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Isolation and Identification of Fungal Diseases Infecting Carrot Plants in Sokoto State of Nigeria

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Carrot plants are often afflicted with myriad of diseases that tend to lessen their eventual yield. With the increasing pressure to provide food for the world sever growing population there is need to curtail the adverse effect of these diseases. This study was aimed at investigating the epidemiological prevalence of some fungal diseases effecting carrot vegetables in selected areas in Sokoto state. To realize these, diseased carrots picked from the study areas were investigated to identify the pathogens affecting them. The carrot samples (leaves and roots) were collected from Moreh, Badageni and Ruggar Liman farm areas for fungal isolation and identification. The result revealed ten fungal species from six genera of *Aspergillus, Fusarium, Penicillium, Mucor, Alternaria* and *Acremonium*. Out of the fungal species isolated, *Fusarium oxysporum* was found to have the highest frequency of occurrence with 31.87% and *Acremonium strictum* had the least with (1.10%). All the farms had one pathogen or the other and are present in both the leaves and roots. Badageni farm had the highest occurrence of the isolates mostly on the leaves followed closely by Moreh farm. The findings revealed that carrots in Sokoto were susceptible to fungal attacks, and *Fusarium species* appeared to be the most active of all pathogens associated with the fungal diseases as such, carrots should be well washed and if possible cooked well before eating.

Keywords: carrot, fungi, infections, isolation, prevalence, species.

1. Introduction

Plant disease has been a major factor affecting food production and human societal development over thousands of years (Palmgren *et al*., 2015; Aisha and Rabi'atu, 2022). Plant disease management faces ever-growing challenges due to increasing demands for total, safe and diverse foods to support the booming global population and improving living standards. In recent times, 20–30% of actual production is lost due to plant diseases per year (Oerke and Dehne 2004; Oerke 2005).

Carrot (*Daucus carota* L.) is one of the most important crops of Apiaceae family. Global production of carrot is estimated at 23,321 metric tons. Africa's global share of production is estimated at 1,054 turmeric tons (World Carrot Museum, 2013). In Nigeria, carrot is commonly grown in the Northern part of the country, particularly in dry season between November and February (Simon *et al*. 2009). The estimated production in Nigeria was reported to have stood at 23,500 metric tons from 27,500 hectares of land (FAO, 2015).

Carrot is attacked by a wide variety of pests and diseases (Hill and Waller, 1990). The major fungal diseases of carrot include *Alternaria* leaf blight (*Alternaria dauci*) and *Cercospora* leaf spot or blight (*Cercospora carotae*), leaf mould, *Fusarium* wilt, target spot or early blight and leaf spots. Some of these diseases are soil borne (Villarreal, 1992). Septoria leaf spot affects plants at any stage of development. Another important fungal disease is powdery mildew caused by *Oidiumlyco persicum.* Some other important soil borne diseases of carrot are bacterial soft rot, cavity spot, cottony rot, crown rot, root dieback, root knot nematode and southern blight (Steven, 2003).

These diseases are harmful to the plants survival and eventual yield. It is therefore important to scout for the diseases that infect plants for the sake of the plants and those that consume them (Douglas, 2005). This study is aimed at isolating and identifying fungi that infect carrot plants in Sokoto State of Nigeria.

2. Materials and Methods

2.1 Plant collection

The study was conducted in Sokoto State. Sokoto State is located in the north western Nigeria, which falls within the dry Sahel Savannah. It also falls between Latitude: 13°05′N to 13.067°N and Longitude: 5°14′E to 5.233°E. Fungal diseases of carrot were identified from the farms selected based on morphological signs and symptoms observed (Drost, 2010). Samples were collected from the selected farms during the 2021 dry season. The three different farms selected were: More, Badageni and Ruggar Liman. They were randomly selected for sampling. Diseased Carrots were collected in sterile polythene bags from 2-3 months old plants. The samples were then transported to the Mycology Laboratory of Usmanu Danfodiyo University, Sokoto, for isolation and identification. The materials used were sterilized and the room and the chamber used inoculated as described by Gupta (2008).

2.2 Media Preparation

Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Carrot Dextrose Agar (CDA) Media were used for bioassay. Sabouraud dextrose agar (SDA), Potato dextrose agar (PDA) and Carrot Dextrose Agar (CDA) were prepared according to manufacturer's instructions (Chessebrought, 2009; Rajam, 2016).

