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Diversity and Abundance of Mycoflora Associated with Sandy Soil from Vegetable Cultivated Farms in Selected Production Areas of Augie, Kebbi, Nigeria.

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ABSTRACT:

Several factors were known to influence the diversity, abundance and the spread of soil bone microflora in the soil among which includes soil pH, temperature, light, moisture and physical and chemical characteristics of the soil. We designed this research work to find out the diversity of soil microflora in vegetables cultivated sandy soils in Augie vegetable farms. A total of 17 isolates of microfungi were isolated from 60 sandy soil samples collected from 10 sandy soil vegetable farms in selected villages in Augie. The soil mycoflora were isolated by using soil plating and soil dilution techniques. Fungal species were identified based on morphological characteristics observed on a microscope slide prepared with lactophenol cotton blue. 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. The finding of this present work indicated that sandy soil used in the cultivation of vegetable crops in the area is a continuum of large number of mycoflora which may interfere with healthy production of these crops. The present investigation will be helpful in documentation and conservation as well as in controlling soil borne pathogens.

KEYWORDS: Diversity, sandy soil, mycoflora, vegetable.

1. INTRODUCTION

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 of 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.*

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.

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Many fungi can be isolated from the soil, with their incidence varying according to geographic, environmental or bioclimatic factors such as collection site, time of the years relative air humidity, rainfall, wind speed and proximity to the source where they were produced Fatima *et al* (2003) Consequently, these factors determine the quality and quantity of soil mycobiota existing in aquatic and terrestrial ecosystems. Fungal diversity of any soil depends on a large number of factors of the soil such as PH, organic content and moisture (Rangaswami *et al*., 1998). The soil pH, organic content and water are the main factors affecting the fungal population and diversity and Organic carbon largely controls microbial growth in the natural soil. Fungal flora may vary

depending on its native soils. Distribution of soil fungi depending upon the nature of the organic content, climatic condition, surface vegetation and soil texture was studied by Srivastava, M.P (2019). Direct relationship is observed between the soil Texture and Moisture content. Silt and Clay soil holds the highest moisture content that's why there is increased population of fungi.

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. Preliminary survey carried out during our previous study show that most of vegetables crops were produce on sandy soil. 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. Apart from our previous work, 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 would provide preliminary data on soil borne fungi in vegetable cultivated sandy soil in Augie local government area, Kebbi State, Nigeria.

2. MATERIALS AND METHODS

Collection of soil Sample:

Soil samples were collected from six selected vegetable production villages. Sixty (60) soil samples were collected; ten (10) samples from each of the selected village and only vegetable farms cultivated under sandy soil were used in the collection of samples. Therefore, soil samples were collected from a depth of 15cm with the help of a sterilized cork. The collected soil samples were was later transferred to a separate well labelled polyethylene bags. All samples collected were taken to the biology laboratory of Kebbi State Polytechnic, Dakingari for proper laboratory analysis.

Isolation of fungi from the soil samples:

Soil dilution and soil plate method on media such as Potato Dextrose Agar were used as isolation techniques. Direct plate techniques adopted from Warcup, 1950 was used as isolation technique for plating method. About 0.005g of soil sample was spread in to a petri dish and 20m of the PDA media was added in to each plates supplemented with chloramphenocol to prevent bacterial growth. For serial dilution (Waksman, 1922), dilution of 10^{-5} was used. 1ml of the diluted soil samples from 10^{-5} dilution factor was poured into each of the petri dishes added with 20ml of PDA media. The plates were incubated for 48 hours at 25ºC for complete fungal growth. However, isolated fungal colonies were sub cultured in replicate to obtain pure culture for proper identification of individual isolate genus and species level.

Identification of the Soil Fungi:

The fungal isolates obtained from pure cultures were stained on clean microscope slides prepared with *lectophenol cotton blue* and observed on microscope for microscopic examination. Fungal isolates were later identified using colony morphology, colour, texture and micro conidia. Identification of all the isolates was made with the help of standard procedure and relevant literature.

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

3. RESULTS

3.1 Soil Physical characteristics in the area.

Table 4.1 show the color characteristics of sandy soils samples collected in the area. Soil colour characteristics ranges from brownish, dark brown, light brown to redish and redish brown. Sample collected from Mera, Mallamawa and Shafarma village has brown, to brownish white and dark brown color while sample collected from Gidan koni, Bayawa and Dankal has light brown, Redish to Redish Brown color. There was no marked variation in soil texture of the sites, the soil texture of different sites were sandy to sandy loam.

