Phytology

Fungal infection of locust beans *Parkia biglobosa* on sale at wudil and darki markets of Kano State, North Western Nigeria

Yahaya SM[1*], Yakasai MA[1], Safwan M[1]

[1] Department of Biology, Kano University of Science and Technology, Wudil, Kano State, Nigeria

Correspondence Author: Yahaya SM

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Abstract

Experiment was carried out for a period of four months (November, 2017 to February, 2018) to determine the prevalence of fungal infection of locust beans on sale at two popular markets in Kano State, Wudil and Darki using standard microbiological methods, which involved plate culture methods. A total of 67 isolates were counted and recorded which belong to the two species of *Aspergillus, Candida albican, Botrytis spp, Penicillium spp and Curvularias spp.* The pathogens occur with the following percentage occurrence *A. fumigatus* (23.88%), *A. flavus* (19.40%), *Candida albican (17.90 %) Penicillium spp* (16.41 %), *Curvularias spp (13.43%), Botrytis spp* (8.95%). More isolates were counted at Wudil market 36 (53.73%), while at Darki market 31 (46.26 %) isolates were recorded. The differences between the isolates counted in the two locations was statistically significant (P>0.001). The result of the study showed that fungal infection of locust beans in the two markets was mainly the result of poor post-harvest handling and also exposure to unfavourable storage conditions.

Keywords: fungal pathogens, incidence, locust beans, post-harvest handling

Introduction

Parkia biglobosa, known as the African locust bean is a dicotyledonous, perennial deciduous tree of the Fabaceae family grows to between 7 and 20 metres high, in some cases up to 30 metres (Ogaraku 2010) [18]. The tree of the locust bean requires "between 0-300 metres of altitude, a mean annual rainfall of between 400-700 millimetres and a mean annual temperature of about 24-28°C." It prefers well-drained, thick clay soils but can also be found on shallow, thin sandy soils. It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Where the tree is grown, the crushing and fermenting of these seeds constitutes an important economic activity. Various parts of the locust bean tree are used for medicinal purposes. As a standing tree, locust bean may have a positive effect on the yield of other nearby crops (Allen 1983; Anyanwu 2003) [2, 3].

The pods of the tree, commonly referred to as locust beans, are brown initially and turn dark brown when fully mature. They are 30-40 centimetres long on average, with some reaching lengths of about 45 centimetres. Each pod can contain up to 30 seeds (Allen 1983; Anyanwu 2003) [2, 3]. Annual production of seeds in northern Nigeria is estimated at around 200,000 metric tons (Allen 1983; Anyanwu 2003) [2, 3]. While the products of the tree are not common in international trade, they form an important part of local and regional trade in West Africa (Anyanwu 2003) [2, 3].

The most valuable parts of the locust bean are the seeds which are high in lipids (29%), protein (35%), and carbohydrate (16%), and therefore are good source of fat and calcium for rural dwellers. The seed provides one of the popular seasonings in African diet. This nutritious and delicious food spice is popularly called "ogiri" in Igbo, "iru" in Yoruba or"dadawa" in Hausa in Nigeria (Fagbohun and Lawal, 2011) [8]. There are two types of seed within each pod – reddish-dark and dark (black). The ratio between these seeds varies from 1:20 to 1:5, with darker seeds outnumbering lighter ones. Reddish-dark seeds have a thinner coat and they germinate earlier than black seeds that haven't first been acid treated. "Dark seeds have a harder seed coat and require various pre-treatment's to ensure good germination rates (Falaye and Fagbohun 2012) [9].

In many African countries the indigenous healers use different parts of the locust bean tree for health benefits. In a report by Oerke (2005) [17] *Parkia biglobosa* was reported to be the most used for treating hypertension in South western Nigeria. The tree was also reported to have wound-healing properties and can influence the proliferation of dermal fibroblasts. In a similar survey conducted in Guinea by Farombi (2003) [10] on use of antimalarial plants, *Parkia biglobosa* was cited among the most successfully used. The plant compared favourably with those of streptomycin, making it a potential source of compounds used in the management of bacterial infections (Farombi, 2003; Guarro, 2013) [10, 11]. In veterinary medicine, a root decoction is used to treat coccidiosis in poultry. Green pods are crushed and added to rivers to kill fish.

However, despite its valuable importance locust beans are attacked by pathogenic fungi resulting in scarcity and high cost of the seed (FAO 2002) [7]. Therefore, the main aim of this study was to isolate and identify pathogenic fungi responsible for losses of locust beans and provide information for the design of control strategies for the fungi.