2.3 Inoculation and isolation of fungi

The infected leaves and roots were cut into small segments (3mm in diameter) with a sterilized blade, surface sterilized with 1% hypochlorite for 2 min, plated on media aseptically and then incubated at 28°C for 5 days. A pure culture obtained was maintained by sub-culturing each of the different colonies that emerged onto the media plates and incubated at 28°C for 5 days. As a control, each of the healthy root and leaves were sterilized with 75% ethanol. The sample was cut into small segments (3 mm in diameter) with a sterile blade, placed on the media and then incubated at 28°C for 5 days (Mailafia *et al*., 2017).

2.4 Preparation of slides

Slides of the mycelium observed from the different isolates were prepared as follows: A portion of growing mycelium from the edge of the culture plate was picked with the help of inoculating needle and placed on a clean glass slide then a drop of sterile water was added followed by a drop of methylene blue, covered with coverslip and was observed with the aid of binocular microscope.

2.5 Identification of fungal pathogens (Phenotype)

The Microscope used was MC30 in the Mycology Laboratory of Usmanu Danfodiyo University Sokoto to examine the colony characteristics to establish identity of the fungi. A sterile needle was used in taking a little portion of the hyphae containing spores on the sterile glass slide, stained with lacto phenol cotton blue and then examined under the microscope for fungal structures. The macroscopic/culture features and the microscopic characteristics observed were then compared with fungal identification atlas for identification of the fungi (Snowdon, 1990). The isolated fungus was identified based on colony and morphological characteristics, such as colour and shape observed with the microscope. The morphological characteristics and appearance of the fungi isolated were confirmed and authenticated with the help of Mycological Atlas Book.

2.6 Pathogenicity test of the isolates

The procedures of Onuorah*et al*. (2015), Chukwuka*et al*. (2010) and Baiyewu*et al*. (2007) were used for Pathogenicity test of the isolates. Healthy carrot roots were inoculated with the fungi isolated from the diseased carrot, incubated at 28^0C for five days and observed spoilage. The carrot that showed sign of spoilage were dissected and subjected to fungal isolation. Fifty healthy roots were properly washed with tap water, rinsed with distilled water and surface-disinfected with ethanol. Sterile cork borer was used to bore holes in each of the roots. Each of the isolated fungi was inoculated into the roots after which the cores of the roots were replaced. Sterile petroleum jelly was used to seal the holes of the root to prevent contamination. Fifty roots of carrot were wounded with the cork borers but were not inoculated with the fungi to serve as controls. The inoculated carrot roots and the controls were placed in sterile polythene bags (one fruit per bag). Each of the roots was moistened with wet balls of absorbent cotton wool to create a humid condition. The roots were incubated at 28^0 for five days and observed for spoilage. The fungi were re-isolated from the root and leaves and compared with the original isolates. The decay rate of each microorganism in the healthy roots was determined by measuring its rot diameter after two weeks of its inoculation into the healthy carrot root.

3. Results and Discussion

3.1 Results

3.1.1 Isolation and Identification of Fungal isolates from the diseased carrot

Table1 shows the Macroscopic and Microscopic features of the fungal isolates from diseased

carrots. Ten different colonies were identified based on the cultural and morphological features of the suspected isolates, out of which four were *Aspergillus* spp., two were *Fusarium* spp., and a species of *Penicillium*, *Mucor*, *Alternaria* and *Acremonium* were also Identified. *Aspergillus* spp. was found to be the most abundant isolates from the samples. Badageni fadama recorded the highest occurrence of all the isolates followed by More fadama and the Ruggar liman fadama had the least percentage of occurrence.