S/No	Village	Coordinates	Sample	Soil Type	Soil colour
		N- 12º 56’ 23.0’’	A	Sandy	Brownish white
1	Mera	E- 004º 31’ 28.1’	**B**	Sandy	Brown
			C	Sandy	Brownish white
			A	Sandy loam	Dark brown
2	Mallamawa	N- 12º 51’ 41.7’’	B	Sandy loam	Brown
		E- 004º 35’ 13.8’’	C	Sandy	Brownish white
			A	Sandy	Light brown
3	Shafarma	N- 12º 47’ 01.7’’	B	Sandy	Brown
		E- 004º 35’ 24.1’’	C	Sandy	Brown
			A	Sandy loam	Dark brown
4	Gidan koni	N- 12º 52’ 06.2’’	B	Sandy	Light brown
		E- 004º 38’ 56.2’’	C	Sandy	Light brown
			A	Sandy	Redish
5	Bayawa	N- 12º 52’ 19.5’’	B	Sandy	Redish
		E- 004º 40’ 37.4’’	C	Sandy	Redish
			A	Sandy	Light brown
6	Dankal	N- 12º 50’ 20.3’’	B	Sandy	Redish brown
		E- 004º 41’ 42.7’	C	Sandy	Redish brown

Fungal Species diversity

Soil physical and chemical characteristics play a vital role in derterming fungal diversity and abundance.. During the investigation period 1765 fungal colonies of 17 fungal species were observed (Table: 2). Among the isolates the genera *Aspergillus* has the highest number of species represented by four species but *fusarium oxysporum* were dominant with a mean total of 249 colonies and with highest colonies observed from sandy soil of Mera. The least abundant species was *A. fumigatus* with a mean number of 23 colonies and was only observed from two localities. (Table: 2). *F.* Sp, *F. oxysporum, Pythium* Sp, *A. niger, Rh. solani, T. hazamani*, *C. phomoides, A.* Sp, *Py. capsici* and *M.* Sp were the most frequent species than, *A. clavatus, A. flavus, A. fumigatus, R. stolonifer, R. oryzae, P. chrysogenum* and *P. digitatum* which were the less frequent fungal species recorded from the six localities. Fungal species diversity was higher from all the isolates of the five sample villages with Simpson diversity index ranging from 0.999 to 1 (Table: 2). However, fungal colonies were higher on samples from shafarma 414 and lower from Dankal samples. The highest species richness was recorded from Mera and Bayawa followed by Mallanawa, Shafarma and Dankal. (table. 2)

Table 2. Mean total count per mg soil (CFU), Species diversity index and number of individual fungal species recorded in sandy soil of different localities. 1. MRA Mera, 2. MLW: Mallamawa, 3. SFM: Shafarma, 4. GKN: Gidan komi, 5. DKL: Dankal and 6. BYW: Bayawa in Augie, Kebbi, Nigeria.

S/No.	CFU	Rcn	No. of colonies of individual species collected on sandy soils															
			Fo	Fs	Ps	Af	Ac	Afg	An	Rs	Ro	Rhs	Pch	Pd	Th	Cp	As	Pc	Ms
1	380	17	52	23	35	12	23	17	28	23	18	15	19	7	27	17	24	38	2
2	237	15	41	20	27	18	10	-	22	13	8	9	-	7	4	8	13	12	25
3	414	15	47	33	40	22	33	-	27	21	28	24	-	15	15	17	20	30	42
4	251	12	45	18	23	18	-	-	28	-	-	9	15	-	19	17	11	17	3
5	137	13	22	7	12	11	-	-	13	9	-	-	10	2	13	4	8	12	14
6	346	16	42	18	33	15	21	6	12	22	26	-	18	12	17	29	4	31	40
	1765	**78**	**249**	**119**	**170**	**96**	**87**	**23**	**130**	**88**	**80**	**57**	**62**	**43**	**95**	**92**	**80**	**140**	**126**
	0.981		0.996	0.999	0.998	0.998	1	0.995	0.998	0.998	0.999	0.999	1	0.998	0.998	0.998	0.994	0.995	

Rcn. Richness, 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

Abundance of fungal species in sandy soils

Table3. Abundance of individual fungal species isolated on vegetable cultivated sandy soil in Augie

The table indicated that the dominant species observed in sandy soil from the area was *F. Oxysporum* with 14.1% followed by *Pythium* Sp. and *Mucor* Sp with 9.6% and 8.7% relative abundance respectively, while *A. fumigatus* was the least recorded species with 1.3% total colony count.

