively. *Penicillium* sp. was predominant only in Samnut 11 (40.74%) as *A. niger* was most frequent in Samnut 10 (40.28%). *Fusarium* sp. was isolated with highest percentage incidence in NUTII 288, Samnut 22, Samnut 23 and Samnut 24 having 33.97%, 34.29%, 34.44%, 53.33% percentage incidence respectively.

Effect of Seed Borne Fungi on Germination Tests

The results of percentage germination, ability and speed of germination as influenced by seed borne fungi are presented in Table 2. Significantly lower percentage germination between 46.67 ± 12.02 and 53.33 ± 14.53 were recorded for NHK 5V8 and MK 373 respectively when

Prevalence of Seed Borne Fungi

Fungi associated with different cultivars of groundnut seeds are presented in Table 1. *Aspergillus niger*, *A. niger, Fusarium* sp. and *Penicillium* sp. were isolated from all the cultivars with varying degree of occurrence except *A. niger* that was absent in Samnut 24. The occurrence of *A. flavus* was highest in NHK5V8, MK 373 and Samnut 21 with percentage incidence of 51.85%, 42.86%and 69.44% respectively. *Penicillium* sp. was predominant only in Samnut 11 (40.74%) as *A. niger* was most frequent in Samnut 10 (40.28%). *Fusarium* sp. was isolated with highest percentage incidence in NUTII 288, Samnut 22, Samnut 23 and Samnut 24 having 33.97%, 34.29%, 34.44%, 53.33% percentage incidence respectively.

Effect of Seed Borne Fungi on Germination Tests

The results of percentage germination, ability and speed of germination as influenced by seed borne fungi are presented in Table 2. Significantly lower percentage germination between 46.67 ± 12.02 and 53.33 ± 14.53 were recorded for NHK 5V8 and MK 373 respectively when

Table 1: Incidence of Fungi Isolates from Different Groundnut Cultivars

Cultivars	*Aspergillus Niger*	*Aspergillus Flavus*	*Penicillium* sp.	*Fusarium* sp.
		%		
NHK 5V8	10.37 ± 2.02	51.85 ± 3.21	18.70 ± 7.03	19.07 ± 7.98
NUTII 288	24.52 ± 4.30	23.65 ± 8.21	17.86 ± 5.57	33.97 ± 5.74
MK 373	34.52 ± 8.52	42.86 ± 10.37	8.93 ± 7.78	13.69 ± 4.32
Samnut10	40.28 ± 8.67	34.72 ± 9.84	5.56 ± 9.62	19.44 ± 4.81
Samnut 11	22.78 ± 7.82	33.15 ± 10.10	40.74 ± 16.04	3.33 ± 0.77
Samnut 21	8.33 ± 3.43	69.44 ± 13.68	11.11 ± 1.25	11.11 ± 1.25
Samnut 22	13.01 ± 2.54	26.19 ± 5.08	26.43 ± 7.92	34.29 ± 3.90
Samnut 23	11.11 ± 19.25	24.44 ± 6.43	30.26 ± 5.46	34.44 ± 5.03
Samnut 24	0 ± 0[c]	23.33 ± 9.17	23.33 ± 5.17	53.30 ± 8.33
Mean	18.33 ± 6.28	36.63 ± 8.45	20.32 ± 7.32	24.74 ± 4.68
SEM	6.81	11.56	9.76	12.83

Values with the same superscript(s) down the column are not significantly different at α=0.05

Table 2. Evaluation of the Percentage Germination, Ability and Speed of Germination of the Different Groundnut Cultivars

Cultivars	Germination (%)	Ability to germinate	Speed of germination
NHK 5V8	46.67 ± 12.02^{c}	33.78 ± 10.05^{b}	40.58 ± 1.18^{b}
NUTII 288	70.00 ± 10.00^{abc}	46.67 ± 6.67^{b}	45.38 ± 2.91^{b}
MK 373	53.33 ± 14.53^{ab}	24.00 ± 4.00^{b}	36.99 ± 2.96^{b}
Samnut 10	83.33 ± 3.33^{ab}	48.44 ± 8.44^{b}	47.45 ± 5.59^{ab}
Samnut 11	70.00 ± 10.00^{abc}	53.33 ± 6.67^{ab}	46.49 ± 7.70^{ab}
Samnut 21	93.33 ± 6.67^{a}	100.40 ± 9.50^{a}	69.49 ± 5.47^{a}
Samnut 22	73.33 ± 16.67^{abc}	83.33 ± 5.44^{ab}	49.61 ± 12.40^{ab}
Samnut 23	100.00 ± 0.00^{a}	77.78 ± 11.11^{ab}	57.59 ± 11.53^{ab}
Samnut 24	90.00 ± 5.77^{a}	100.04 ± 7.81^{ab}	55.67 ± 4.43^{ab}
Mean	75.56	68.22	49.93
P-value	0.01	0.02	0.03
CV (%)	29.90	78.58	27.82