Table 1: Identification of fungal Isolates from the diseased carrots

S. No	Observed Isolates	Macroscopy	Microscopy
1.	*Aspergillus flavus*	White-yellow consists of dense felt yellow-green conidiophores.	Conidiophores hyaline, coarsely roughen, phialides borne directly on the mutilate.
2.	*Aspergillus niger*	Colony consisting of a compact white-yellow basal medium feet with dense to black conidiophores	Conidial head radiate tending to splint in loose column with edge conidiophores stipes smooth walled, by a fine but often in a brown radicle born on the metaled.
3.	*Aspergillus fumigatus*	The colonies have distinct margin and appeared greenish brown in colour. The surface has powdery appearance	Conidial head typically columnar. Conidiophores are short, smooth walled green particularly in the upper part.
4.	*Aspergillus nidulans*	It initially appeared yellow and gradually turned orange and completely brown on matured culture.	Conidial heads are columnar, conidiophores are brown, shorts (60-150m in length), and smooth-walled vesicles are hemispherical, small with metulae and phialides occurring on the upper portion.
5.	*Fusarium oxysporum*	Aerial mycelium sparse or floccose, becoming felly, whitish or peach, usually with a purple tinge, more intense near the medium surface. Reverse in shades.	Micro-conidia 0 (-2) septate, borne on lateral, simple (often reduced) phialides.
6.	*Fusarium proliferatum*	Produced white villous colonies with a diameter of 7mm and produced light purple pigment after 7 days on the media.	Produces microscopic, long, canoe-shaped spores called conidia. These asexual conidia have 3 to 7 cells and are produced on specialized hyphae called conidiophores.
7.	*Penicillium notatum*	Texture velvety to powdery; green, blue, gay-green, white. The plate reverse is usually pale to yellowish.	Septate hyphae with branched or un branched conidiophores that have secondary branches known as metulae, on the metulae, arranged in whorls, are flask-shaped sterigmata that bear un branched chains of round conidia.
8.	*Mucor hiemalis*	Colonies creamish yellow in daylight, more yellowish in darkness.	Sporangiophores simple at first, later slightly sympodially branched, often with yellowish bearing dark brown up to (85) m in diameter with deliquescent walls.
9.	*Alternaria alternate*	*Alternaria* sp. grows as long chains with dark brown conidiophores.	Pale or dark brown conidiophores that may be straight or flexuous in appearance. Brownish conidia with short beak or no beak at all.
10.	*Acremonium strictum*	Colonies are flat, with smooth, wet, velvety or floccose texture, sometimes resembling there cottony mounds.	It shows long slender phialides, and conidia are cylindrical or ellipsoidal, formed in slimy bundles at the tips of the phialides.

3.1.2 *Distribution of the Fungal Isolates of Diseased Carrots from More, Badageni and Ruggar Liman farms*

Table 2 shows that ten different species of fungi were isolated from both the leaves and roots of the carrot plant. The result revealed that *Fusarium oxysporum* present in all the three sampled areas. The highest rate of occurrence was found in Badageni farm with 15 for leaves and 9 for the roots. It was also noted that in almost all the cases, the leaves were the most

affected. *Acremonium strictum* had least occurrence and was found only on the leaves of the sample collected from Badageni farm.

Table 2: Distribution of fungal isolates of diseased carrots from More, Badageni and Ruggar Liman farms

Fungal species	Locations					
	More		Badageni		Ruggar Liman	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Fusarium oxysporum	12	7	15	9	5	10
Fusarium proliferatum	3	_	_	_	_	_
Aspergillus niger	9	5	13	_	7	3
Aspergillus flavus	9	5	6	3	3	3
Aspergillus nidulans	4	_	_	_	_	_
Aspergillus fumigatus	_	3	3	3	5	_
Alternaria alternate	6	_	5	7	_	_
Penicillium notatum	4	6	3	_	_	_
Mucor hiemalis	_	_	_	_	3	_
Acremonium strictum	_	_	2	_	_	_

3.1.3 Prevalence of fungal Isolates of Diseased Carrots from More, Badageni and Ruggar Liman farm Sokoto during the 2021 dry season

Table 3 below revealed the prevalence of fungal isolates from the diseased carrot. The result shows that *Fusarium oxysporum* had the highest frequency of occurrence with 58 (31.87%), followed by *Aspergillus niger* with 38 (20.88%). *Acremonium strictum* had the least frequency of occurrence 02 (1.10%).

T**able 3:** Prevalence of fungal isolates in diseased carrot from More, Badageni and Ruggar Liman Fadama in Sokoto during the 2021 dry season.

Fungal species	Frequency of Occurrences	Percentage of Occurrences (%)
Fusarium oxysporum	58	31.87
Fusarium proliferatum	03	1.65
Aspergillus niger	38	20.88
Aspergillus flavus	29	15.93
Aspergillus nidulans	04	2.20
Aspergillus fumigatus	14	7.69
Alternaria alternate	18	9.89
Penicillium notatum	13	7.14
Mucor hiemalis	03	1.65
Acremonium strictum	02	1.10
Total	**182**	**100**

3.1.4 Results of Pathogenicity Test of the Isolates

Signs and symptoms for spoilage after pathogenicity test of the isolates were observed and recorded in Table 4.

