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Original Research Article

Diversity and Selectivity of Mycotoxin Fungi Affecting Arachis hypogaea Seed Quality in Western Kenya

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ABSTRACT

Decline in ground nut seed quality due to seed-borne fungi contributes to 30 - 50% potential yield loss in Kenya. Seed infections by mycotoxin fungi not only reduce seed quality but also make nuts unsafe for human consumption. The objective of this study was to assess the morphological diversity and selectivity of mycotoxin producing fungi causing groundnut seed quality deterioration in Western Kenya**.** The seeds of groundnuts were collected from three markets. Fungal isolation was done using full-strength PDA (39 g/l) under clean airflow and ultraviolet light conditions in the laminar flow. The prepared cultures in petri-dishes were then incubated under continuous white fluorescent at 15- 25 ^{o}C inside a Gallenkamp incubator. This was repeated till pure isolates were obtained. Morphological diversity assessment and identification was based on mycelial and substrate colour and published references at the plant pathology laboratory, University of Eldoret. Ten morphologically diverse mycotoxin producing fungi were identified to be responsible for low groundnut seed quality in Western Kenya. The most frequently isolated pathotypes were three morphologically diverse strains of *Fusarium oxysporum* and three strains of *Penicillium corylophilum*. Two *Aspergillus* species (*A. niger* and *A. sulphures*) and other two unidentified were also isolated. Red Valencia variety of groundnut were the most affected by most mycotoxin fungi but *A. niger* showed preference to Homa-bay variety while *A. sulphures* was preferred Red Valencia. The existence of more than one strain of mycotoxin fungi provides the evidence on evolution and differentiation of the fungi alongside the resistant varieties of groundnuts being released by researchers. The gene-for-gene phenomena could be the reason behind the specificity of *A. niger* and *A. sulphures* to Homa-bay and Red Valencia varieties respectively. In conclusion, *Aspergillus*, *Fusarium* and *Penicillium* species are responsible for groundnut seed quality deterioration in Western Kenya.

Keywords

Arachis hypogaea, mycotoxin fungi, morphological diversity, variety-pathotype specificity

Introduction

Groundnut (*Arachis hypogaea)* is the sixth most important oilseed crop in the world. It is grown on 26.4 million ha worldwide with a total production of 37.1 million metric t and an average productivity of 1.4 metric t/ha (FAO, 2003). Over 100 countries worldwide grow groundnut and due to its economic and nutritive value, this crop is a beneficial enterprise for rural farmers in Kenya. The production of groundnut is concentrated in Asia and Africa but in Kenya, its production is mainly concentrated in Western region (Kipkoech *et al*., 2007). Despite being tolerant to a number of biotic and abiotic stress factors, groundnut responds differently to a number of seed borne pathogens (Burnett and Hunter, 2000; Rasheed *et al.*, 2004).

More than 50% yield loss has been reported in Western Kenya due to a number of factors including fungal diseases. Decline in ground nut productivity in Western Kenya has been attributed by a number of factors. This include seed-borne fungal diseases such as *Aspergillus flavus* with farmers obtaining less than 30 - 50% of the potential yields (Kipkoech *et al*., 2007). *.A .flavus* infects germinating groundnut seed and is associated with grains in storage of which aflatoxins is of utmost concern (Waliyar *et al*., 2013). This is due to their carcinogens and immunosuppressive effects in both humans and domestic animals (Wu *et al*., 2011).

Most researchers have detected different mycotoxin fungi and their toxin production ability in stored grains, which deteriorate the quality of stored produce (Vedahayagam *et al*., 1989). However, very little has been done towards detecting the presence and number of mycotoxin producing fungi that affect seed quality in Western Kenya despite the numerous reports of low yields due to seed-borne pathogenic fungi (Nafula, 1997).

Due to lack of current and reliable scientific data on mycotoxin producing fungi and their involvement in seed quality deterioration Kenya, the study was set to assess major fungal pathotypes, morphological diversity and pathotype-variety specificity of mycotoxin producing fungi responsible for ground nut seed quality deterioration in Western Kenya.

Materials and Methods

Study location

Infected groundnut seeds of two different varieties (Red Valencia and Homa-bay) were obtained from three markets (Kamkuywa, Kimini and Chwele) in Bungoma County, Western Kenya.

