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RESEARCH ARTICLE

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A Survey of Mycoflora of Garlic Cloves (*Allium sativum L.*) In Sokoto Metropolis, Nigeria

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ABSTRACT

A survey of mycoflora associated with garlic cloves (Allium sativum L.) was conducted in five different markets site in Sokoto metropolis, Sokoto State. A total of 135 garlic (cloves) were collected, isolated and identified based on their morphological and cultural characteristics. Result shows that out of these 135 samp1es, six different types of fungal species were isolated and identified. These fungal species includes: Aspergillus niger, Absidia crymbefera, Mucor racemoses, Rhizopus stolonifer, A. flavus, and A. terrus. Among these fungal species, Aspergillus niger was found to have higher frequency of occurrence with 20(24.7%), Absidia crymberfera followed with 18 (22.2%), followed by Mucor racemoses having a frequency of 15 (18.5%), then Rhizopus stolon with 12 (14.8%) which is also followed by A. flavus and A. terrus having appearance of 9(11.1%) and 7(8.6%) respectively. Further research work should be carried out to be able to determine whether these fungi can be transmitted to seedling on the field.

Keywords: Mycoflora, Garlic cloves, *Allium sativum*, Fungal species.

INTRODUCTION

Allium sativum L. commonly known as garlic, is a species in the onion family *Alliaceae*. Its close relative includes the onion, shallot, leek and chive. Garlic has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, spicy flavor that mellows arid sweetens considerably with cooking [1]. A bulb of garlic, the most commonly used part of the plant, is divided into numerous flashy sections called cloves. Single clove garlic (also called pearl garlic or solo garlic) also exists, it originate in the Yunan Province of China.

Nutritionally, garlic has been known to 'thin the blood', much in the same way as fish oils. It can help in lowering h pressure and evidence is building in its use in lowering blood cholesterol levels [2]. Briethaupt reported that garlic cloves contain proteins 8%, starch 15%, potassium 620mg/100g and vitamin C I7mg/100g [2].

Properties of garlic

When crushed, *Allium sativum* yields allicins, a powerful antifungal compound (phytoncide). In some cases, it can be used as a home remedy to help speed recovery from strep throat or other minor ailment because of its antibiotic properties. It also contains the sulfur containing compounds allin, ajoene, diallyisulfide, dithim, S-allylcysteine, and enzymes, vitamin B_2, proteins, minerals, saponin, flavonoids arid maillard reaction products, which are non-sulfur containing compounds. Furthermore a phtoalexin called allixin (3- hydroxy-5-methoxy-6-methyi-2-penthyl-4H-pryan-4-one) was found, a non—sulfur compound with a γ-prone skeleton structure with anti-oxidative effects, [1], anti-microbial effects, [3], anti-tumor promoting effects, [4], inhibition of aflatoxin B_2 DNA binding, [4], and neurotrophic effects. Allixin showed an anti-tumor promoting effect in vivo, inhibiting anti-tumor formation by TPA

in DMBA initiated mice. Analogs of this compound have exhibited anti-tumor promoting effects in in-vitro experimental conditions. Here in, allixin and its analogs may be expected useful compounds for cancer prevention or chemotherapy agents for other diseases.

The composition of the bulbs is approximately 84.09% 13.3 8% organic matter, and 1.53% inorganic matter, while the leaves are 87.14% water, 11.27% organic matter, and 1.59% inorganic [4].

The phytochemicals responsible for the sharp flavor of garlic care produced when the plant's cells are damaged. When a cell is broken by chopping, chewing or crushing, enzymes stored in cell vacuole triggers the breakdown of several sulfur-containing compounds stored in the cell fluids. The resultant compounds are responsible for the sharp of hot taste and strong smell of garlic. Some of the compounds are unstable and continue to evolve over time. Among the members of the onion family, garlic has by fear the highest concentration of initial reactions productions, making garlic much more potent than onions, shallots, or leeks, [5]. Although, people have come to enjoy the taste of garlic, these compounds are believed to have evolved as a defensive mechanism, deterring animals like birds, insects, and worms from eating the plant [6].

