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Research Paper

Isolation and Identification of Pathogenic Soil-borne fungi Associated with Vegetable Cultivated Soil in Augie Local Government area of Kebbi State, Nigeria

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ABSTRACT: *The investigation was conducted to find out pathogenic soil borne fungal diversity in vegetables cultivated soil collected from Augie local government area of Kebbi state, Nigeria between the month of November to December 2022. Samples were collected from six major vegetables cultivated areas namely, Mera, Mallamawa, Shafarma, Gidan koni, Dankal and Bayawa. A total of 54 soil samples were collected from 18 Vegetable lands, 3 samples from each field. Potato Dextrose Agar (PDA) medium supplemented with Chloramphenocol using soil dilution method and soil plate method was used in the isolation of soil borne fungi. In our investigation a total of 17 isolates were obtained from the soil samples and were identify to genus and species level using morphological features observed on a microscope slides stained with Lectophenol cotton blue and subsequently by means of relevant literatures. From the fungal isolates, species identified belong to 11 genera and 17 species. Three species were identified from the genus Aspergillus, two species from each of the genus Fusarium, Rhizophus and Pennicilium, while one species from each of the genus Pythium, Rhizoctonia, Trichodema Collectotrichum, Pythoptera, Alternaria and Mucor. The identified soil fungi were, Fusarium Sp, Fusarium oxysporum, Pythium Sp, Aspergillus niger, Aspergillus clavatus, Aspergillus flavus, Aspergillus fumigatus, Rhizopus stolonifer, Rhizopus oryzae, Penicillium chrysogenum, Penicillium Sp, Rhizoctonia solani, Trichodema Sp, Colletotrichum Sp, Alernaria Sp, Pythoptera capsici and Mucor sp. Among the identified species Fusarium oxysporum was found to be maximum numbers with 14.1% percentage colony contribution out of the total 1765 colonies isolated from all samples. All the isolates have potential pathogenic effects on certain vegetables plants.*

KEYWORDS: ***Isolation, identification, soil borne, pathogenic, fungi, vegetable, soil***

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I. INTRODUCTION

Large number of organisms can be found in the soils as soil biota making it as a reservoir of many living fauna. The most important among the organisms living in the soil are the soil microflora. This makes soil a large ecosystem inhabiting a variety of living organisms. The important soil microbes are the bacteria, viruses, fungi, protozoan and nematodes (koike, *et al.*, 2003). Soil fungi are the second most abundant soil microbes after bacteria. Fungi are microscopic cells that usually grow a long threads or strands called hyphae, which push their way between soil particles, roots, and rocks. Fungi are very successful inhabitants of soil, due to their high plasticity and their capacity to adopt various forms in response to adverse or unfavorable conditions (Sun et al., 2005). Hyphae are usually only several thousandths of an inch (a few micrometers) in diameter. Single hyphae can span in length from a few cells to many yards. A few fungi, such as yeast, are single cells. They can survive in the soil for many years even in the absence of host plant and can therefore be present in both in cultivated soils; some also in virgin soils. Soil inhabiting fungi play a variety of ecological role as decomposers, mutualist, mycorhiza and pathogens. The pathogenic fungi are usually the dominant organism in the soil. The term soil borne pathogen therefore, can be define as pathogen that can cause plant diseases via inoculum that comes to the plant by way of the soil (koike, *et al.*, 2003). Several group soil borne pathogenic fungi exist in a variety soils ranging from moist to dry soil and from virgin to previously cultivated soil (Villalta, 2012). The most important group of soil borne fungi that cause plant diseases especially vegetables includes *Ascomycetes, Zygomycetes and oomycetes* consisting of different genera and a number of species. Important genera include the well known

fungi such as V*erticillium, Phytophthora, Rhizoctonia* and *Pythium.* These organisms penetrate the plant and decompose the living tissue, creating a weakened, nutrient deficient plant, or death (Jenkins, 2005). Kumar *et al,* (2015) observed a maximum number of fungal colonies belonged to deuteromycotina and few to zygomycotina among which *Aspergillus flavus, A.fumigatus, A.nidulans, A.niger, A.terreus, Penicillium chrysogenum, P.frequentens* were predominant. There is a greater diversity of fungi in the soil of most agricultural fields including vegetable fields. In general soil pathogens are found in the uppermost 12 to 18 inches of the soil. They may occur at greater depths than 18 inches but in fewer numbers than at the shallower depths. For example, Verticilliumb albo-atrum has been found in soils to depths of 36 inches, but the first 12 inches of the soil were found to contain 3 to 4 times the amount of inoculum found in the 12 to 36 inch layer.