Materials and Method

Study Sites

Wudil Market

The market is located in the Wudil local government area of Kano state. It is one of the largest markets in Kano state, has

no good storage facilities. Some merchants store their grains in rusted basins. Merchants rarely use chemicals on their grains.

Darki Market

The market is also located at Wudil local government area of Kano state. It is another big grains market in Kano state and has similarities in form, structure and functions with Wudil markets.

Experimental procedures

A survey was conducted to provide information on the incidence of fungal species responsible for the losses of locust beans on sale at Wudil and Darki markets. The investigation period was from November 2017 to February, 2018.

Isolation and identification of fungi on Locust beans

The method used for isolation and identification of fungi associated with losses of quality and quantity of locust beans.

Sample collection and collection site

Twenty Samples of locust beans seeds were obtained twice a week on Mondays and Thursdays directly from grain sellers each at Wudil and Darki markets and transported to the laboratory at Kano University of science and technology in polyethylene bags for handling. Locust bean seeds were surface sterilized by immersion in 3% (v/v) sodium hypochlorite and then rinsed three times in running tap water and allowed to dry. Samples were placed in onto four PDA plates (five seed per plate) and incubated at 25.7^0c for three days. Each week, growth of fungal isolates was monitored and the number of isolates that appeared was counted and recorded. Each distinct isolates were sub cultured into fresh PDA until pure.

Pathogenicity tests

Pathogenicity tests were conducted to prove Koch's postulates. Diseases- free locust beans were surface sterilized with 10% (v/v) sodium hypochlorite solution and rinsed three times in running tap water and allowed to dry. A sterilized needle was used to inoculate fungal hyphae isolated from the locust bean sample and then placed on fresh samples onto the surface of steririlized locust beans. Controls were inoculated with sterile distilled water. Materials were placed on the laboratory bench. Re isolation from the disease areas were done on the 4^{th} day post inoculation and onto PDA plates and incubated at 25.7^oc for three days. Symptoms of fungal growth were recorded.

Microscopic examination

Fungi were stain with cotton blue lactophenol and observed with a compound microscope at a magnification of ×10, ×40. Morphological characteristics of the isolated fungi were determined as described by Dorothea *et al.* (1976) [6]. Length of the hyphae was determined with eyepiece graticule.

Statistical Analyses

Data were analyzed statistically using one way analysis of variance (ANOVA) and differences among the means was tested for significance at $P \leq 0.05$ using SSPSS, 16.0.

Results

Identification, characterization and frequency of occurrence of the isolated organisms

Fungal infection was recorded from all the locust bean samples. A total of 67 isolates were identified which belong to the two species of *Aspergillus, Candida albican, Botrytis spp, Penicillium spp and Curvularias spp.* Thus were *A. fumigatus* 16 (23.88%), *A. flavus* 13 (19.40%), *Candida albican 6(17.90 %) Penicillium spp* 11 (16.41 %), *Curvularias spp 9 (13.43%), Botrytis spp 6* (8.95%). While control plates showed no growth (Table 1).

Variation in isolates counted in Wudil and Darki markets

The highest number of isolates 36 (53.73%) was recorded at Wudil market, while at Darki market 31 (46.26 %) isolates were recorded (Table 1). The highest number of isolates was recorded on the fourth week with 17(25.35%). While the least isolates were recorded on the second and fifth weeks 11 with (16.41%) recorded for each week (Table 2).

Variation in isolates counted on Mondays and Thursdays

More isolates were counted on Monday 40 (59.70 %) than Thursday 27 (40.29 %) (Table 3). The differences between fungi isolated on Mondays and Thursdays was statistically significant ($P>0.001$).

Pathogenicity tests

The results of the pathogenicity tests confirmed the Koch's postulates for identification of the causative agent of a particular disease. The pathogen where present in all cases of the disease. All the fungi isolated were pathogenic on the beans (Table 4).

Table 1: Number of fungal isolates counted at Wudil and Darki Markets

Identified Fungi	Wudil Market	Darki Market	Total	Mean	%
A. fumigatus	10	6	16	8	23.88
A. flavus	6	7	13	6.5	19.40
C. albicans	5	7	12	6	17.9 0
Botrytis spp	4	2	6	3	8.95
Penicillium spp	7	4	11	5.5	16.41
Curvularias spp	4	5	9	4.5	13.43
Total	36	31	67	33.5	99.99

Table 2: Total Number of Fungal isolates counted on weekly basis at Wudil and Darki Markets