A large number of sulfur compounds contribute to the smell and taste of garlic. Dialyl disulfide is believed to be an important odour component. Allicin has been found to be the compound most responsible for the 'hot' sensation of raw garlic. This chemical opens thermo TRP (Transient Receptor Potential) channels that are responsible for the burning sense of heat in foods. The process of cooking, garlic removes allicin, thus mellowing its spiciness [6].

Garlic has been cultivated for such countless ages and it is believed to have grown in the wild areas where it has become naturalized, it probably descended from the species *Allium longicuspis* which grows wild in South Western Asia. [7]Reported thatof about 700 species of genus AIlium, many are native to central Asia. The wild garlic, crow garlic and field garlic of Britain are the species *Allium ursinum*, *Allium vineale*, and *Allium oleraceum* respectively, in North America, *Allium vineale* (known as wild garlic or crow garlic) and *Allium canadense*, known as meadow garlic or wild garlic and wild onion are common weeds in fields [5].

In Africa, garlic was found in Egypt around 3000 BC, fromEgypt, it spread to other African countries, among them are Uganda (East Africa) and Nigeria in West Africa around 1842 [8]. Nigeria was among the top 20 (twenty) producers of garlic in Africa, garlic are mainly grown in Kano and Sokoto State. In Sokoto, garlic is grown extensively in Goranyo, Gwadabawa, Kware and part of Wamakko (Kwalkwalawa and Dundaye) local government areas of the Sokoto.

MATERIALS AND METHODS

Study Area

The samples were collected from kwalkwalawa river, in Sokoto, North-western Nigeria. Sokoto lies between longitudes 4^0 8'E and 6^0 5'E, and latitudes 12^0 N and 13^0 58'N [9]. The climate of Sokoto is tropical continental, with much of the rains between June and September, while the long dry season is from October and May [10].

Sample Collection

Samples of apparently healthy and mouldy garlic cloves were collected from 5 (five) different markets in Sokoto Metropolis. They were separated and placed in a sterile polythene bags and then labeled appropriately. They were then brought to the Mycology Laboratory of Biological Sciences Usmanu Danfodiyo University, Sokoto for fungal isolations and identification.

Media Preparation

The medium used in this study was Sabouraud's Dextrose Agar (SDA). The media was prepared in accordance to the manufacturer's instructions sixty-four grams (64g) of SDA was weighed and dissolved in 1000mls of distilled water. The pH was adjusted to 5.6, the media was well shake and then autoclaved at 121°C for fifteen (15) minutes. The sterile medium was allowed to cool down to 45°C before it was poured into sterile Petri dish.

ISOLATION

Surface Sterilization

The samples were surface sterilized for 60 seconds in 1% sodium hypochiorite and rinsed in three changes of sterile distilled water. Segments of the surface sterilized garlic cloves about 5mm in diameter were placed on SDA [11] and incubated at room temperature (28±2°C) for 5 days at which time, the development and growth o f the fungi were evident on the medium.

Sub-Culturing

More SDA plate were prepared and allowed to solidify. A small portion of each of different fungal colony was singly placed in the center of the SDA plate and allowed to be inoculated at room temperature (28±2^0C) for 5 days. Sub-culture was done to obtain the pure isolate. The developing fungal colonies were sub-cultured repeatedly on fresh SDA plates until pure cultures of the isolates were obtained [12].

Fungal Identification

The pure cultures of the isolates obtained were subjected to microscopic examination with the view to identify the organism present in the garlic cloves. Clean grease-free glass slide was used for the identification. A drop of water was placed in the center of the slide, a small portion of the fungal culture was cut out with a sterile inoculating needles. The 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, damped with the chips. The viewing was first done with the lower magnification (X_4) then the higher magnification (X_{10}) objective. The nature of the mycelium, the types of fruiting body and the spore structure served as the criteria or the identification of the isolates. The isolates were identified and confirmed with the mycological atlas: (Fisher, 1988).