Values with the same subscripts down the column are not significantly different at p< 0.0**5**

Table 3. Evaluation of the Seedling Length, Seed Vigor, Germination Index and Conductivity Test of Different Groundnut Cultivars

Cultivars	Seedling length (mm)	Seed vigor Index	Germination index	Conductivity test (µS cm^{-1} g^{-1})
NUTII 288	2.35 ± 0.73^{e}	166.00 ± 34.32^{e}	2.72 ± 0.23^{b}	9.00 ± 1.53^{bc}
MK 373	2.39 ± 0.36^{e}	126.10 ± 40.94^{e}	2.22 ± 0.49^{b}	12.67 ± 2.33^{bc}
Samnut 10	5.45 ± 0.87^{cd}	449.67 ± 58.92^{d}	4.39 ± 0.71^{ab}	11.33 ± 2.40^{bc}
Samnut 11	7.56 ± 0.80^{bc}	513.80 ± 19.46^{cd}	4.33 ± 0.85^{ab}	19.67 ± 0.88^{a}
Samnut 21	10.71 ± 0.58^{a}	997.33 ± 77.03^{a}	7.28 ± 0.62^{a}	7.00 ± 3.21^{bc}
Samnut 22	7.77 ± 1.03^{bc}	554.67 ±100.33^{cd}	4.66 ± 1.63^{ab}	16.33 ± 2.19^{c}
Samnut 24	9.24 ± 1.31^{ab}	924.00 ±100.31^{ab}	7.36 ± 0.57^{a}	-54.57 ± 3.76^{d}
Samnut23	8.33 ± 0.93^{ab}	738.80 ± 41.41^{bc}	5.94 ± 1.59^{a}	6.34 ± 2.60^{c}
Mean	6.38	500.15	4.55	4.48
P-value	< 0.001	< 0.001	0.003	< 0.001
CV (%)	49.37	66.32	51.54	493.08

Values with the same subscripts down the column are not significantly different at p<0.05

DISCUSSION

Aspergillus niger, *A. flavus, Fusarium* sp. and *Penicillium* sp. were isolated from all cultivars and this agreed with the work of Mohammed and Chala (2014) who reported that *A.niger*, *A. flavus, A. ochraceus, A. parasiticus* and *Penicillium* sp. were associated with groundnut in Ethiopia. *Aspergillus* is a common mould in tropical and subtropical countries and usually associated with stored agricultural produce such as groundnut, cereal and cotton seeds (Janardhan *et al*., 2011). *A. flavus* is known to produce a form of mycotoxins called aflatoxin. *A. flavus* produces aflatoxin B_1 and B_2. Other toxic compounds produced by *A. flavus* are cyclopionic acid, kojic acid, nitropropionic acid, aspenoxin, aflam and aspergillic acid (Guchi, 2015). Aflatoxins are highly toxic metabolites may lead to Reye's syndrome, Kwashiorkor and acute hepatitis (Wild and Turner, 2002).

Groundnut propagates only by seed, and this constitutes a major component of production (Grigoleto *et al*., 2012). Germination tests carried out in this study have shown that the quality of groundnut seeds after harvest could be deteriorated depending on the prevalence of seed borne fungi. Germination was generally low in groundnut cultivars with high incidence of seed borne fungi, most importantly those infested with the species of *Aspergilus, Penicillium* and *Fusarium.* A similar observation has been reported by Mukherjee *et al*. (1992), Chavan (2011) and Oladipupo (2011). Groundnut cultivars such as Samnut 21, 23 and 24 with high seed vigour index were found to record the highest percentage germination, greater ability as well as speed of seed to germinate. Akonda *et al.* (2016) reported that fungi such as *Aspergilus niger* and *Fusarium* spp. are associated with damage of plumule, radicle and hypocotyl of germinating seedling which consequently lead to the reduction of percentage germination and seedling vigour. The higher germination percentage recorded in Samnut 21, 23 and 24 with low incidence of seed borne fungi could also be linked to better integrity of their membrane permeability. These cultivars therefore leached less of water soluble chemicals as compared to those cultivars with low percentage germination. Seeds with high incidence of fungi attack had been found to show higher electrical conductivity values, lower seedling vigour resulting from loss of weakening of cell membrane and faster leakages of water soluble compounds like sugars, amino acids and electrolytes (Colete *et al*., 2004; Panobianco, 2007).

Conclusion

The results of this study have shown that all the nine cultivars of groundnut were infested by the isolated fungi and such infestation had varying effect on the germination potential of the seeds. Groundnut cultivars such as Samnut 24, 23 and 21 and NHK 5V8 could be said to be rugged and resistant against fungal attack when compared to other varieties. Given the findings of this research it is recommended that concerted efforts should be put in place to screen seeds before distribution to the farmers.