Table3. Abundance of individual fungal species isolated on vegetable cultivated sandy soil in Augie

S/N	Species	% number of individual species
1	*Fusarium oxysporum*	**14.1**
2	*Fusarium solani.*	**6.7**
3	*Pythium* Spp	**9.6**
4	*Aspergillus flavus*	**5.4**
5	*Aspergillus fumigates*	**4.9**
6	*Aspergillus clavatus*	**1.3**
7	*Aspergillus niger*	**7.3**
8	*Rhizopus stolanifer.*	**4.9**
9	*Rhizopus oryzae*	**4.5**
10	*Rhizoctonia solani*	**3.2**
11	*Penicillium chrysogenum*	**3.5**
12	*Penicillium digitatum*	**2.4**
13	*Trichoderma harzianum*	**5.3**
14	*Collectriticum pormoides*	**5.2**
15	*Alernaria* Spp.	**4.5**
16	*Pythoptera capsici*	**7.9**
17	*Mucor* Spp.	**8.7**

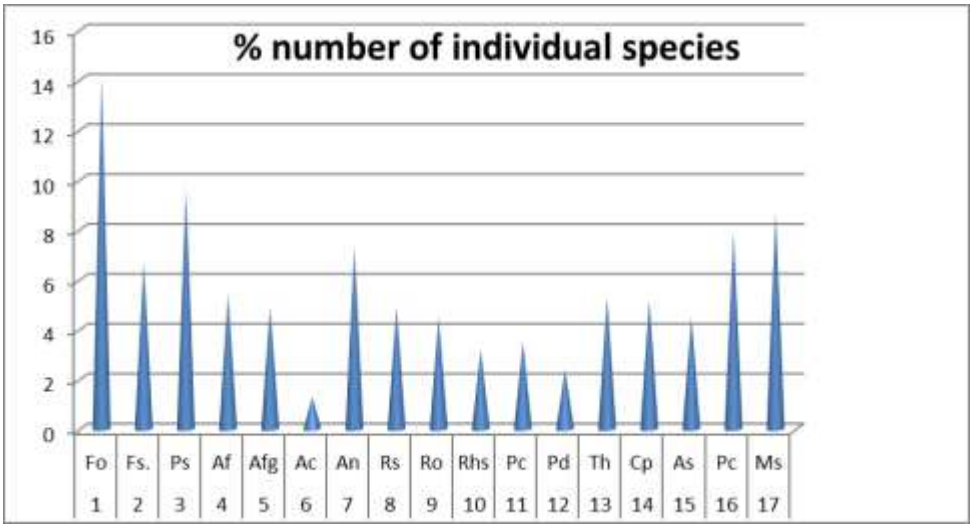
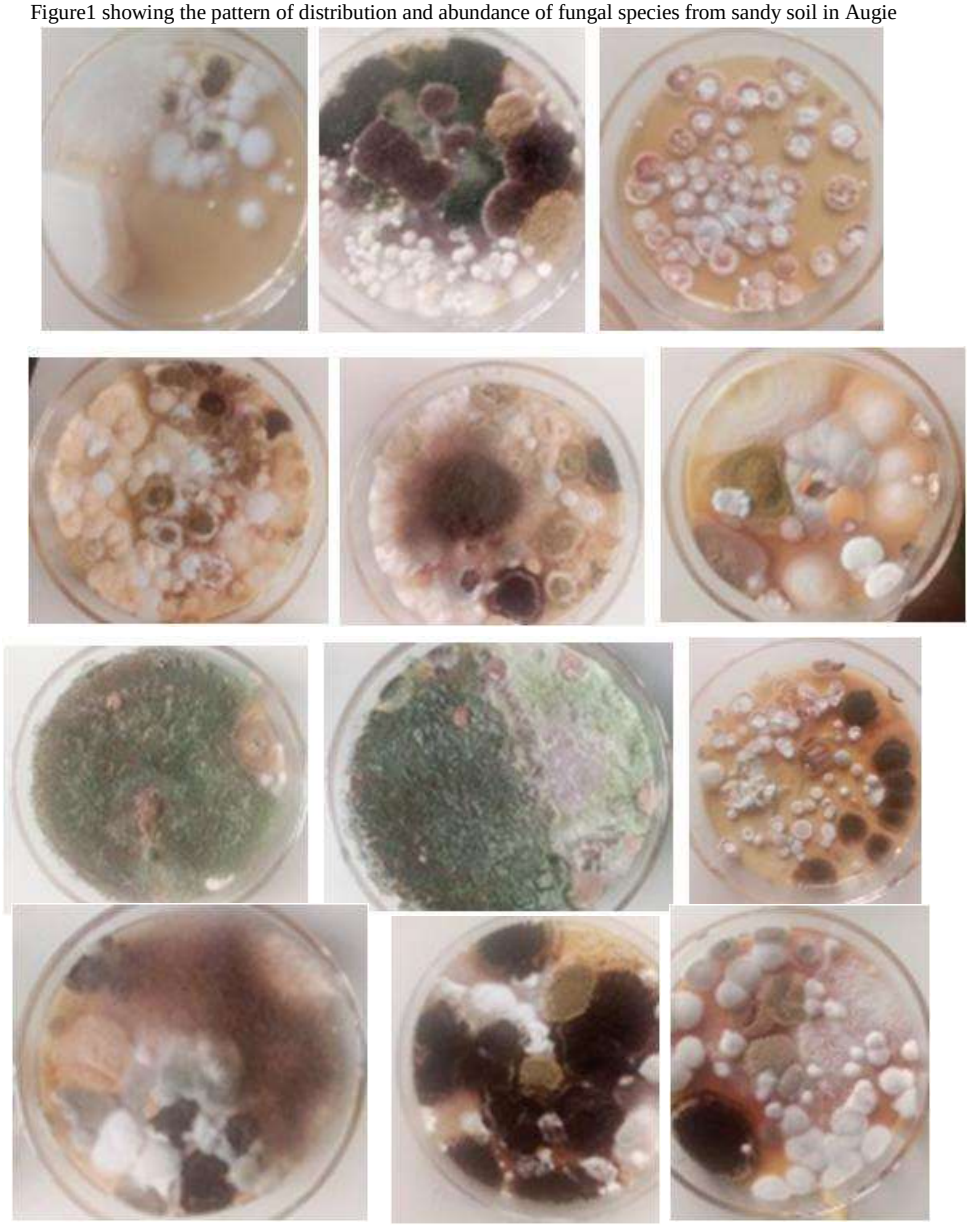


