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Research Article

Identification of Microflora Associated with Groundnut (*Arachis hypogaea* L.) Seeds and Its Impact on Physical Impairment on Seeds Germination Percentages

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Abstract

This study was to investigate the incidence of seed-borne fungi on the groundnut seeds isolate and identify the seed-borne fungi associated with some of groundnut seeds and obtain information on the pathological effect of these fungi on the peanut seed germination. The results of these investigations revealed the existence of four notable seed-borne fungal pathogens, specifically A. niger, A. flavus, A. tamre, and one Aspergillus pp., in the designated research area. A. niger was identified as the predominant fungus affecting groundnut seeds among these pathogens. This finding suggests that the presence of A. niger might inhibit the growth of other fungi through competitive interactions in the environment. Previous research by Ohave. The findings are consistent with the results of my study, which demonstrate that these fungi play a role in causing pathological effects on groundnut seeds, resulting in issues such as seed shriveling, discoloration, and decreased germination rates due to seed-borne fungal infections. The conducted study emphasizes the importance of treating seeds purchased from markets with fungicides prior to planting to prevent seedborne diseases like seed rot, decay, and other related pathologies. Furthermore, it is very important to take steps to reduce the spread of seed-borne pathogens and the production of mycotoxins in groundnut seeds by improving storage conditions, which include maintaining low temperatures, humidity levels, and moisture content. Additionally, preventive measures should be implemented to avoid damage during post-harvest processes at storage and during the sale at the market.

Keywords

Fungicides, Groudnut, Microflora, Seed Damage Germination

1. Introduction

Groundnut, (Arachis hypogaea L.,) is an annual plant that belongs to the leguminosae family. It is grown in 108 countries across approximately 22.2 million hectares of land. Specifically, 13.69 million hectares are in Asia (with India accounting for 8 million ha and China for 3.84 million ha), 7.39 million hectares in Sub-Saharan Africa, and 0.7 million hectares in Central and South America [1]. This crop ranks as the 13th most important food crop for edible oil and the 3rd most significant source of vegetable protein. Groundnut seeds are rich in high-quality edible oil (about 50%), easily

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digestible protein (25%), and carbohydrates (20%) [2]. The agronomic practice of cultivating groundnuts is predominantly aimed at procuring its seeds, which serve as a source for oil extraction, direct human consumption due to their protein content, as well as vitamins A and B. The by-product generated after the oil extraction process from the seeds holds significant value for poultry and livestock feed. The shells derived from the seeds can be repurposed as fuel for the production of coarse boards and can also act as a substitute for cork materials. The kernels possess the versatility to be consumed in their raw state, roasted, or sweetened forms. Moreover, the oil extracted from groundnuts exhibits a range of applications, including but not limited to soap manufacturing, cosmetics formulation, and lubrication [3]. Groundnut, scientifically referred to as Arachis hypogaea, constitutes one of the five principal oilseed crops cultivated in the arid landscapes of Ethiopia [4]. The introduction of this crop to the Hararghe region was facilitated by Italian explorers who transported it from Eritrea during the early 1920s [5]. Prominent locales for groundnut cultivation within Ethiopia encompass Babile, Gursum, Beles, Didessa, Gambella, and Pawe. Furthermore, regions such as Gamu Gofa, Illubabor, Gojam, Wello, and Wellega have been recognized as possessing favorable conditions for enhanced production. In the cropping season of 2013/2014, the estimated area dedicated to groundnut cultivation in Ethiopia was 79,947.03 hectares, resulting in a total yield of 112,088.72 tons [6]. Notwithstanding its considerable contribution to exports and the national economy, a myriad of challenges persists that obstruct the effective production and utilization of groundnut. Among the various obstacles encountered, toxigenic fungal infections are identified as the most significant, adversely affecting seed quality through the processes of spoilage and mycotoxin contamination. The domesticated groundnut (Arachis hypogaea L.) represents an ancient agricultural product of the New World, originating from South America (specifically the southern regions of Bolivia and northwestern Argentina), where its cultivation can be traced back to as early as 1000 B.C. The dissemination of this agricultural product to the regions of Africa, Asia, Europe, and the Pacific Islands is posited to have occurred during the sixteenth and seventeenth centuries, coinciding with the exploratory maritime expeditions undertaken by the Spanish, Portuguese, British, and Dutch. The nomenclature Arachis is etymologically derived from the Greek term "arachos," which denotes a weed, while hypogaea signifies an underground chamber; in botanical parlance, this refers to a weed that produces its fruits subterraneously. The two predominant appellations commonly employed for this agricultural product are groundnut and peanut. The designation "groundnut" is predominantly utilized in numerous nations across Asia, Africa, Europe, and Australia, whereas in the regions of North and South America, it is frequently identified as "peanut." The two principal designations employed for this agricultural product are thus "groundnut" and "peanut." The designation "groundnut" is predominantly utilized in numerous nations across Asia, Africa, Europe, and Australia, whereas in the regions of North and South America, it is frequently identified as "peanut." The term "groundnut" pertains to the pods containing seeds that develop subterraneously; the appellation "peanut" arises from the fact that this crop is classified within the leguminous family, which encompasses other species as well [7]. Groundnut cultivation occurs within a variety of agro-ecological systems and is practiced in diverse socioeconomic contexts. Its agricultural activities are primarily restricted to tropical, subtropical, and temperate warm climate zones situated between 40 °N and 40 °S latitude. Presently, it is cultivated over an area of 25.2 million hectares globally, yielding a total production of 35.9 million metric tons and exhibiting a productivity rate of 1.425 tons per hectare, with developing nations in Asia (66%) and Africa (25%) acting as the predominant producers [6]. In the year 2009, China, India, and the United States emerged as the three foremost producers of groundnut [7]. The crop is presently cultivated across approximately 108 nations worldwide. Asia, encompassing 63.4% of the land mass, contributes to 71.72% of global groundnut production, followed by Africa with an 18.6% share and North-Central America, which accounts for 7.5% [8].