Pathogenic soil fungi are responsible for important plant diseases such as damping off, stem and root rots and the vascular wilt diseases. These fungi attack the underground parts of the plant causing damage in a number of different ways.

Today farmers in the area engaged in large scale vegetable productions which now remain the main sources of income to the people and a potential source of revenue to the government. Vegetables crops produce in the area include hot pepper, cucumber, onion, tomato, okra, and groundnuts. Most of these crops produced are transported to the southern, eastern part of the country and neighboring Niger republic. Poor knowledge of the soils and soil microbiota is common among many farmers in Nigeria especially Augie where soil diagnosis has not been given any value in the production process. Document on soil microbiota of Kebbi state and Augie has not been found as at the time of filing this report. Thus, the current investigation was conducted to provide preliminary data on soil borne pathogenic fungi in vegetable cultivated soil in Augie local government area, Kebbi State, Nigeria.

II. MATERIALS AND METHODS

Study site:

The study was conducted in Augie local government area of Kebbi state. Five major vegetable producing villages were involved in the study namely Mera, Nallamawa, Shafarma, Gidan koni, Dankal and Bayawa. From each of the selected villages, three vegetables cultivated fields were randomly selected and three samples were randomly collected from three locations in the field.

Collection of soil Sample:

The soil samples were collected from five different crop fields at five different locations of each of the selected village. Most of the fungi are microscopic and show vast variation quantitatively and qualitatively in different sites of collection and at different depths. Therefore soils samples were collected from a depth of 15cm with the help of a sterilized cork borer pushed horizontally into the ground. The soil caught was emptied into sterilized polyethylene bags. Each sample bag was labelled appropriately by indicating the site of collection, time, date and place of collection. The samples were then taken to the laboratory for laboratory analysis.

Isolation of fungi from the soil samples:

The soil dilution and soil plate method on media such as Potato Dextrose Agar were used as isolation techniques.

Soil Dilution Plate Method (Waksman, 1922):

Soil dilution was made by suspending 1g of soil of each sample in 10ml of sterile distilled water. Dilution of 10-5 was used as a dilution factor to isolate fungi in order to avoid over-crowding of the fungal colonies. 1ml of the suspension of each concentration was added to sterile Petri dishes, in triplicates of each dilution, containing 20ml of sterile Potato Dextrose Agar medium. 1% chloramphenocol solution was added to the medium for preventing bacterial growth, before pouring into Petri plates. The plates were incubated for 24 hours at 25 temperatures until the colonies were formed.

Soil Plate Method (Warcup, 1950): About 0.005g of soil was scattered on the bottom of sterile Petri dish and molten cooled (40-45oC) agar medium PDA was added, which was then rotated gently to disperse the soil particles in the medium. The plates were incubated. One isolate of each fungal genus from each soil sample was selected at random for further sub culturing and experiments. The subcultures were also maintained on Potato Dextrose Agar Slants.

Inoculating Techniques:

The working benches in the laboratory were thoroughly swapped with methylated spirit soaked in cotton wool, and also a burning blue flame was allowed to sterilize the surrounding air before the inoculation proper. The conical flasks were corked tightly with cotton wool and the Petri dishes were fully autoclaved

Identification of the Soil Fungi:

Generally identification of the fungal species was based on morphological characteristics of the colony and microscopic examinations. The colony growth which includes length and width of the colony, the presence or absence of aerial mycelium, the color, wrinkles furrows and any other pigment production were the macro

morphological characters evaluated. Although molecular methods continue to improve and become more rapidly available, microscopy and culture remain commonly used and essential tools for identification of fungal species. The fungi were identified with the help of standard procedure and relevant literature.

