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Fungal Pathogens of Postharvest Rot of Groundnut (*Arachys hypogaea* L.) in Hong Local Government Area of Adamawa State Nigeria

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Research Journal of Biological Sciences Copy Right: Medwell Publication **Abstract:** Fungi such as Aspergillus niger (brasiliensis) Aspergillus flavus, Alterneriadia nthocola, Curvularia lunata, Curvulari apellesecens, Fusarium oxysporum, Fusarium equiseti, Microphomina phaseolina, Rhizopus stolonifer, Penicillium digitatumare associated with heavy losses of seeds, fruits, grains, vegetables and other plant products in transit and storage rendering them unfit for human consumption. The research sought to identify fungal pathogens of groundnut rot in storage in Hong Local Government Area of Adamawa State, Nigeria a major groundnut producing area. A survey was carried out using random sampling on incidence of groundnut rot in the seven districts of Hong Local Government Area in the month of July 2016. Isolation and identification, frequency of occurrence, virulence as well as effect of pathogens on germination of groundnut seeds and seedling growth were carried out. Incidence of rot occurred in all 7 districts with the highest in Hong, Hildi and Gaya, pathogens associated with the rot were identified as follows; Aspergillus niger (brasilensis) Aspergilus flavus, Penicillium chrysogenum, Rhizopus stolonifer, Paecilomyces lilacinus, Pseudallescheria boydii, Cylindrocarpon lichenicola and Scedosaporium prolificans. The frequent occurring fungi were Aspergillus niger (brasilensis) and Aspergillus flavus while the most virulent was Aspergillus flavus. There was significant reduction in seed germination and seedling growth at 99.99% probability level for both the Valencia (Kampala) and Peruvian (Kwathrumthrum) from 33.33% for uninoculated to 11.00% for inoculated. Proper storage practices to reduce groundnut rot as well as enhance seed viability are therefore, suggested.

INTRODUCTION

Agriculture remains significant in the Nigerian economy and involves small scale farmers scattered over wide expanse of land with small holding ranging

from 0.5-3.0 ha per farm land, characterized by rudimentary farm systems, low capitalization and low yield per hectare^[1]. The roles of agriculture remain significant in the Nigerian economy despite the strategic importance of the oil sector.

Groundnut (*Arachis hypogea* L.) is an annual, self-pollinated, growing plant found in many tropical, subtropical and temperate countries of the world^[2]. It is now grown in about 108 countries of the world^[3]. Asia with 63-66% land mass produces 71.72% of world groundnut followed by Africa with 18.6% production and North-Central America with 7.5%^[4].

The major food crops of Adamawa State according to Adebayo and Tukur^[5] are mainly cereals, legumes and root crops while the cash crops are mainly cotton, groundnut and sugar cane. The variable climatic and edaphic factors of the state as well as cultural and socio-economic factors are adduced distribution of food and cash crops in the State. In the North-East Zone, groundnut is a major cash crop produced, especially in Hong^[6]. Reported that seed yield in Northern Nigeria is about 3000 kg ha⁻¹. Adamawa Agricultural Development Programme, ADADP^[7] enumerated groundnut types commonly grown in Adamawa State to include; "Ordaaji" (2 nuts/shell), "Kwamakuni", (3 nuts/shell), "Kwathrumthrum" (2 nuts/shell larger) "Kwanyambi" or Ex Dakar and Kampala (brown/white striped nuts).

Stored food commodities are severely damaged by different groups of fungi including *Aspergillus* spp. *Fusarium* spp. and *Penicillium* spp. *Aspergillus flavus* is common in tropical and subtropical countries and cause contaminationas a result of moulding of badly stored commodities such as groundnut, cereal and cotton seeds^[8].

Fungi such as Aspergillus niger (brasiliensis) Aspergillus flavus, Alterneriadi anthocola, Curvularia lunata, Curvulari apellesecens, Fusarium oxysporum, Fusarium equiseti, Microphomina phaseolina, Rhizopus stolonifer, Penecillium digitatum and Penecillium chrysogenum cause discolouration, rotting, shrinking, seed necrosis, loss in germination capacity and toxification to oil seeds^[9]. These fungi are associated with heavy loss of seeds, fruits, grains, vegetables and other plant products during picking, transit and storage rendering them unfit for human consumption even by producing mycotoxins and affecting their total nutritive value^[10,11]. The tropical climate with high temperature and high relative humidity along with unscientific storage condition adversely affect the storage of cereal grains and oil seed and this can lead to the total loss of seed quality^[12].