Optimal growth conditions for this crop include light, sandy loam soil. It necessitates a warm climate for five months and an annual precipitation range of 500 to 1000 mm. The pods mature between 120 to 150 days subsequent to the planting of the seeds. A temperature range of 25 °C to 30 °C is deemed ideal for the development of the plant [9]. On a global scale, 50% of groundnut production is allocated for oil extraction, 37% for confectionery applications, and 12% for seed purposes. Groundnut haulms serve as an excellent source of hay for livestock feed. Additionally, groundnut cake represents a high-protein feed option for livestock. This crop is also utilized in the production of margarine and soap. Furthermore, high-quality oil derived from groundnuts finds applications within the pharmaceutical sector. The husk of the groundnut can be combusted for energy generation or employed in the production of particle board [10]. Fungal proliferation on stored seeds, including groundnuts, can significantly diminish germination rates while concurrently causing losses in carbohydrate, protein, and total oil content, as well as inducing increased moisture levels, free fatty acid content, and other biochemical alterations. The tropical climate characterized by elevated temperatures and high relative humidity, coupled with inadequate storage practices, adversely impacts the preservation of cereal grains, oilseeds, and similar commodities, ultimately resulting in the complete deterioration of seed quality [11].

Fungi can be classified as one of the most detrimental microorganisms, with a total of 46 fungal diseases documented in groundnut and 67 fungi identified in association with various symptomatic manifestations [12].

Groundnut is subject to attacks from numerous economically significant pathogenic fungi. Aspergillus niger, Fusarium, Penicillium, and Cladosporium represent the predominant fungal genera linked to stored grains, with aflatoxins among all mycotoxins being of paramount concern [13]. This concern arises from their carcinogenic properties and immunosuppressive effects on both humans and domestic animals [14]. Aspergillus is a prevalent mold in tropical and subtropical regions, leading to aflatoxin contamination because of the moulding of inadequately stored commodities, such as groundnuts, cereals, and cotton seeds [15].