Isolation and incubation of mycotoxin fungi

Isolation and purification of the mycotoxin fungi from diseased groundnut seeds was done as described by previous researchers (Owino *et al*., 2013). The groundnut seed samples with symptoms of fungal infection were cut into 1 cm^2 pieces and surface-sterilized in 70 % ethanol for 10 sec and with 1% sodium hypochlorite solution for 30 sec, then rinsed in sterile distilled water for 10 sec three times. Seed pieces were blotted dry and aseptically transferred to freshly prepared potato dextrose agar (PDA Oxoid, 3.9 %) containing streptomycin sulphate (0.39 %) in petri dishes to prevent bacterial growth.

Petri dishes were incubated at 15-25 ºC under continuous white fluorescent light inside a Gallenkamp incubator to induce vigorous mycelial growth. Ten days later, a single conidium representing each collection was transferred on freshly prepared potato dextrose agar medium and incubated for two weeks under the same conditions of continuous light and temperature range.

Diversity assessment and species identification

Data on morphological characteristics including mycelium colour (upper surface) and colour of substrate (under surface), texture and growth of aerial mycelium (luxuriant or scanty) were recorded and used to morphologically assess the diversity of mycotoxin fungi affecting groundnut seed quality. Species identification of the mycotoxin producing fungi was done using plant pathology books and referred journals (Agrios, 2005; Pandey and Sharma, 2010).

Result and Discussion

Morphological diversity of mycotoxin fungi

Ten morphologically diverse species of mycotoxin fungi were observed to lower groundnut seed quality in Western Kenya, Bungoma County. The genus *Fusarium* and *Penicillium* was the most frequently isolated across the varieties and regions from which the seeds were sourced. This implies that the two genus of mycotoxin fungi are the major seed-borne pathogens lowering seed quality in the region. For instance, isolates 4, 5 and 6 were identified as the major pathotypes of *Fusarium oxysporum* with distinct magenta substrate colour turning violet, resembling those identified by Pandey and Sharma, 2010 (Plate 1 and 2).

Isolates 1, 3, 7, 8 and 9 were identified to belong to the genus *Penicillium* and this genus of mycotoxin fungi had highest number of morphologically diverse pathotypes. The higher number of diverse strains indicates the higher frequency at which *Penicillium* evolves to form new strains and this could be due to its evolutionary advantage of massive gene swapping (Ropars *et al*., 2014). Isolates 3, 7 and 9 were identified to be strains of *Penicillium corylophilum* while isolates 1 and 8 were only identified as *Penicillium* species, showing that the two isolates are still unnamed in terms of species (Plate 1 and 2).

The third genus of the mycotoxin fungi isolated in this study was *Aspergillus* and two isolates were identified as *A. Niger* (isolate 2) and *A. Sulphures* (isolate 10). These two species of *Aspergillus* expressed contrasting morphological difference with *A. Niger* showing black mycelia and greyish substrate colour. However, *A. sulphures* showed dirty white mycelial growth with orange substrate colour (Plate 1 and 2).

Variety-pathotype specificity of mycotoxin fungi

For the variety-pathotype specificity, seed infection by *Aspergillus* species and *Fusarium oxysporum* was observed to occur in both Red Valencia and Homa-bay varieties. However, higher incidences of *Penicillium corylophilum* were observed in Red Valencia variety while Homa-bay variety did not have any incidence (Table 1).

Aspergillus niger only infected seeds of Homa-bay variety while *A. sulphures* showed specificity to Red Valencia variety in terms of the isolates from infected seeds. The observed specificity and selectivity of the pathotypes could be due to the influence of environmental, host plant or pathogenic strain factors (Agrios, 2005).

In conclusion, *Fusarium oxysporum, Penicillium* sp, *Penicillium corylophilum, Aspergillus sulphure* and *Aspergillus niger* are the major mycotoxin producing pathogenic fungi affecting groundnut seed quality in Western Kenya but the frequency of infection seems to depend on the pathotype-variety interaction and selectivity of the pathogen. Therefore, there is need for advanced microscopic assessments and genetic diversity studies to give detailed morphogenetic diversity results on the identified mycotoxin producing fungi.