Figure1 showing the pattern of distribution and abundance of fungal species from sandy soil in Augie



Isolated colonies from direct and pure culture on PDA

4. Discussion

Fungal diversity of any soil depends on large number of factors of the soil such as pH, organic content as well as texture. In this study we investigated fungal diversity in sandy soil from Augie vegetable farming localities. 1765 colonies were isolated from samples collected in six farming villages. The results indicated a greater diversity of fungal flora in sandy soil with Simpson diversity index ranging from 0.99 – 1. Gupta *et al.* (2012) also observed a great diversity of fungal mycoflora in agricultural fields of Bareilly with sandy texture. Maranha *et al* (2007) attributed fungal diversity to the heaviness of soil texture, acidity and increasing organic matter content which led to progressively higher percentages of samples harbouring entomopathogenic fungi. Irshad *et al.* (2012) studied the Microbial Diversity in the Sandy Soil of Nara Thar Desert Khairpur, Sindh, Pakistan and observed a significance influence of soil texture on fugal diversity. Large number of fungal species was recorded with higher species diversity and richness. There is similarity of fungal diversity from all samples investigated which is closely related to the findings of Chandini *et al*, (2017) who reported similar diversity of fungal flora from Mattavara forest, Chikamagalur, Karnataka. Chandini *et al* (2017) concluded from their findings that the soil which has large amounts of organic matter due to the accumulation of more litter in scrub jungle and moist deciduous forest, acidic soil pH and silt and clay soil texture holds good amount of moisture content harboured a good qualitative and quantitative mycoflora in the soil for the purpose of recycling of dead organic matter thus making them available for the next generation and maintained the ecological balance in the environment with dominant and sporulating genera.

The most frequent species recorded in the area was the genus *Aspergillus* represented with four species *A. flavus, A. clavatus, A. niger and A. fimigatus.* This is closely related to the findings of

Chandini *et al* (2017), Mohsen *et al*, (2017) who recorded several species from the genus *Aspergillus.* This may suggest that the genera may be one of the dominant local fungal floras in the area. The most dominant species were observed from sandy soil in the area was *Fussaruim oxysporum* with 14.1%. Fusarium species are very diverse and are found in all soil types around the world (Peter *et al*, 2019) and is known to cause serious diseases in vegetables.

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.

5. CONCLUSION

There is greater diversity of soil borne fungi in sandy soil in the area with similar diversity pattern recorded from all samples. The abundance of fungal species observed un the area may due to local fungal fauna in the area and frequent cultivation process. Among the 17 isolate *F.* Sp, *F. oxysporum, Pythium* Sp, *A. niger, Rh. solani, T. hazamani, C. phomoides, A.* Sp, *Py. capsici* and *M.* Sp were the most frequent species than, *A. clavatus, A. flavus, A. fumigatus, R. stolonifer, R. oryzae, P. chrysogenum* and *P. digitatum* which were the less frequent fungal species recorded from the six localities.

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