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Isolation and Identification of Pathogenic Fungi Causing Deterioration of Lettuce Plant (*Lactuca sativa*) A Case Study of Yankaba and Sharada Vegetables Markets

Research article

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Abstract

Experiment was carried out to determine the fungal pathogens responsible for post-harvest losses of Lettuce on sale at Yankaba, and Sharada, market for a period of three months (September, 2014 to December, 2014). Sample of lettuce was collected and analyzed for the presence of fungal species using standard methods. Fungal deterioration in the two markets were mostly due to *Candida albican, occur with 30.1%, Aspergillus niger* with the rate of 31.1%, while *Aspergillus fumigatus and Aspergillus flavus* had occurrence rates of 25.5% and 13.8% respectively. The differences in fungal deterioration of the lettuce was found to be statistically significant (P>0.05) between the two sampling dates. The results of this study found that a post-harvest loss of the lettuce in the two vegetable markets was due to attack by fungal species.

Keywords: Post-harvest, Mould, Irrigated areas, Incidence

Introduction

Lettuce, *lactuca sativa*, is a member of the family Asteraceae (Compositae) [1]. Lettuce is annual herbaceous plant with broad leaves of variable sizes and form arranged in a rosette. It has a single main root with broad fibrous lateral branches. The stem is cylindrical and contains latex. Lettuce is one of the UK vegetable crops [2,3]. It is important in most temperate climate production systems and in other regions like West Africa where it is the most popular salad crop [2,4]. In addition to its main use as a leafy green, it has also gathered religious and medicinal significance over centuries of human consumption.

Lettuce cultivars can be grouped into four of five types, based on the plant form and use. The crisphead (or iceberg) forms form a closed heads which is resistant to mechanical damage. These are the most important type grown and form the bulk of production in Europe and America [5]. The butter head type forms loose open heads and soft leaves easily damage in handling. Leaf lettuce also shares this fragile nature. Cos or romaine lettuce has erect, elongated leaves that forms a loose leaf-shaped head. Stem lettuce is grown for its thickened parenchymatous stem harvested when the plant is still in the vegetative stage [6,7].

Lettuce has very high water content, 94-95% in the various forms but with some variation in the nutritional content. Crisphead lettuce supplies amounts of ascorbic acid (7 mg/100g), vitamin A (470 I. U. /100g) and calcium (22 mg/100g). On the other hand butterhead lettuce contributes 8 mg of ascorbic acid, 1065 I. U. of vitamin A and 35 mg of calcium per 100 g of edible product. Cos lettuces are

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more nutritious. They supply 22 mg of ascorbic acid, 1925 I. U. of vitamin A and 44 mg of calcium in 100 g of edible product [8,1]. Leaf lettuce supplies 18 mg ascorbic acid, 1900 I. U. of vitamins A, 68 mg of calcium in 100 g of edible product. Lettuce, regardless of type, also supplies some phosphorus, iron, sodium and potassium [9,1].

Diseases causes a serious problem for lettuce growers and marketers; and for many diseases there are no opportunities for their control once the crop has been infected. Lettuce diseases may result from attack by viruses, fungi, bacteria, nematodes or from a non-pathogenic source. Some diseases are seed-borne [10], others are air-borne [5], while others are transmitted by insects or microorganisms [5].

Large quantities of vegetables are produced in Nigeria. However, accurate production figures are not available. In previous studies [11] reported that production of vegetables is seasonal resulting in abundant supply during the rainy season and scarcity at dry seasons. Due to their soft texture they are easily wounded as a result of harvesting, and other post harvest handling operations such as packaging, transportation and storage [12,13] reported that the long chain of marketing system of vegetables between the producer and consumer makes it difficult to accurately assess the level of damage in many crops in Nigeria. In [14] FAO report indicated that Nigerian vegetables have not been able to meet world standard because of poor harvest handling. The present study was aimed at identifying fungal species associated with post-harvest losses in Lettuce with a view to providing baseline information for the development of control strategies for reducing post-harvest losses which will increase availability and reduce cost of the produce.

Material and Methods

Sampling Site

Sharada: is located at Gwale local government area of Kano state a distance of about 50 Klm away from Kano University of Science and Technology, Wudil. It is one of the large vegetable markets in Kano state. There are no fruits and vegetables grown in Kano state that are not found at Sharada. Despite being one of the largest vegetable growing and selling markets at Kano metropolitan, there are no good storage facilities at Sharada market. Some marketers store their fruits and vegetables on the floor of the stores, while others kept theirs packed in baskets. Marketers hardly used chemicals on their fruits and vegetables. They however washed them either with water or detergents.