Groundnut seed is susceptible to a wide range of pathogens and pests which cause a lot of damage to the crop, thereby reducing yield^[13].

Groundnut (*Arachis hypogea* L.) is an important oil seed crop in Nigeria and is widely grown in the tropics and sub-tropics^[14]. It is one of the most important crops that have the ability to thrive on newly reclaimed sandy soils as a legume of high nutritive value as well as being a source of edible oil^[15]. Moulds are associated with heavy loss of seeds, fruits, grains, vegetables and other plant products during picking, transit and storage

rendering them unfit for human consumption even by producing mycotoxins and affecting their total nutritive value^[10,11] there is therefore, need to identify the pathogens prevalent in the area and assess their threat to this crop that is of utmost importance to the community (Kilba) and come up with an alternative control method other than the conventional un eco-friendly fungicides to control the fungi associated with the postharvest rot of groundnut. This research seeks to assess the incidence of postharvest rot of groundnuts as well as the fungal pathogens responsible in all the eight districts of Hong Local Government Area.

MATERIALS AND METHODS

Study area: A survey for the incidence and severity of groundnut rot in storage was carried out in Hong Local Government Area of Adamawa State (Fig. 1). The Local Government is divided into 7 districts which served as the locations for the survey. The districts are namely Hong, Pella, Gaya, Kulinyi, Dugwaba, Hildi and Uba (Fig. 2).

The isolation and identification of fungi associated with groundnut spoilage, determination of the pathogenicity of the isolates were conducted in the Medical Laboratory of Microbiology Department, Modibbo Adama University of Technology (MAUTECH) Yola from 18th July 2016-24th October 2016.

Hong Local Government Area is situated on Latitude 10.3° and Longitude 12.40° and 13° E it has a tropical climate marked by the dry and rainy seasons, the rainy season commences in April and ends in October, the average rainfall is 759 mm, the wettest months are August and September, the dry season starts from November and ends in April, the temperature is moderately hot ranging from $25\text{-}35^{\circ}\text{C}^{[5]}$.

Adamawa State is located at the North-Eastern part of Nigeria and lies between Latitudes 7° and 11°N of the equator and between Longitudes 10° and 14°E of the Greenwich Meridian. It shares boundaries with Taraba State in the South and West, Gombe in its North West, Borno to the North and Cameroon Republic along its Eastern borders. It lies in the Northern Guinea Savanna ecological zone^[5].

Source of samples: Samples of unshelled groundnut seeds of 2 genotypes namely Kampala and "Kwathrumthrum" were collected from 1 major market in each of the 7 districts namely Hildi, Kulinyi, Dugwaba, Uba, Gaya, Pella and Hong. 50 of the samples of each genotype were purchased from seller (2 randomly selected sellers/traders within the selected market) in each district making a total of 700 being collected from the various districts, the samples were conveyed to the laboratory in a dry clean polythene bag. Groundnut samples were labelled according to location and then photographed (Fig. 3 and 4a, b).

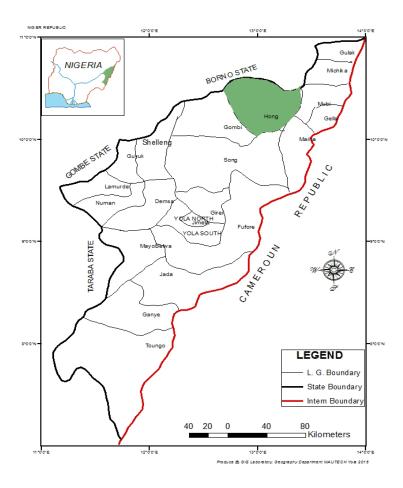


Fig. 1: Map of Adamawa State showing the study area

Incidence of rot on groundnut in storage: Incidence of rot of groundnut in storage was carried out. The samples purchased from the markets were assessed by determining the number of groundnut seeds showing rot out of the total number of groundnut seeds purchased from the market. The incidence of fungal spoilage/rot was expressed in percentage using the equation:

$$Incidence \ of fungal \ disease = \frac{seeds \ collected}{Total \ number of \ the} \times 100$$

$$groundnut \ seeds \ collected$$

Sterilization of inoculation room and instruments: Sterilization of laboratory environment was carried out in order to avoid contamination of the bench and tables used for inoculation were swapped clean using 95% ethanol and UV light switched on for 30 min before carrying out inoculation. Petri-dishes were sterilized at 160°C for 1 h in the oven, forceps, needles used for inoculation were sterilized by flaming and dipping into methylated spirit to cool.