The previous research indicated that, groundnut seeds exhibit a significant vulnerability to pathogenic diseases, as they act as a reservoir of stored nutrients for fungi, resulting in discoloration, deterioration, shrinkage, seed necrosis, diminished germination capacity, and toxification of oil seeds [12, 15]. The incidence of aflatoxin contamination in groundnuts may transpire prior to harvest during the maturation phase in the field, throughout the harvesting process, and during the post-harvest storage period. This contamination hinders groundnut producers from accessing larger western markets, escalates reliance on foreign food assistance, constrains economic prospects, and detrimentally impacts consumer health. According to the Food and Agriculture Organization [12], developing nations constitute approximately 95% of global groundnut production yet face challenges in selling substantial quantities of groundnut on the international stage due to aflatoxin contamination. For example, a food processing enterprise in Ethiopia procured groundnuts from India, while groundnut cultivators in Gursum and Bablie struggled to secure a market for their produce [16]. In Ethiopia, a prior investigation indicated mean aflatoxin B1 concentrations of 34.7 and 105 μg/kg in samples of groundnut and peanut butter, respectively [16]. documented aflatoxin concentrations ranging from 5 to 250 μg/kg in groundnut seeds originating from eastern Ethiopia [17]. The total aflatoxin concentrations in Aspergillus flavus-positive groundnut seed samples fluctuated between 15 and 11,865 μg/kg [18].

There for the objectives of this study was to investigate the incidence of seed-borne fungi on the groundnut seeds isolate and identify the seed-borne fungi associated with some of groundnut seeds and obtain information on the pathological effect of these fungi on the peanut seed germination.

2. Material and Method

2.1. Collection of Seed Sample

Groundnut seeds were collected from four different sellers all within Jimma central market. These seed samples were investigated at Jimma University College of agriculture and veterinary medicine plant pathology laboratory.

2.2. Detection Agar Plate Method (APM)

Peanut seed specimens were subjected to an analysis regarding their association with seed mycoflora through the application of the standardized Agar plate methodology (ISTA, 1985). From each seed sample, a working subset comprising 400 seeds was established, from which 70 seeds were randomly selected. During the isolation process, the samples underwent surface sterilization utilizing a 5% Chlorox aqueous solution for an approximate duration of one minute. Subsequently, these seeds were rinsed with sterilized distilled water and then subjected to blot drying. A total of five seeds were placed in each Petri dish. The inoculated seeds were then incubated at a temperature of 25 ±2 ℃ for a period of seven days, followed by the implementation of pure culture techniques.

2.3. Identification of Fungal Isolates

Petri plates having diseased specimens were observed to identify the seed borne fungi on the basis of colony color. Further confirmation was made by preparing the slides and observed under microscope with low as well as high magnifications (4, 10, 40, and 100X). A drop of water was placed in the center of slide, the small portion of fungi culture was cut out with the aid of sterilized inoculating needle. The cut piece was put directly in the water droplet and tease out, a cover slip was then covered over the teased portion, it was mounted on the microscopic stage and viewed. The viewing was first done with lower magnification (x4) and later with higher magnification (x40). The nature of mycelium, the types of fruiting body and spore structure served as criteria for the identification of isolate. The isolates were identified and confirmed with mycological atlas as described by fisher 1988.

2.4. Data Collected

Fungal species found growing on the surface of seeds, Type and frequency of occurrence of identified fungal species was recorded. Percentage frequency (PF) of occurrence of fungal was calculated by using the following formula: PF= (No. of seeds on which fungus appears / Total number of seeds) X 100. Percent of germination (PG) of seed varieties are determined as proportion of germinated seed over the total number of seed and computed by using the following formula: PG= No. of seeds germination/ Total number of seeds) X 100.

3. Results

3.1. Occurrence of Seed Borne Fungi on Groundnut Seeds

The agar plate method technique has proven more suitable for the detection of *Aspergillus niger*, *Aspergillus flavus* and another *Aspergillus spp*. (Table 1 & figure 1. *A. niger*, *A.*

flavus and A. tamrii were predominant across the market. A total of four fungi species comprising one genus namely *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tamrii*, *other Aspergillus spp.* were isolated from peanut seed samples taken from Gimma central market (Kulober and bishishe) (Table 1). Among the fungal species that were isolated, *Aspergillus niger show the highest mean* frequency flowed by *Aspergillus tamrri Aspergillus flavus and other Aspergillus spp*. across the hole seller in Jimma central market (bar figure 1). The highest average frequency of fungal pathogen was absorbed the sample taken from kullober2, then followed by Kullober1, Bishisha1 and 2 respectively (table 1).