Table 4: Pathogenicity test Symptoms of the Isolates

Place of sample collection	Symptoms of Spoilage
More	i. Brownish-black areas with yellow centres on the infected areas.
	ii. Green-brown water-soaked lesions on the leaves with enlarge and turn dark brown or black
	iii. Sunken lesions on the roots
	iv. Small necrotic flecks on the leaves
	v. small water soaked, soft, lesions on crown and roots
	vi. White fluffy growth all over the affected tissues
Badageni	i. Soft and decaying tissues developing
	ii. Brownish-black areas with yellow centres on carrot foliage
	iii. Sunken lesions on the roots
	iv. Green-brown water-soaked lesions on the leaves
	v. The lesion produced distension in the roots surface and changed in colour in the affected area. vi. Loss of weight.
Ruggar Liman	i. Sunken grey lesions on the infected area
	ii. Soft and decaying tissues developing
	iii. White mould appeared and watery discharge
	iv. Loss of weight and change in colour
Control	Watery discharged and loss of weight.

3.1.5 Distribution of fungal Isolates in the diseased carrots from More, Badageni and Ruggar liman Fadama from pathogenicity test

Table 5 present the results of the pathogenicity of the fungal isolates from More, Badageni and Ruggar Liman farms respectively. *Aspergillus* spp. has the highest frequency of occurrence from both the leaves and the roots and the *Mucor* spp. had the least.

Table 5: Distribution of fungal Isolates in the diseased carrots from More, Badageni and Ruggar Liman farm from pathogenicity test

Fungal species	Locations					
	More farm		Badageni farm		Ruggar Liman farm/	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Aspergillus spp	5	3	4	2	3	_
Fusarium spp	2	1	3	1	1	2
Alternaria spp.	1	_	_	_	_	_
Penicillium spp.	1	1	1	_	_	_
Acremonium spp.	_	_	1	_	_	_
Mucor spp.	_	_	_	_	1	_

3.1.6 Distribution of the fungal isolates using Carrot Dextrose Agar (CDA), Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA).

Table 6 showed the distribution of the fungal isolates based on the media used the carrot dextrose agar was able to grow colonies of *Fusarium* spp., *Aspergillus* spp., *Alternaria* and *Acremonium* spp. the Sabouraud dextrose agar

and revealed the presence of *Fusarium* spp., *Aspergillus* spp. and *Mucor* spp. The distribution of the fungal isolates using potato dextrose agar also revealed the presence *Fusarium* spp. and *Aspergillus* spp.

Table6: Distribution of the fungal isolates using Carrot Dextrose Agar (CDA), Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA).

Fungal species	Media					
	CDA		SDA		PDA	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Fusarium oxysporum	+	+	+	+	+	+
Fusarium proliferatum	+	_	_	_	_	_
Aspergillus niger	+	+	_	_	+	+
Aspergillus flavus	_	_	+	+	+	+
Aspergillus nidulans	_	_	+	+	_	_
Aspergillus fumigatus	_	_	+	+	+	+
Alternaria alternate	+	+	_	_	_	_
Penicillium notatum	+	_	+	+	_	_
Mucor hiemalis	_	_	+	_	_	_
Acremonium strictum	+	_	_	_	_	_

3.2 Discussion

Isolation and identification of fungal pathogens associated with carrot in this research revealed ten fungal species from six genera of *Aspergillus, Fusarium, Penicillium, Mucor, Alternaria* and *Acremonium*. Out of the fungal species isolated, *Fusarium oxysporum* was found to have the highest rate of occurrence in all the three areas of sample collection. This is in conformity with the findings of Hong and Pam (2001), that, the most common diseases on carrots produced in Tasmania were crown rot and cavity spot which are caused by *Fusarium* spp. *Acremonium* spp had the least occurrence with (1.10). Different organisms have been isolated from diseased carrot ranging from *Pythium* sp. (McElroy *et al*., 1971), *Thielaviopsis basicola* (Punja and Gaya, 1993), *Fusarium* sp. (Piling and Cox, 1999), *Alternaria* sp. (Saude and Hauberk, 1997). The variations in the isolates obtained from different researchers may be connected to the fact that different varieties of carrots may be used as well as different experimental procedures (Mildenhil, 1975). Difference in the location of sample collection can also be accountable for this.

Determination of the Pathogenicity of fungal species isolated in this research revealed that the isolated fungi are pathogenic to the carrot plant with *Aspergillus niger* exhibiting the highest degree of virulence. This is similar to the findings of Balogun *et al*. (2003) who reported the same result with *Aspergillus niger* having the highest level of virulence in Ilorin, North central Nigeria.

4. Conclusion

This study has investigated the epidemiological prevalence of some diseases effecting carrot vegetables in some selected areas in Sokoto State. The study considered samples from three different farms in Sokoto State. The results revealed that carrots from the areas under consideration were infected by different pathogens. The study recommends that carrot plants should be well treated before consumption.

Conflict of Interest

The author declares that there is no conflict of interest.

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