Preparation of Potato Dextrose Agar (PDA): About 39 g of Potato Dextrose Agar (PDA) was dissolved in 1 L of distilled water, the Potato Dextrose Agar was then poured into two 500 mL conical flask then plugged with cotton wool and wrapped with aluminium foil before autoclaving it 121°C for 15 min at 10 l bs. pressure and 6 mL (0.1%) of streptomycin was added to the litre of sterilized media and swirled gently to mix properly, just before pouring into Petri dishes to prevent bacterial growth and allowed to cool and solidify according to the method of Suleiman and Michael^[16].

Isolation of pathogens: Under aseptic conditions the diseased sample of groundnut seeds showing rot was picked with a pair of sterile forceps. The seeds were then immersed into 1% sodium hypochlorite solution contained in a sterile 90 mm diameter petri-dish for surface sterilization for 30 sec, the sterilized seeds were then rinsed in three changes of sterile distilled water and then blotted dry between sterile filter papers. With a flamed and cooled pair of forceps, each sterilized seed was then

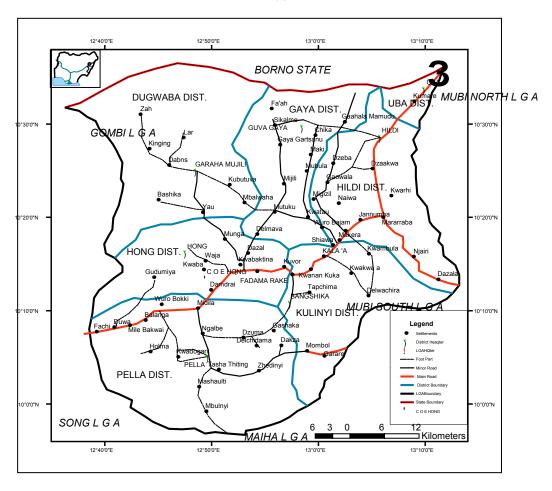


Fig. 2: Hong Local Government Area showing the study sites; GIS Laboratory Department of Geography, Mautech, Yola 2015



Fig. 3(a-b): a) Sample of healthy "Kwathrumthrum" groundnut seeds and b) Sample of "Kwathrumthrum" diseased groundnut seeds

split and plated aseptically on 90 mm diameter Petri-dish containing sterile solidified Potato Dextrose Agar (PDA) and incubated at an average room temperature of 33 \pm 20°C

for 7 days (i.e., immediately when new colonies begin to grow) before sub-culturing on fresh sterilize PDA using the method of Klich and Pitt^[17] and Robert *et al.*^[18]



Fig. 4(a, b): a) Sample of healthy Kampala groundnut seeds and b) Sample of diseased Kampala groundnut seeds

Pure colonies were photographed, pure colonies were obtained from hyphal tip transfer to fresh PDA, pure isolates of fungal species obtained were stored on solidified sterile PDA in Mc-Cartney bottles and these were appropriately labelled according to organism. The slant was initially corked loosely to allow the content fungus to grow and then was tightly corked and stored in a freezer to serve as stock culture.

Identification of the isolated pathogens: Microscopic examination was carried out to observe the structure and characteristics of the fungal isolates. A sterile needle was used to pick a portion of the hyphae/spore and placed on a sterile glass slide stained with lacto-phenol blue and examined under the photographic microscope using the method of Fawole and Oso^[19]. Micrograph of the isolates showing (conidia etc.) and the morphological and cultural characteristics observed were compared with structures in the identification guides of the center for research on pathogenic fungi and of Barnett and Hunter^[20].

Pathogenicity test: Pathogenicity test was carried out using techniques of Fajana^[21]. Healthy dried groundnuts seeds were surface sterilized with 1% sodium hypochlorite (NHCL) solution for 30 sec to remove surface contaminants and rinsed in 3 changes of sterile distilled water and then dried between a 12 cm diameter Whatman filter paper. The cultured isolates were dissolved into sterile distilled water and poured into 9 cm diameter petri dish laid with cotton wool and filter paper (Whatman No. 1). The 5 healthy seeds of *A. hypogea* were spread on each plate and incubated. The control set up seeds were treated with sterile distilled water and incubated the level of rot was measured^[21].