Colonies	Weeks					Total	Mean	%
	1	2	3	4	5			
A. fumigatus	3	2	4	5	2	16	8	23.88
A. flavus	3	2	3	2	3	13	6.5	1940
C. albicans	2	3	2	3	2	12	6	17.90
Botrytis spp	2	2	3	4	2	6	3	8.95
Penicillium spp	2	2	3	3	1	11	5.5	16.41
Curvularias spp	2	2	3	2	1	9	4.5	13.43
Total	12	11	16	17	11	67	33.5	99.99

Table 3: Number of fungal isolates counted on Mondays and Thursdays at Wudil and Darki Markets

Identified fungi	Mondays	Thursdays	Total	Mean	%
A. fumigatus	9	7	16	8	23.88
A. flavus	8	5	13	6.5	19.40
C. albicans	9	3	12	6	17.9 0
Botrytis spp	5	1	6	3	8.95
Penicillium spp	6	5	11	5.5	16.41
Curvularias spp	3	6	9	4.5	13.43
Total	40	27	6 7	33.5	99.99

Table 4: Pathogenicity test after inoculation for 4days on fresh Locust beans

Fungi	*A. fumigatus*	*A. flavus*	*C. albicans*	*Botrytis* spp	*Penicillium* spp	*Curvularias* spp
Pathogenicity	+	+	+	+	+	+
Test	+	+	+	+	+	+

Discussion

All samples collected from the two markets showed fungal infection. A total of 67 fungal isolates were isolated during the study. Fungi isolated included *A. fumigatus, A. flavus and C. albicans* (yeast), *Botrytis* spp, *Penicillium* spp and *Curvularias* spp. The fungus *A. fumigatus* was the most frequently isolated from the two markets while *Botrytis* spp was the least common fungus. Pathogenicity tests confirmed the fungi as originally isolated pathogen of locust beans. More isolates were recorded at Wudil markets then Darki markets. Also higher numbers of isolates were recorded on Monday exposure than Thursday exposure.

In a related study Kumasi *et al* (2009) [12], Molnár *et al*. (2015) [14] and Yahaya *et al*., (2018) [23] observed that many of the post-harvest diseases of grains and legumes are the result of infections by pathogens in the field which continue to develop after harvest Omafuvbe *et al.,* (2000) [20] and Bigneil (2010) reported that species of bacteria and fungi affect locust bean seeds and its products causing spoilage when stored. Grains readily spoil due to microbial activities and are generally short lived unless steps are taken to remove, kill or prevent growth of associated microorganism (Omafuvbe *et al.* 2000; Richard1 *et al*. 2017) [20, 21]. The pathogens can occur on growing crops as well as harvested commodities leading to damage ranging from rancidity, odour and flavour changes and germ layer destruction. In a related study on maize grain Adamu (2002) [1] concluded that the high number of fungal colonies isolated on crushed and uncrushed maize could be due to physical damage and harvesting puncture, which could occurred during harvesting and handling.

The study showed that *A. fumigatus and A. flavus* had the highest frequency of occurrence on locust beans sampled from Wudil and Darki markets, although it was clear that other pathogens were present. This was similarly reported by Lassois *et al*., (2010) [13].

The high colony counts obtained at Wudil could be attributed to poor storage facilities and exposure to harsh environmental conditions. At Wudil market it was clear that merchants stored their locust beans in dirty sacs, on the ground near gutters, open stalls and dirty areas. This promotes fungal growth due the conducive environment to infest the locust beans seeds. The longevity of the infected pods and seeds varies considerably depending on the environmental conditions as reported by (Mordue 1971) [15].

The higher count obtained on Monday could be due to heavy activities with high influx of customers from different locations for buying and selling on Fridays which is the market day at Wudil (Two days before each Monday sample collection at Wudil and Three days before sampling at Darki). A statistical significant (P = 0.001) was obtained between the two sampling days.

The four fungal species namely *A. fumigatus, A. flavus Candida albicans*, *Botrytis* spp, *Penicillium* spp and *Curvularias* spp are the common post harvest fungi associated with locust beans on sale at the studied markets. The results obtained in this study indicated that the Darki, market is the more suitable for selling of lucust beans. This is likely because there are less buyers and sellers at Darki market than at Wudil markets. More over the merchants at Darki markets use relatively better storage facilities than at Wudil markets. Wudil market is also one of the leading cattle markets in Northern Nigeria. So animal faeces contribute to the contamination of the market result in infection of legumes, grains and other products in the area surrounding the market. This may have accounted for the high isolate count at Wudil. To safe guard consumers from buying produce which may be a health hazard effort should be made to ensure that good storage facilities are enforced in all the markets in Kano State.