Staining Technique for Fungi:
Inoculating needles were flamed over the burning Bunsen burner. Then using the needle, a small portion of the growth on the culture plate was transferred into the drop of lacto phenol cotton blue on the slide. The specimen was tease carefully using inoculating wire loops to avoid squashing and over-crowding of the mycelium. The specimens were observed under the microscope for microscopic identification.

III. Statistical Analysis:

Simpson diversity index was used to determine the diversity of fungal species in the area. Simpson diversity index is a range of value between 0 and 1. The greater the index, the higher the diversity of a species
The percentage contribution of each isolates was assessed.

$$\text{Percent contribution} = \frac{\text{No of colonies of an individual sps in a sample}}{\text{Total number of all colonies of all sps in a sample}} \text{ X } 100$$

Simpson Diversity Index equation

$$D = 1 - \left(\frac{\Sigma\, n(n-1)}{N(N-1)} \right)$$

Where, D = is the diversity index
n = total number of individual species in a sample
N = to number of all species in the whole sample sample
1 = is constant value

IV. RESULTS

4.1 Soil Physical characteristics in the area.
Table 4.1 show the type soil, soil color and soil pH of different soil samples collected in the area. Vegetable cultivated soil in the area includes sandy, loamy, clay loam and sandy loam. Soil colour characteristics ranges from brownish, dark brown, light brown to redish.

Table 4.1 Soil Physical characteristics in the area.

s/no	Village	Sample	Soil Type	Soil colour
1	Mera	A	Sandy	Brownish white
		B	Laomy	Dark brown
		C	Clay loam	Dark brown
2	Mallamawa	A	Sandy loam	Dark brown
		B	Sandy loam	Brown
		C	Sandy	Brownish white
3	Shafarma	A	Sandy	Light brown
		B	Loamy	Brown
		C	Clay/loam	Dark
4	Gidan koni	A	Sandy loam	Dark brown
		B	Loamy	Brown
		C	Sandy	Redish
5	Bayawa	A	Sandy	Redish
		B	Sandy	Redish
		C	Sandy	Redish
6	Dankal	A	Loamy	Redish
		B	Sandy	Redish brown