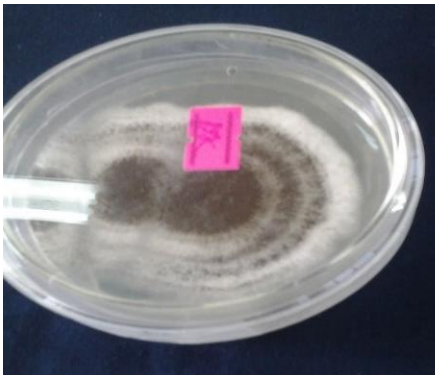

Figure 1. *A.niger.*

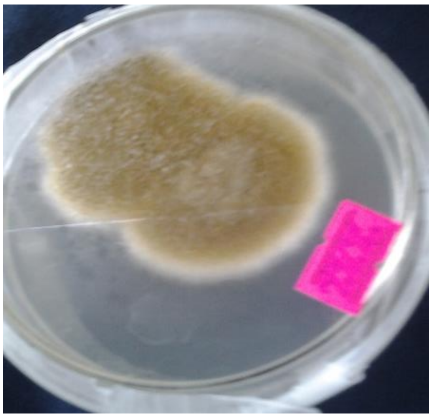

Figure 2. *A.tamri.*

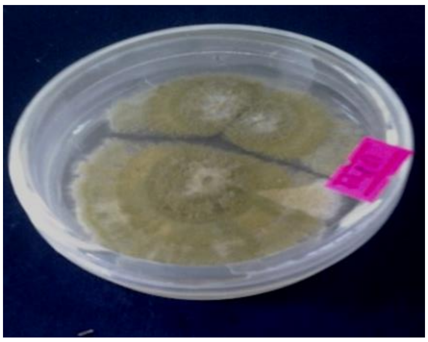

Figure 3. *A.flavus.*

Figure 4. *All mycoflora isolated from ground nut seed.*

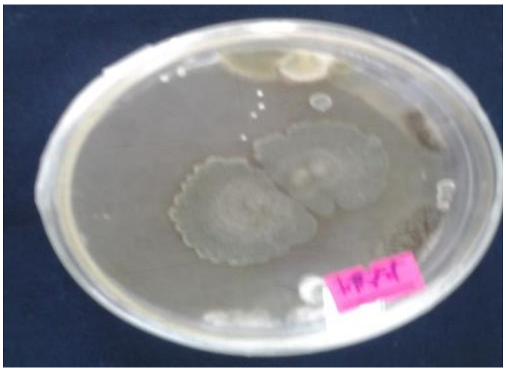

Figure 5. *Another genus aspergillus.*

Figure 6. *Fungal microflora on the groundnut seed plated on agar.*

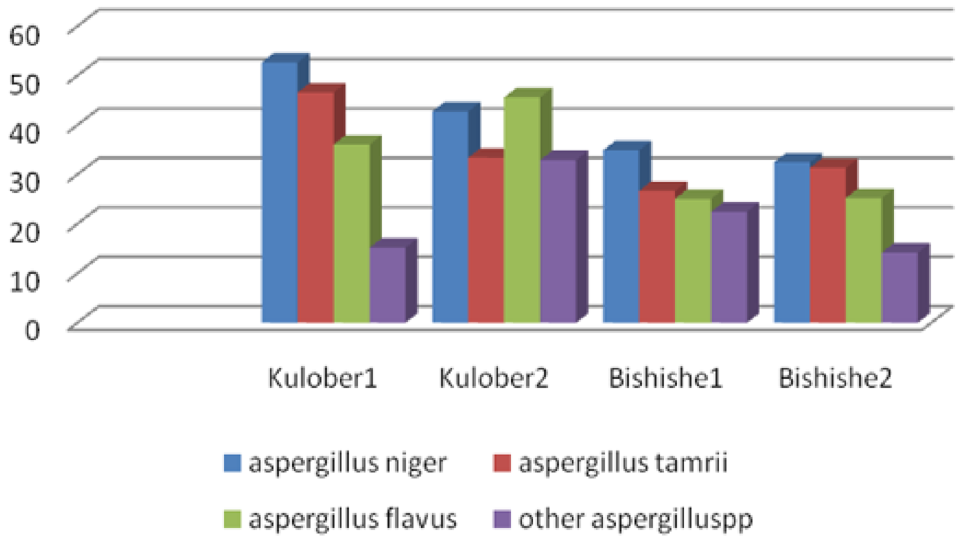

Figure 7. *Frequency of fungal species across the market.*

Table 1. *Frequency of fungal pathogen associated with Ground nut seed.*

	Fungal species%				
Market name	**aspergillus niger**	**aspergillus tamrii**	**aspergillus flavus**	**other aspergilluspp**	**Mean**
Kulober1	52.68	46.6	36.05	15.2	35.98
Kulober2	42.78	33.42	45.6	32.93	36.27
Bishishe1	34.91	26.7	25.1	22.47	27.30
Bishishe2	32.46	31.33	25.24	14.19	25.81
Mean	40.68	34.26	29.2	21.2	

3.2. Seed Physical Damage and Germination %

As different authors wrote about seed born fungi associated with the groundnut seed that affected the germination percentage and seed imrgence, these results also showed that, the germination percentage across the whole seller in Gimma central market were not more than 50%. The highest and lowest germination percentage was observed the sample taken from Bishishe1 and Kullober2 respectively (bare Figure 2). As many reporters also wrote the physical damage (seed shriveling and discoloration) of seed also good indicator for the presence of mycoflora associated with the seed. In these results the highest and lowest physical seed damage was absorbed from the sample taken from Kullobere2 and Bishishe1 respectively (Figure 2).

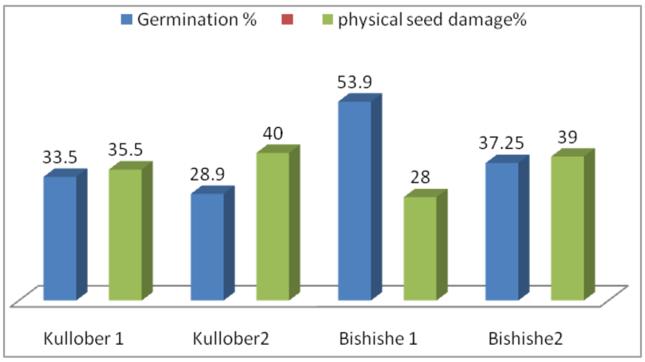

Figure 8. *Physical damage and Germination %.*

Figure 9. *Seed damage kulober 1&2.*

Figure 10. *Seed damage Bishisha1&2.*

Figure 11. *Effect of mycoflora on ground nut seed germination.*

4. Discussion