The set up was arranged in 3 replicates and incubated for 7 days. On establishment of rot, innoculum from the infected seed was re-isolated and compared with the first

isolate. Result was analysed using Statistics for Agricultural Sciences (SAS). Isolate's virulence was assessed based on Da silva and Bettiol^[22] pathogenic potential of grouping isolates: 0-20 low virulent group, 21-40 moderately virulent group, 41-60 highly virulent group, 61-80 very highly virulent group, above 80 totally virulent group.

Effect of pathogen on germination of seeds: The effect of pathogen on germination of seeds was carried out using the techniques^[21]. The 3 healthy seeds were placed on a plate and inoculated with each of the pathogen, the control seeds were with sterile distilled water. Germination was determined through the equation:

Number of seeds ger min ated
Total number of seeds plated

Effect of pathogen on growth of seedlings: Effect of the pathogen on the growth of the seedlings was carried out following the inoculation procedure. The 3 healthy seeds of *A. hypogea* inoculated with eachpathogen were spread on each plate and incubated in the dark. The control seeds were treated with sterile distilled water. The lengths (mm) of the radicles and plumule emerging from the seeds were measured for 7 days using a measuring ruler. The set up was arranged in 3 replicates and kept for 7 days. Result was analysed using statistical analysis system.

RESULTS AND DISCUSSION

Incidence of groundnut rot in Hong Local Government Area: Results of the study showed that incidence of rot varied among all the 7 districts at 99.99% level of probability in the case of Kampala, the highest

incidence of rot occurred in Hong (8.67%) with the least in Dugwaba (5.33%) district Table 1. The percentage incidence on the "Kwathrumthrum" showed the highest incidence occurring in Hong (6.67%) with the least in Uba (2.67%) district Table 2.

Fungal isolates of groundnut in Hong Local Government Area: Fungi found associated with groundnut rot in the 7 districts of Hong Local Government Area were Aspergillus niger (A. brasiliensis), Aspergillus flavus, Penicillium chrysogenum, Rhizopus stolonifer, Pseudaiiescheria boydii, Paecilomyces lilacinus, Cylindrocarpon lichenicola and Scedosporium prolificans.

Frequency of fungal isolates (%) on groundnut in Hong Local Government Area: Occurrence of the isolates on rotten groundnut seeds in Hong Local Government Area (p = 0.05) showed a wide variation with in the 7 districts Table 3. The highest occurrence of Aspergillus niger was in Kulinyi (15.17), Dugwaba (12.83) and Hildi (11.83%) followed by Pella (11.17), Gaya (8.83) and Hong (8.50%) and then by Uba (6.83%). For Aspergillus flavus its highest occurrence was in Gaya (11.67%) followed by Pella (10.50%), Dugwaba (10.50%), Uba (9.17%), Kulinyi (6.83%), Hildi (4.33%), Hong (2.83%). Penicillium chrysogenum occurrence was high at Hildi (5.67), Pella (5.50), Uba (5.00), Hong (4.33), Gaya (4.17), Kulinyi (2.67) and Dugwaba (1.17%). The highest occurrence for Rhizopus stolonifer was at Hildi (5.00), Gaya (2.83), Uba (2.00), Dugwaba (1.17) and Hong (1.33%) followed by Kulinyi (0.00) and Pella (0.00%). Pseudaiiescheria boydii ranked high in Dugwaba (2.83) and Hong (2.83%) followed by Gaya (1.50), Hildi (1.33) and Uba (1.00%) followed Kulinyi (0.00) and Pella (0.00%). For Paecilomyces lilacinas high occurrence was in Uba (7.00%) followed by Dugwaba (2.83) then Pella (1.67) Gaya (1.50) and Hong (1.50%)

Table 1: Groundnut variety used for the study

		Botanical		Pod size
Subspecies	Variety	types	Seed coat colour	cm
Fastigiata	Kampala	Valencia	Red-white (var)	3-4
Hirsuta	Kwathrumthrum	Peruvian	Brown	3-4

and then Hildi 0.00 and Kulinyi 0.00%. There was a higher occurrence of *Cylindrocarpon lichenicola* was in Pella 3.83, Hong 1.33 and Hildi 1.33% followed by Uba 0.00, Gaya 0.00, Dugwaba 0.00 and Kulinyi 0.00%. *Scedosporium prolificans* recorded a higher occurrence in Hong 2.83 and Dugwaba 2.83 followed by Hildi 1.33 and Uba 1.33 then Kulinyi 0.00, Pella 0.00 and Gaya 0.00%.