	C	Sandy	Redish brown

4.2 Fungal species isolated and identified on vegetable soils in Augie Local Government area, Kebbi State, Nigeria

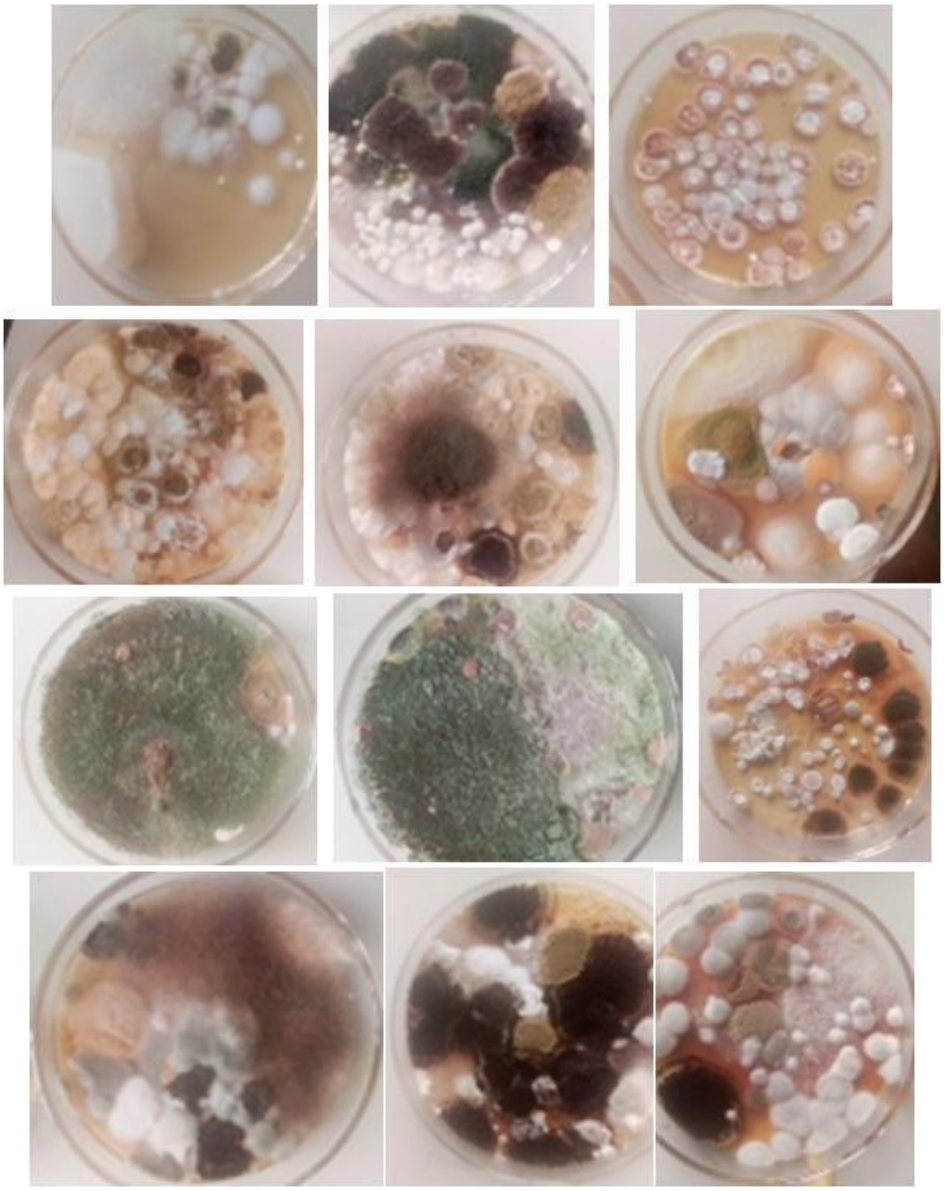
Table 4.2 indicated the fungal species isolated and identified in the area as the major group of pathogenic soil borne fungi associated with vegetable soils in the area. A total of 17 species isolates and 11 genera were recorded in the area. Fungi of the group Ascomycota was the dominant group of fungi recorded in the area. Oomycota, zoomycota and basidiomycota were the least recorded fungal fauna in the area.

Table 4.2 Fungal species isolated and identified on vegetable soils in Augie Local Government, Kebbi state, Nigeria

S/N	Species	Divisions
1	*Fusarium oxysporum*	Ascomycota
2	*Fusarium solani*.	Ascomycota
3	*Pthium* Spp	Oomycota
4	*Aspergillus flavus*	Ascomycota
5	*Aspergillus fumigates*	Ascomycota
6	*Aspergillus clavatus*	Ascomycota
7	*Aspergillus niger*	Ascomycota
8	*Rhizopus stolanifer*.	Zygomycota
9	*Rhizopus oryzae*	Zygomycota
10	*Rhizoctonia solani*	Basidiomycota
11	*Penicillium chrysogenum*	Ascomycota
12	*Penicillium digitatum*	Ascomycota
13	*Trichoderma harzianum*	Ascomycota
14	*Collectriticum pormoides*	Ascomycota
15	*Alernaria* Spp.	Ascomycota
16	*Pythoptera capsici*	Oomycota
17	*Mucor* Spp.	Zygomycota

4.3 Number of individual species in each village and percentage colony contribution

Table 4.3 indicated the total number of individual species isolated in each and their percentage colony contribution. In total 1765 colonies was isolated from all samples with highest isolates obtained from Shafarma Village (414/23.4%) and the lowest isolates was obtained from Dankal (137/7.7%). *Fusarium Oxysporum* has the highest percentage colony contribution of 14.1% and *Aspergillus clavatus* has lowest percentage colony contribution of 1.3%. Simpson index of diversity shows higher fungal species diversity in the area with a diversity index ranging from 1 to 0.99 which is significantly similar from the entire sample.