Yankaba: is located at Nassarawa Local Government area with a distance of about 50klm away from Kano University of Science and Technology, Wudil. It is one of the large vegetable markets in Kano state. There are no fruits and vegetables grown in Kano state that are not found at Yankaba. Despite being one of the largest vegetable growing and selling markets at Kano metropolitan, there are no good storage facilities at Yankaba market. Some marketers store their fruits and vegetables on the floor of the stores, while others kept theirs packed in baskets. Marketers hardly used chemicals on their fruits and vegetables. They however washed them either with water or detergents.

Sample Collection

Lettuce was collected from Sharada and Yankaba markets Kano State. Samples were collected two times in a week, Mondays and Thursdays respectively which lasted for three months. The samples were collected directly from the market inside polyethene bag (container) to the laboratory in (K U S T Wudil) for analysis.

Isolation Media

Potato dextrose agar was the media used for isolation of post-harvest fungi in lettuce sample. Thirty nine grams of PDA were weighed using weight balance and suspended in 1litre of distilled cool water. The contents were sterilized by autoclaving machine at 1210c for 15 minutes. These were allowed to cool for 5 minutes on a laboratory bench until the temperature fell to 450oc.ten milliliters of lactic acid were added to inhibit bacterial growth. The media was dispensed into sterilized Petri dishes of 9cm diameter.

Procedure for Isolation of Fungi

The isolation was carried out according to the method described by [14-16] as follows 39g of powdered PDA was weighed using weighing balance and dispense in one liter of distilled water, stirred thoroughly before been place inside the flask bottles and autoclaved inside the machine. The media was later poured into sterilized petri dishes and allowed to solidify. The sample obtained from Kwakwachi irrigation area was sterilized by washing in running tap water and allowed to dry. Portions (2mm) were cut with a sterilized cork borer. Cut pieces were placed on PDA and incubated at 25.7 ±2 c for 3 days.

Colony Count and Subculture

Fungal colonies that grown were observed accounted and recorded. Each distinct colony was sub cultured into fresh PDA media to facilitate possible identification.

Determination of Pathogenicity of Isolated Micro Organism

Pathogenicity tests were conducted to prove Koch postulate. Healthy leaves of lettuce vegetable surface sterilized in 10% (v/v) sodium hypochlorite solution and rinsed in 3 changes of running tap water and allowed to dry. A ruler was used to mark a (2mm) diameter circle on each sample and sterilized needle was used to streak fungal hyphae on the marked portions. Controls were inoculated with distilled water. Both were placed on the laboratory bench. Sterilized forceps were used to remove portions from the diseased areas on the 4th day and placed on freshly prepared PDA plates and incubated at 25.7 ±2oc for 3 days. Fungal growth that appeared was recorded.

Microscopic Examination

For each examination, a streak of fungal mycelium was placed on a clean glass slide. One drop of cotton blue lacto phenol was added and the cover slip placed. The slide was mounted on the microscope and observed at magnification of ×10, ×40 and ×100. Morphological characteristics of fungi isolated were determined and identified using method described by [17]. Lengths of the hyphae were determined with eyepiece graticule by using colonial and morphological characteristics.

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Photography

Photographs of fungal mycelia were taken from mounted slide using camera Lucida at Biology Department, Kano University of Science and Technology, Wudil.

Statistical Analysis

Data collected on fungal colonies was analyzed using s Chi-square analysis. This was achieved using computer program Grap PAD insert Version 1.15 copyright 1990 [18].

Results

Infection of fungal *species* was recorded on all the leaf samples. A total of 987 colonies were counted and recorded which belong to the *C. albican* and the three species of *Aspergillus*. Thus were *Candida albican 294(%) A. niger {307 (31.1%}), A. flavus {135 (13.8%}), A. fumigatus {252 (25.5%)}*. Control plates showed no growth (Table 1).

Variation of the colony counted in Sharada and Yankaba market

Sharada area recorded the highest colony count with 617 (62.5%). Where a total of 211 (56.1%) and 406 (66.4%) colonies was recorded on Monday and Thursday respectively. The least colony count was recorded at Yankaba, market area with a total of 370 (37.4%), comprising 165 (43.8%) and 205 (33.5%) on Monday and Thursday respectively.

Variation in the colony counted on Monday and Thursday

A total of 376(38.0%) were recorded in the Monday, while 611 (61.9%) were recorded during Thursday exposure (Table 2).