Results of the these investigation revealed that at least four important seed-borne fungal pathogens namely *A. niger*, *A. flavus*, *A.tamre* and one Aspergillus pp.in the study area. Out of all those pathogen, A. niger was the predominate fungi on groundnut seed. This observation suggested that, A. niger inhibit the growth of other fungi due to the competition for in the site. The findings of the present investigation agree with the findings of other investigators the association of similar fungi with peanut seeds. *A. niger* and *A. flavus* were predominant in groundnut and seed coat was greatly infected by fungi followed by cotyledon and axis. Such similar reports have been made on groundnut seed. It was also reported that *A. flavus* was the important mycotoxins producer and produce aflatoxin B1, B2, G1 and G2 which are hepatocarcinogenic species have been to reduce the germination of seed and damage the seeds in the storage. these studies agree with my study that showed those fungi were associated with the pathological effect on groundnut seed, such seed shriveling, seed coloration and low percentage germination was as a result of infection of seed-borne fungi on those seeds.

5. Conclusion and Recommendation

The experiment has helped to show that seed collected from open markets for the purpose of planting must be treated with fungicide to avoid seed disease such as seed rote, seed decay, and other pathological effect. There is also needed to reduce the growth of seed born pathogen and mycotoxin production in ground nut seed by improving the storage condition, that is low temperature, low humidity and low moisture content. The damage done during the post-harvest operation should also be avoided.

Abbreviations

APM	Agar Plate Methodology
PF	Percentage Frequency
PG	Percent of Germination

Conflicts of Interest

The authors declare no conflicts of interest.

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