Isolated colonies from direct and pure culture on PDA

Table 4.3 Number of individual species in each village, percentage colony contribution and species diversity

S/No.	Villages	Total No. of CFU	Average number of individual colonies																
			Fo	Fs	Ps	Af	Afg	Ac	An	Rs	Ro	Rzs	Pc	Pd	Th	Cp	As	Pc	Ms
1	MRA	380/21.5%	52	23	35	12	23	17	28	23	18	15	19	7	27	17	24	38	2
2	MLW	237/13.4	41	20	27	18	10	0	22	13	8	9	0	7	4	8	13	12	25
3	SFM	414/23%	47	33	40	22	33	0	27	21	28	24	0	15	15	17	20	30	42
4	GKN	251/14.2	45	18	23	18	0	0	28	0	0	9	15	0	19	17	11	17	3
5	DKL	137/7.7%	22	7	12	11	0	0	13	9	0	0	10	2	13	4	8	12	14
6	BYW	346/19.6%	42	18	33	15	21	6	12	22	26	0	18	12	17	29	4	31	40
Total		**1765**	**249**	**119**	**170**	**96**	**87**	**23**	**130**	**88**	**80**	**57**	**62**	**43**	**95**	**92**	**80**	**140**	**126**
% contribution			**14.1**	**6.7**	**9.6**	**5.4**	**4.9**	**1.3**	**7.3**	**4.9**	**4.5**	**3.2**	**3.5**	**2.4**	**5.3**	**5.2**	**4.5**	**7.9**	**8.7**
Diversity index			**0.99**	**1**	**0.99**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.99**	**1**	**0.99**	**0.99**

Fs-Fusarium Sp, Fo-*Fusarium oxysporum, Ps-Pythium* Sp, An-*Aspergillus niger, Ac-Aspergillus clavatus, Af-Aspergillus flavus, Afg-Aspergillus fumigatus, Rs-Rhizopus stolonifer, Ro-Rhizopus oryzae, Pc-Penicillium chrysogenum, Ps-Penicillium* Sp, Rs-*Rhizoctonia solani, Ts-Trichodema* Sp, Cs-*Colletotrichum* Sp, As *Alernaria* Sp, Pc*Pythoptera capsici* and Ms-*Mucor* sp

4.4 Percentage colony contribution of individual species from six villages in Augie.

Table 4.4 indicates the percentage colony contribution of individual fungal species isolated from six sampling villages in the area. *Fusarium oxysporum* show the highest colony from all the villages with 17.9, 17.2, 16, 13.6, 12.9 and 11.3 for Gidan koni, Mallamawa, Dankal, Mera, Bayawa and Shafarma respectively. The percentage colony contribution is an indication of the fungal population in the soil. This indicated that there may be higher risk of *Fusarium* related plant diseases in all the six villages. The percentage colony contribution for all isolates ranges from 0.5% to 17.9% from all the villages and the lowest percentage colony contribution was observed from Mera, Bayawa, and Dankal with 0.5% for *Mucor* Sp, 1.1% *Alternaria* Sp and 1.2% for *Pennicilium digitatum* respectively.

4.4 Percentage colony contribution of individual species from six villages in Augie.

S/N	Species	% Colony contribution of individual species in each village					
		MRA	MLW	SFM	GDK	DKL	BYW
1	*Fusarium oxysporum*	13.6	17.2	11.3	17.9	16	12.9
2	*Fusarium solani.*	6	8.4	7.9	7.1	5.1	5.2
3	*Pythium* Spp	3.2	11.3	9.6	9.1	8.7	9.5
4	*Aspergillus flavus*	9.2	7.5	5.3	7.1	8	4.3
5	*Aspergillus fumigates*	3.1	4.2	7.9	0	0	6
6	*Aspergillus clavatus*	6	0	0	0	0	1.7
7	*Aspergillus niger*	4.4	9.2	6.5	11.1	9.4	3.4
8	*Rhizopus stolanifer.*	6	5.4	5	0	6.5	6.3
9	*Rhizopus oryzae*	4.7	3.3	6.7	0	0	7.5
10	*Rhizoctonia solani*	3.9	3.7	5.7	3.5	0	0
11	*Penicillium chrysogonium*	5	0	0	5.9	7.2	5.2
12	*Penicillium digitatum*	1.8	2.9	3.6	0	1.2	3.4
13	*Trichoderma harzimani*	7.1	1.6	3.6	7.5	9.4	4.9
14	*Collectriticum pormoides*	4.4	3.3	4.1	6.7	2.9	8.3