Pathogenecity test

The results of the pathogenicity test confirmed all the four criteria outline in Koch postulates for identification of the causative agent of a particular disease. The pathogen where present in all cases of the disease. The same pathogens was isolated from the diseased host and grown in pure culture. When inoculated into a healthy sample of lettuce plant the pathogen from the pure culture causes the same disease. The same pathogen was reisolated from the new host and shown to be the same as the originally isolated pathogen (Table 3).

Table 1: Number of colonies isolated from the two markets on Monday and Thursday.

	Location					
Colony	Sharada	Yankaba	Total		Mean	% abundance
A. niger	41	266	307		102.3	31.1
A. flavus	80	55	135		45.0	13.8
A. fumigatus	25	227	252		84	25.5
C. albican	35	259	294		98	30.1
Total			987		329.3	100

Table 2: Variation in the colony count of all the species for the two selected sites on samples collected on Monday and Thursday.

Location	Monday	Thursday	Total	% abundance
Sharada	211	406	617	62.5
Yankaba	165	205	370	37.4
Total	376	611	987	100

Table 3: Pathogenicity Test.

Effect of fungal isolates on Lettuce *Lactuca sativa*				
	Inoculated		Control	
Test organisms	Diameter on 4th day	Texture on 4th day	Diameter on 4th day	Texture on 4th day
A. niger	2.8	Rot	1	Turgid not soft
A. flavus	2.2	Soft	1	Turgid not soft
A. fumigatus	1.6	Soft	1	Turgid not soft
Candida albican	2.5	Rot	1	Turgid not soft

Discussion

A total of 387 colonies were isolated during the study. Fungi isolated include *A. niger, A. flavus and C.albican* (yeast). The genera *A. niger* was the most frequently occurring with the percentage occurrence of 31.1% followed by *C. albican* with percentage occurrence of 30.1%, *A. fumigatus* with percentage occurrence of 25.5% the least occurring colony was *A. flavus* with percentage occurrence of 13.8%. Pathogenicity test confirmed the pathogens as originally isolated pathogen of lettuce sample from Sharada and Yankaba markets. More colonies where recorded at Sharada then Yankaba markets. Also high number of colonies was recorded on Monday exposure than Thursday exposure.

Alao [19] reported that many of the post-harvest diseases of fruits and vegetable, grains and legumes are the result of infections by pathogens in the field which continue to develop after harvest. In a related study [20] reported that fungi are the most common cause of spoilage on fruits and vegetables and several fungi like *penecillium* spp, *cladosporium* spp, and *altternaria phomopsis* are known to cause large scale storage loss of fruits and vegetables from harvest to storage.

The finding of this research support the report of [13] who studied fungal deterioration of some vegetables in northern Nigeria and found that losses in spinach is attributed to the activities of *A. niger, A. fumigatus , Mucor, and Rhizopus stolonifer*. The finding also agrees with the work of [21,20] who isolated, *A. niger, A. flavus, Rhizopus, and Mucor* from samples of vegetables grown at Nassarawa local government area of Kano state.

The high colony counts obtained at Sharada could be attributed to the nutrients effluent discharge in the surrounding household and industries around the area, which were used for infecting these fields. Such effluents could contain some nutrients that might favour the growth of the fungi as against the lower number of colonies isolated at Yankaba market where area is free from household and industrial effluents [21,22].

The higher count obtained on Monday could be due to heavy activities with high influx of customers from different locations on the day for buying and selling. Statistical difference of (P >0.05) was obtained between the two seasons (Table 2).

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It can be concluded that the four fungal species namely *Candida albican*, *A. niger, A. fumigatus*, and *A. flavus,* are the common post harvest fungi associated with lettuce on sale at the studied markets. The results obtained in this study indicate that Yankaba, site is the most suitable for marketing of fresh and healthy vegetables. This is because in Yankaba area there is total absence of household and industrial effluents in the area surrounding the market this accounted for the least colony count. Sharada site is the least suited for marketing of vegetables because, effluents from household and industries in the surrounding area were the source of infection. The effluents might contain toxic chemicals that on long time exposure could pose serious health hazards to the consumers of these vegetables. Therefore, to safe guard the consumers from buying produce which may be of health hazard effort should be made ensure that all effluents from household and industries be adequately treated before discharge into the surrounding environment. Likewise marketing of crops should be prohibited in any area close to household and industrial effluents discharge [23].

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