References

1. Adamu B. Post-harvest losses of Maize grain Grown at Gombe State, Nigeria. Unpublished Msc. Thesis. Abubakar Tafawa Balewa University, 2002.
2. Allen DJ. The pathology of tropical food legumes: disease resistance in crop improvement. Chichester, UK: John Wiley & Sons, 1983.
3. Anyanwu AC. Principle of crop production 6th edition, 2003, p160–166.
4. Bigneil E. Molecular biology and genomic: Caister Academic Press. Clark T.F, 1966.
5. Bukar A, Mukhtar MD, Adamu S. Isolation and identification of postharvestspoilage fungi associated with sweet oranges (*citrus sinensis*) traded in Kano metropolis. Bayero Journal of Pure and Applied Sciences, 2009; 2(1):122–124.
6. Dorothea F, Ronald J, Ronald CB. *A Colour Atlas of Pathogenic Fungi*. Wolfe Medical Publication Ltd., 1976, p12-53.
7. FAO. Database, Food and Agriculture Organisation, Roma, Italy, 2002. URL: http://apps.fao.org/lim500/nph wrap.pl.
8. Fagbohun ED, Lawal OU. Effect of Storage on nutrient composition and mycoflora of sundried soyabean (Glycine max). African Journal of Food Science, 2011; 5(8):473-477.
9. Falaye OS, Fagbohun ED. Effects of storage on the proximate, mineral composition and mycoflora of “tinco” Dried meat sold in Oshodi, market Lagos State, Nigeria Global. Journal of Biological Science and Biotechnology, 2012; 1(1):54-58.

10. Farombi EO. African indigenous plants with Chemotherapeutic Potential and Biotechnological, 2003, p12-29.
11. Guarro J. Fusariosis, a complex infection caused by a high diversity of fungal species refractory to treatment. European Journal of Clinical Microbiology Infection and Disease, 2013; 32:1491–1500.
12. Kumasi UN, Umar K, Faruk G. Review of Losses of grains on sale on some selected markets of Keffi. Unpublished, Msc. Thesis, University of Sokoto, 2009, p10-23.
13. Lassois L, Jijakli MH, Chillet M, de Lapeyre de Bellaire L. Crown rot of bananas: preharvest factors involved in postharvest disease development and integrated control methods. Plant Disease, 2010; 94:648–658.
14. Molnár O, Bartók T, Szécsi Á. Occurrence of *Fusarium verticillioides* and *Fusarium musae* on banana fruits marketed in Hungary. Acta Microbiol Immunol Hung, 2015; 62:109–119.
15. Mordue JEM. Glomerella cingulata. CMI Descriptions of Pathogenic Fungi & Bacteria No. 315. Wallingford, UK: CAB International, 1971.
16. Mordue JEM. Colletotrichum lindemuthianum. Descriptions of Pathogenic Fungi and Bacteria Set 32, Sheet No. 316. Wallingford, UK: CAB International, 1971a.
17. Oerke EC. Crop losses to pests. J. Agric. Sci, 2005; 144:31-43.
18. Ogaraku AO. Deleterious effects of fungi on Post-Harvest Crops and their management Strategies. Arun A. and Analia E.P. (Eds.). In: Management of Fungi Plant Pathogens. CAB international U.K, 2010, 28-35.
19. Ogbadu LJ, Okagbue RN. Fermentation of African locust bean parkia biglobosa seeds. Involvement of different species of Bacillus. Foods Microbiology, 1988; 5:195-199.
20. Omafuvbe BO, Shonukan OO, Abiose SH. Microbiological and Biochemical changes in the Traditional Fermentation of Soya beans – dawadawa Nigerian food condiment. Food Microbiology, 2000; 17:469-474.
21. Richard1 BI, Ukwela MU, T Avav T. Major Diseases of Groundnut (*Arachis hypogaea* L.) in Benue State of Nigeria. Asian Journal of Advances in Agricultural Research, 2017; 4(3):1-12.
22. Yahaya SM, Fagwalawa LD, Ali MU, Lawan M, Mahmud S. Isolation and Identification of Pathogenic Fungi Causing Deterioration of Lettuce Plant (*Lactuca sativa*) A Case Study of Yankaba and Sharada Vegetables Markets. Journal of Plant Science & Research, 2016, 3(1).
23. Yahaya SM, Abubakar Y, Ali MU, Lawan M, Ajingi YS, Haruna M, *et al*. Fungal Infection of Banana (Musa sapientum) Sold at Wudil and Yanlemo Markets of Kano State. Dutse Journal of Pure and Applied Sciences, 2018, 4(1).