15	*Alernaria* Spp.	6.3	5.4	4.8	4.3	5.8	1.1
16	*Pythoptera capsici*	10	5	7.2	6.7	8.7	8.9
17	*Mucor* Spp.	0.5	10.3	10.1	12.3	10.2	11.5

MRA-Mera, MLW-Mallamawa, SFM-Shafarma, GDK-Gidankoni, GKL-Dankal and BYW-Bayawa

V. DISCUSSION

The study was conducted to isolate and identify pathogenic soil borne fungi in vegetables cultivated soils in Augie local Government area of Kebbi state Nigeria. Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content, and moisture. Physico- chemical analysis of soil showed that pH range of soil conditions ranging from 5.1 to 7.5 and soil textures determined the fungal population and their diversity in agricultural fields.

The current study observed a total of 17 pathogenic soil borne fungi belonging to 11 genera from the 1765 fungal colonies isolated in the area. This is closely related to the findings of Kumar *et al,* (2015) who studied fungal soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam District and recorded about 18 isolates from 6 genera. Jadhav, *et al,* (2017) also recorded 18 isolate from Soil Fungi from Kadegaon Tehsil, Sangli District, Maharashtra, India. The findings of this research is different with work of Gaddeyya *et al.* (2020) who observed a maximum number of fungal isolates belonging to Deuteromycotina and Zygomycotina as opposed to our own finding where 11 out of 17 isolates belong to Ascomycetes. This also confirmed to the findings of Raja *et al,* (2017) who observed that a gram of garden soil can contain around one million fungi such as yeasts, and moulds.

There is higher diversity of soil borne fungi in the area because the diversity index of all the isolates recorded range from 0.99 - 1 Simpson index. The highest percentage colony contribution was recorded on *Fusarium oxysporum* with 14.1%, followed by *Pythium* Sp 9.6%, *Mucor* Sp 8.7%, *Pythoptera capsici* 7.9%, *Aspergillus niger* 7.3%, *Fusarium* Sp 6.7%, *Aspergillus flavus* 5.4%, *Trichodema* Sp 5.3%, *Colletotrichum* Sp 5.2%, *Aspergillus fumigates* and *Rhizopus stolonifer* 4.9%, *Rhizopus oryzae* and *Alernaria* Sp 4.5%, *Penicillium chrysogenum* 3.5%, *Rhizoctonia solani* 3.2%, *Penicillium digitatum* 2.4%, and *Aspergillus clavatus* 1.3%. This indicated that there is higher diversity of *fusarium oxyporum* in the area which may result in fusarium related diseases such as wilt and root rot diseases among many vegetables plant. Vegetable diseases may alarming due to the higher diversity of soil borne pathogenic fungi in the area.

The results of this current work also coincided with findings of many researchers in the field. Thilagam, *et al,* (2018) recorded five fungal species on different plant parts to includes *Alternaria, Fusarium solani, Fusarium oxysporum, Aspergillus flavus,* and *colletotricum spp*. Salau *et al,* (2015) conducted a review on fungal diseases of vegetables in Sokoto State, Nigeria and reported similar species isolated in this investigation. Samaila *et al,* (2018) isolated five fungal species on selected vegetables plants in Kankara, Katsini State to include those recorded in our investigation. Danish *et al,* (2017) study soil borne fungi associated with groundnut, pearl millet and sorghum crops in sub zone Hamelmalo, Eitrea and isolated fungal species similar to those isolated in this research.

VI. CONCLUSION

Based on the preliminary research results, it can be concluded that isolation results have obtained 17 pure isolates of the fungi, presumably associated with certain pathological diseases among the vegetable crops. We therefore, conclude that vegetable production in the area may be affected by fungal diseases. This research will be extended in our next couple of research to ascertain the type of fungal diseases of vegetable crops in the area and their prevalence.

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