Table.1 Mycotoxin fungi affecting groundnut seed quality based on variety and seed source

SEED SOURCE	VARIETY	MYCOTOXIN FUNGUS	DIVERSE FUNGI
Chwele	Homa-bay	*Aspergillus niger*	**3**
Kamkuywa		*Fusarium oxyporum*	
Kamkuywa		*Penicillium sp.*	
Chwele	Red valencia	*Penicillium sp.*	**4**
Kamkuywa		*Penicillium corylophilum*	
Kimini		*Fusarium oxyporum*	
Chwele		*Penicillium corylophilum*	
Kimini		*Penicillium corylophilum*	
Kamkuywa		*Aspergillus sulphures*	

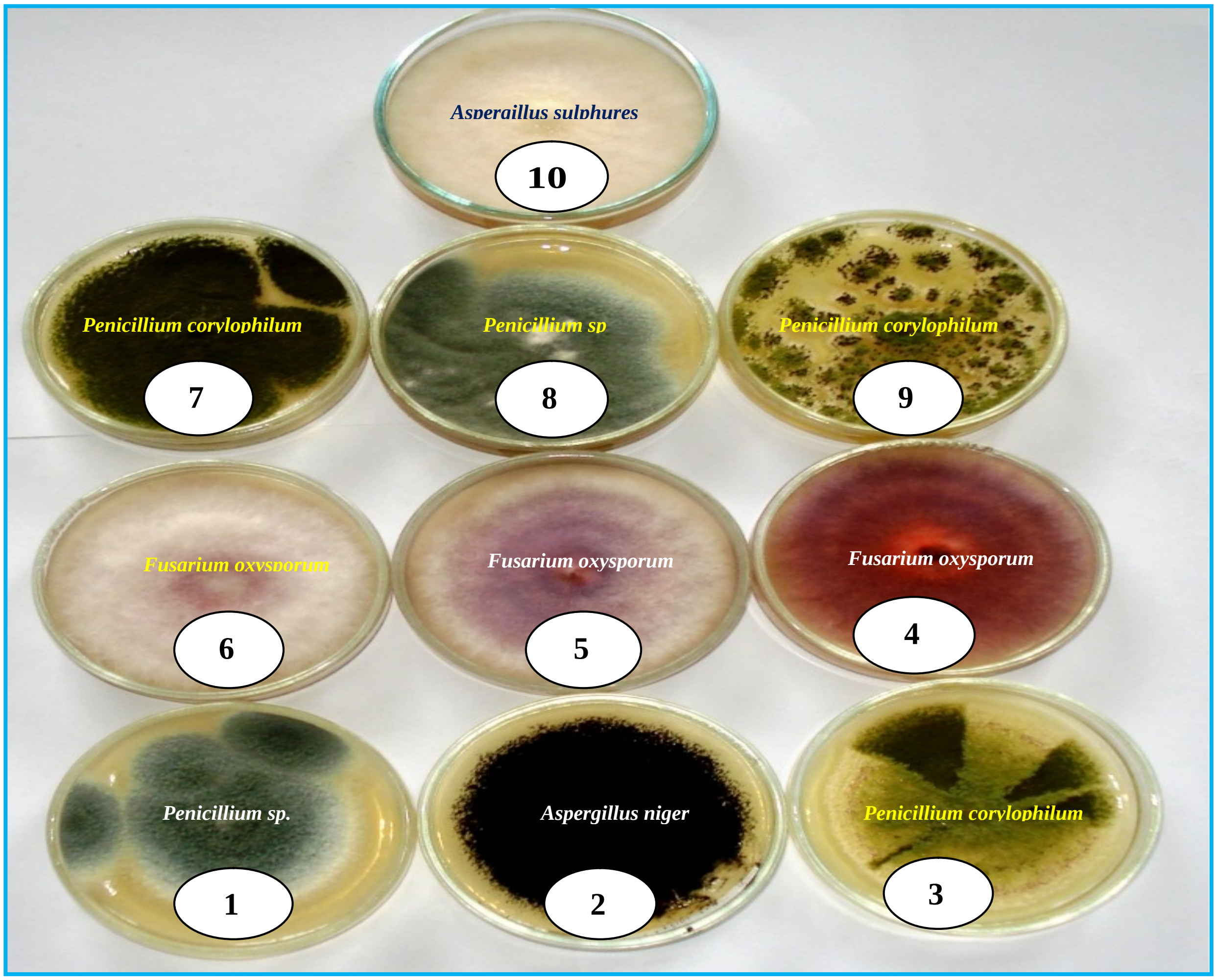

Plate 1: Morphological diversity (UPPER SURFACE) of pathogenic fungi infecting Red Valencia Homa-Bay varieties of Arachis hypogea L. in Bungoma county. ***The isolate numbers correspond to those in plate 2***