Virulence of rot of pathogens: The eight isolates were confirmed to be pathogenic as they produced rot lesions when inoculated to both the healthy genotypes of the groundnut seed. Virulence of the isolates also varied. Aspergillus flavus was rated as totally virulent, Aspergillus niger (brasiliensis) as very highly virulent, Penicillium chrysogenum and Rhizopus stolonifer as highly virulent, Pseudaiiescheria boydii as moderate virulent group, Paecilomyces lilacinus, Cylindrocarpon lichenicola and Scedosporium prolificans were the low virulent group Table 4 (Fig. 5-12).

Effect of pathogens on groundnut seed germination:

There was a significant reduction in the rate of germination of seeds inoculated with all the pathogens except for *Pseudallescheria boydii* and *Cylindrocarpon lichenicola* as compared to the control, the highest reduction in germination was by *Rhizopus stolonifer* and *Scedosporium prolificans* (11.00% in the Kampala). The highest reduction in the "Kwathrumthrum" cultivar was by the *Aspergillus flavus* 11.00%). However, there was no significant variation for reduction in germination between the Kampala and "Kwathrumthrum" (Table 5).

Table 2: Incidence (%) of groundnut rot in Hong Local Government Area of Adamawa State, Nigeria

Districts	Kampala	Local
Kulinyi	7.33	6.00
Hong	8.67	6.67
Pella	6.67	5.33
Dugwaba	5.33	4.67
Gaya	7.33	4.00
Hildi	8.00	6.67
Uba	6.00	2.67
LSD (0.05)	1.68	1.31

LSD: Least Significant Difference

Table 3: Frequency of occurrence (%) of fungi isolated from groundnut seeds in Hong Local Government Area of Adamawa State, Nigeria

	Pathogens							
Location	Aspergillus brasiliensis	Aspergillus flavus	Penicillium chrysogenum	Rhizopus stolonifer	Pseudaiiescheria boydii	Paecilomyces lilacinus	Cylindrocarpon lichenicola	Scedosporium prolificans
Kulinyi	15.17	6.83	2.67	0.00	0.00	0.00	0.00	0.00
Hong	8.50	2.83	4.33	1.33	2.83	1.50	1.33	2.83
Pella	11.17	10.50	5.50	0.00	0.00	1.67	3.83	0.00
Dugwaba	12.83	10.50	1.17	1.17	2.83	2.83	0.00	2.83
Gaya	8.83	11.67	4.17	2.83	1.50	1.50	0.00	0.00
Hildi	11.83	4.33	5.67	5.00	1.33	0.00	1.33	1.33
Uba	6.83	9.17	5.00	2.00	1.00	7.00	0.00	1.00
LSD (0.05)	3.42	2.31	0.93	0.64	0.26	0.19	0.30	0.35

Table 4: Virulence of fungal pathogens on groundnut seeds (%)

	Pathogenic potential		
Pathogen	Kwathrumthrum	Kampala	
Aspergillus flavus	Totally virulent	Totally virulent	
Aspergillus brasilensis	Very highly virulent	Very highly virulent	
Penicillium chrysogenum	Highly virulent	Very highly virulent	
Rhizopus stolonifer	Highly virulent	Very highly virulent	
Pseudaiiescheria boydii	Moderately virulent	Moderately virulent	
Paecilomyces lilacinus	Low virulent	Low virulent	
Cylindrocarpon lichenicola	Low virulent	Low virulent	
Scedosporium prolificans	Low virulent	Low virulent	

0-20%-Low Virulent Group, 2-40%-Moderately Virulent Group, 41-60%-High Virulent Group, 61-80%-Very High Virulent Group, 80% and Above-Totally Virulent Group

Table 5: Effect of pathogen on groundnut seed germination (%) in Hong
Local Government Area of Adamawa State. Nigeria

Organism Covernment Are	Kampala	Kwathrumthrum
Aspergillus niger	22.33	22.33
Aspergillus flavus	22.33	11.00
Penicillium chrysogenum	22.33	22.33
Rhizopus stolonifer	11.00	22.33
Pseudaiiescheria boydii	33.33	22.33
Paecilomyces lilacinus	22.33	22.33
Cylindrocarpon lichenicola	0.00	33.33
Scedosporium prolificans	11.00	22.33
Control	33.33	33.33
LSD (0.05)	0.81	0.93

LSD; Least Significant Difference

Table 6: Effect of pathogens on growth of seedlings of "Kwathrumthrum" groundnut (mm) in Hong Local Government Area of Adamawa State, Nigeria