REFERENCES

Adamu, S., Bukar, A., and Mukhtar, M. D. (2009). Isolation and identification of postharvest spoilage fungi associated with sweet oranges (*Citrus sinensis*) traded in Kano Metropolis. *Journal of Pure and Applied Sciences*, 2(1):122 – 124.

Akonda, M. R., Yasmin, M. and Hossain, I. (2016). Incidence of seedborne mycoflora and their effects on germination of maize seeds. International Journal of Agronomy and Agricultural Research (IJAAR), 8(1), 87-92.

Almeida, F. A. C., Moraes, J. S., Santos, R. C., Almeida, R.P. and Araújo, E. (1998). Influence of processing, packing and storage environment on the sanitary quality of peanut seeds. *Rev. Oleag. E. Fibr*. 2(2): 97-102.

Amadi, J. E., Nwaokike, P., Olahan, G. S. and Garuba, T. (2014). Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava*) in Awka metropolis. *International Journal of Engineering and Applied Sciences*, 4(10), 7-12.

Chavan, A. M. (2011). Nutritional changes in oilseeds due to *Aspergillus* spp. *J. Exp. Sci.*, 2: 29-31.

Chavan A. M. and Kakde, R. B. (2008). Studies on abnormal oilseeds mycoflora from Marathwada region. *Bionano Frontier,* 2(2):101-104.

Colete, J. C. F., Vieira, R. D. and Dutra, A. S. (2004). Electrical conductivity and soybean seedling emergence. *Scientia Agricola* 61: 386-391.

Dezfuli, P. M., Sharif-zadeh F. and Janmohammadi, M. (2008). Influence of priming techniques on seed germination behavior of maize inbred lines (Zea mays L.*). J. Agric. Biol. Sci*., 3: 22-25.

Elwakil, M. A. and El-Metwally, M. A. (2001). Seed-borne fungi of peanut in Egypt; pathogenicity and transmission. *Pakistan journal of Biological Science*, 4: 63 – 68.

Fernandez, E, Rosolem C. A, Maringoni, A. C. and Oliveira D. M. T (1997). Fungus incidence on peanut grains as affected by drying method and Ca nutrition. *Field Crops Res*. 52(1): 9-15.

Grigoleto M. R. P., Medina, P. F. and Parisi J. J. D. (2012). Levantamento da germinação e de fungos e insetosemsementes de amendoimproduzidas e armazenadas no Estado de SÃO PAULO. In: Cong. Inter. deInic. Cient. Pp.1-8.

Guchi, E. (2015). Aflatoxin Contamination in Groundnut (*Arachishypogaea*L.) Caused by *Aspergillus* Species in Ethiopia. *Journal of Applied & Environmental Microbiology* 3.1: 11-19

Janardhan A. D., Subramanyam, A. Praveen, K., Pradeep, M. and Narasimha, G. (2011). Aflatoxin impacts on germinating seeds. *Manuals of Biological Research*. 2(2):180-188.

Kotowski, F. (1926). Temperature relations to germination of vegetable seeds. *Proc. Amer. Soc. Hort. Sci.*, 23: 176.

Labouriau, L.G. (1983). *Seed germination*. Washington DC, USA: Organization of American States.

Masselink, A. K. (1980). Germination and seed population dynamics in Melampyrum pretense L. *Acta Botanica*, 29: 451 – 468.

Mohammed, A. and Chala, A. (2014). Incidence of *Aspergillus* contamination of groundnut *(Arachishypogaea*L.) in Eastern Ethiopia. *African Journal of Microbiology*, 8(8): 759-765.

Mukherjee, P. S., Nandi, S.K. and Nandi, B. (1992). Deteriorative changes in groundnut seeds in storage. *Journal of Mycopathological Research*, 30(2): 113-119

Nwokolo, A. and Smartt, J. (1996). Response of Groundnut to the distribution of rainfall or irrigation. Annual Report No. 13: University of Nottingham, Nottingham, U.K. pp. 49-63.

Oladipupo, O. A. (2011). Effect of seed-Borne fungi on percentage germination and health of Groundnut seedlings. Department of crop Protection, College of Plant Science and Crop Production, M. Sc studies *in Nigam SN (ed.) International* Arachis Newsletter, No. 21. Pp 55–57

Panobianco, M. Vieira, R. D. and Perecin, D. (2007). Electrical conductivity as an indicator of pea seed aging of stored at different temperatures. *Scientia Agricola,* 64: 119-124.

Pitt, J. I., Dyer, S. K and McCammon, S. (1991). Systemic invasion of developing peanut plants by *Aspergillus flavus*. Lett. *Appl. Microbiol*. 13(1):16-20.

Perry, B. A. (1984 b). Commentary on ISTA Vigour Test Committee Collaborative Trials. *Seed Sci. & Technol.*, 12: 301-308.

Wild, C. P. and Turner, P. C. (2002). The toxicology of aflatoxins as basis for public health decisions. *Mutagenesis*. 17: 471-481