The isolates were identified based on morphological andcultural characteristics in accordance with [13] and [14].

RESULTS AND DISCUSSION

Mycological analysis of the garlic cloves revealed that mouldy and healthy garlic cloves were infected with fungi. Six different fungal species were isolated from the mouldy and healthy garlic cloves. These isolated fungal species are:-*Aspergillus niger*, *A. terreus*, *Mucor* sp., *Rhizopus* sp., *A. flavus*, and *Absidia Crymbera* which is shown in Table 1 below.

Table 1: Incidence of fungi associated with spoiled garlic cloves

Fungi identified	Frequency of occurrence				
	G.N	T.K	O.M	N.M	K.D
Aspergillus niger	4	5	2	3	6
Absidia crymbefera	2	4	3	5	4
Mucor racemoses	2	5	2	3	3
Rhizopus stolonifer	1	2	0	5	4
Aspergillus niger	0	3	3	3	0
Aspergillus terreus	2	1	1	0	2

Key:- *G. N: Gawon Nama-T.K: Ungwan Tasha Kura- O.M: Old Market- N. M: New Market- K. D: Kasuwan Daji*

Table 2 below shows the percentage occurrence of the fungi isolated Out of these isolated fungi, *A. niger* was the frequently isolated fungus with 24.691% frequently of occurrence, followed by *Absidiacrymbera* 22.222% and the least encountered fungal species is *Aspergillus terreus* with the frequency of 8.64 1%.

Table 2: Percentage of occurrence of fungi isolated

Fungi identified	Percentage of occurrence (%)	
	Total Frequency of occurrence	%
Aspergillus niger	20	24.7
Absidia crymbefera	18	22.2
Mucor racemoses	15	18.5
Rhizopus stolonifer	12	14.8
Aspergillus niger	9	11.1
Aspergillus terreus	7	8.6

From the results, six genera of fungi which include; *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp., and *Absidia* sp. were isolated from the garlic cloves collected from five different market sites in Sokoto Metropolis.

Results from the work were in conformity with the reports ol previous work by [15] who isolated *Aspergillus*, *Mucor* and *Rhizopus* from other crops such as pepper, tomatoes and Onion. *Selerotium cepivorum* (white rot) was also isolated from garlic by [16]. In the same manner, [17] also reported several cases of *Fusarium cepa* and *F. oxysporum* (Basal or bottom rot) which were found to affect the bulbs of garlic which he considers as secondary invader. Furthermore, *Absidia* sp. and *Puccinia alli* also called *Puccinia porri* is also isolated and it is found to cause rust to vegetable crops in the field, the organisms were isolated from other crops like onion, leeks as well as garlic [18].

Several species of fungal isolates have attracted attention because of their ability to produce toxic metabolites which may constitute a health hazard to animals feeding on the infected substrates as well as decline in crop yield [19]. Differences in the frequency of occurrence of the fungal isolate in this study may be due to environmental factors or variation in prevailing inocula.

CONCLUSION

Although no attempt was made to investigate the possible sources and dispersal mechanism of the pathogens in the present work, but it may be suggested that temperature, exposure tounfavorable and mishandling, storage using unsterilized containers arid inappropriate storage facilities and storage methods may be important in the dissemination of inoculums leading to the infection of the garlic.

However, certain cultural practices can help to reduce the incidence of diseases in garlic. Garlic will keep for 6 to 7 month if it is stored at 32°F and at 65 to 70% relative humidity. High relative humidity will keep the bulbs from dehydrating [17]

Recommendations

Further research work should be carried out to able determine whether these fungi can be transmitted to seedling on the field. Garlic had often been associated with so many fungi therefore proper rotation and planting disease-free seed, should be practiced. Culls and diseased foliage should also be removed either by burning or burying away from the field after harvest. However, further research need to be carried out to determine the made and action of infestation of garlic cloves by these pathogenic pests.

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