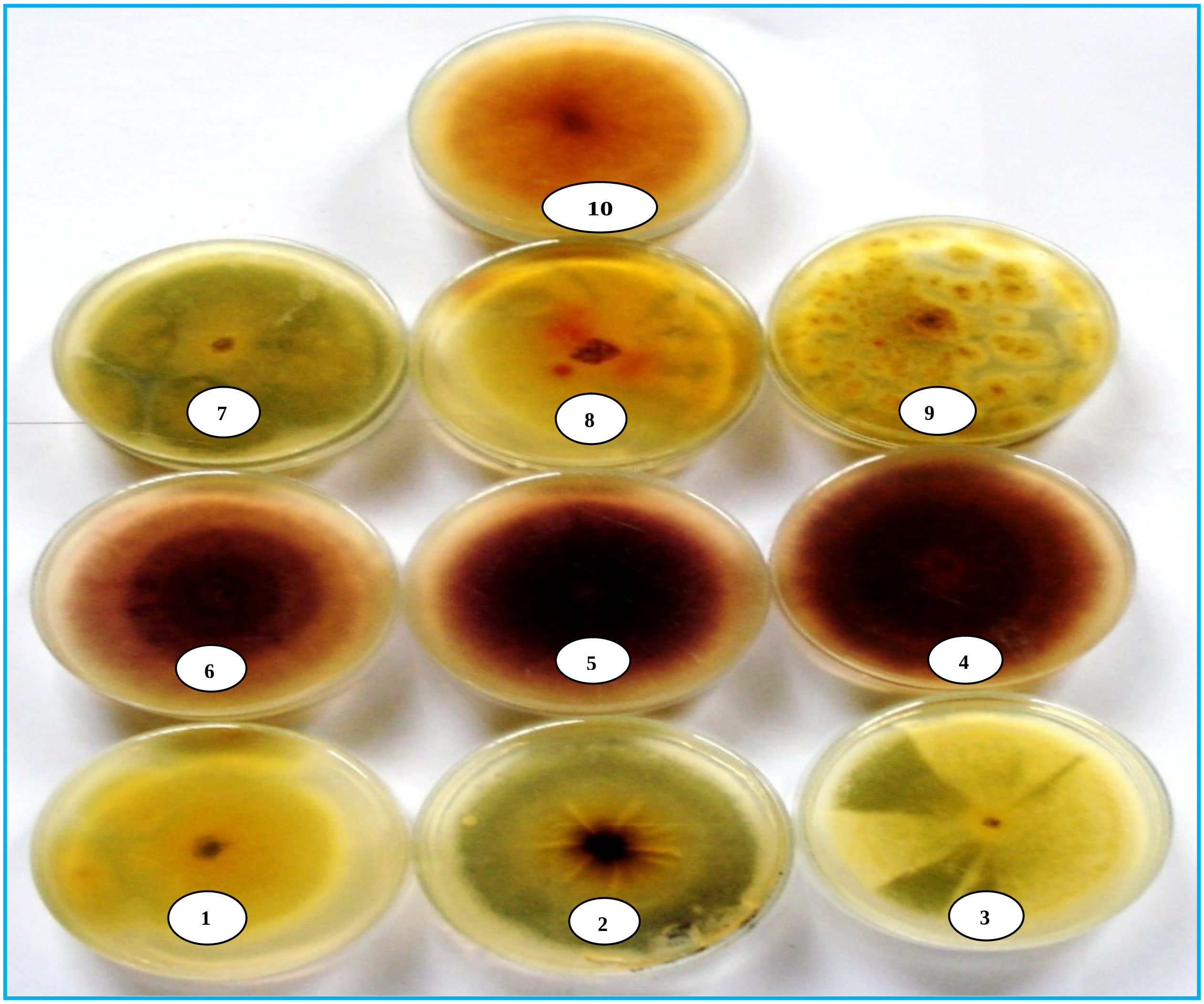

Plate 2: Morphological diversity (SUBSTRATE) of pathogenic fungi infecting Red Valencia Homa-Bay varieties of Arachis hypogea L. in Bungoma County. ***The isolate numbers correspond to those in plate 1***

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