Pathogen	Radicle	Plumule
Aspergillus niger	20.00	16.67
Aspergillus flavus	7.33	3.33
Penicillium chrysogenum	21.67	10.33
Rhizopus stolonifer	19.67	10.67
Pseudaiiescheria boydii	26.00	24.33
Paecilomyces lilacinus	12.67	22.67
Cylindrocarpon lichenicola	24.33	14.00
Scedosporium prolificans	13.33	9.67
Control	39.33	33.67
LSD (0.05)	22.70	16.00

LSD; Least Significant Difference

Effect of pathogens on growth of seedlings: There was no significant reduction in the growth of seedlings (plumule and radicle) for the Kampala, however, for the "Kwathrumthrum" growth of radicle was significantly reduced for all pathogen-inoculated seeds except *Pseudallescheria boydii* and *Cylindrocarpon lichenicola* for the plumule all seedings showed a significant reduction in growth except *Pseudallescheria boydii* and *Paecillomyces lilacinus* (Table 6).

Incidence of the groundnut rot was universally spread in all the seven districts of Hong Local Government Area of Adamawa State, there are reports according to Natarajan^[23] who reported that the method of storage



Fig. 5: 7 days old culture of Aspergillus niger syn Aspergillus brasiliensis

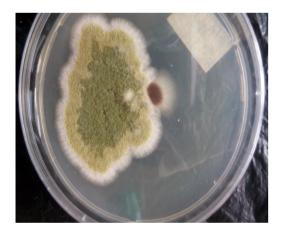


Fig. 6: 7 days old culture of Aspergillus flavus



Fig. 7: 7 days old culture of Penicillium chrysogenum



Fig. 8: 7 days old culture of Rhizopus stolonifer



Fig. 9: 7 days old culture of Pseudaiiesceria boydii



Fig. 10: 7 days old culture of Cylindrocarpon lichenicola



Fig. 11: 7 days old culture of Paecilomyces lilacinus



Fig. 12: 7 days old culture of Scedosporium prolificans

of the seeds and the initial seed quality affect the deterioration of the seed and would be subjected to invasion by fungi. Nakail *et al.*^[24] recorded the susceptibility of groundnut to colonization of *Aspergillus flavus* especially during storage.

The 8 fungal pathogens were found on the groundnuts in Hong Local Government Area of Adamawa State, the presence of these pathogens agrees with the report of Sullivan who reported that *Aspergillus flavus, Rhizopus stolonifer* and *Penicillium* spp. are storage pathogens of groundnut seeds which are highly susceptible to a number of disease causing pathogens because the seeds are very rich in nutrients for the fungi to thrive. Vikas and Mishra^[25] isolated 9 species of fungi which include *Aspergillus flavusPenicillium* spp. *Fusarium* spp., *Aspergillus niger* from the seeds of different varieties of groundnut during storage of 1 year.

According to Chavan and Kakde^[9] the most occurring moulds of post-harvest products in storage were commonly the *Penicillium*, *Aspergillus*, *Rhizopus* and *Fusarium* and this agrees with the findings of the research as the most occurring pathogens in all the seven districts are *Aspergillus flavus and Aspergillus niger* (*brasilenesis*). Chavan^[26] found that the species of *Aspergillus*, *Penicilium*, *Fusarium*, *Rhizopus* and *Alternaria* were commonly occurring post-harvest moulds in storage conditions.

Aspergillus flavus as recorded from the research was totally virulent and as found in other reports that most of the species of Aspergillus were dominant and play a vital role in the seed bio-deterioration, Ibiam and Egwu^[27] reported that among different species of fungal infection, Aspergillus flavus was the most predominant one.

Most pathogens caused a significant reduction in seed germination rate and growth of seedlings in both the Kampala and "Kwathrumthrum". This agrees with reports by Chavan and Kakde^[9] fungi such as Aspergillus niger (brasiliensis) Aspergillus flavus, Alterneria anthocola, Curvularia lunata, Curvularia apellesecens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penecillium digitatum and Penecillium chrysogenum cause shrinking, seed necrosis, loss in germination capacity and toxification of oil seeds. Soil-borne fungal pathogens are a major constraint in crop production by infecting roots^[28, 29].

CONCLUSION

The frequent occurring fungi were Aspergillus niger (brasilensis) and Aspergillus flavus while the most virulent was Aspergillus flavus. There was significant reduction in seed germination and seedling growth at 99.99% probability level for both the Valencia (Kampala) and Peruvian (Kwathrumthrum) from 33.33% for un-inoculated to 11.00% for inoculated. Proper storage practices to reduce groundnut rot as well as enhance seed viability are therefore, suggested.

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