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Isolation, Identification and Characterization of *phyllosphere* Fungi from Vegetables

I.D. ISIAKA [1], Z. TIAMIYU[2], L. A. OBIDELE[3], A.M. ABDULLAHI[4], H.A. MUHAMMAD[5]
[1](Department of Microbiology, College of Biosciences/ Federal University of Agriculture, Abeokuta, Nigeria)
[2](Department of Biochemistry and Molecular Biology, Faculty of Life Sciences, Federal University Dutsin-ma, Katsina, Nigeria
[3](Department of Microbiology, College of Biosciences/ Federal University of Agriculture, Abeokuta, Nigeria)
[4](Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, Nigeria)
[5](Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria)

Abstract:- Diverse group of microorganism colonize phyllopshere and performvarious but definite ecological functions. The phyllosphere of five different vegetables namely; Common Okra) *Abelmoschusesculentus*L., (Fluted Pumpkin) *Telfariaoccidentalis*, (African Spinach) *Amaranthuscruentus* ,(Jew's Mallow) *Corchorusolitorius*and (Lagos Spinach) Celosia*argentea}*, each from two different locations, (Student Union Building and the Fadama of Federal University of Agriculture, Abeokuta, Ogun State) and were examined Microbiologically for fungal growth using culture-dependent techniques. A total of 18 fungal species covering 5 genera respectively were isolated and characterized as *Fusarium, Penicillium, Aspergillus, Acremonium* and *Geotrichium.*

The fungi genera isolated from this study showed that both human and plant pathogen can colonize plant's phyllosphere, since most of the edible leafy vegetables have less waxy phyllosphere which permit microbial growth. It is recommended that they are washed and cooked properly before consuming them to avoid food poising and food borne illness.

Keywords:- *Epiphyte, Locations, Fungi, Vegetable Types.*

I. INTRODUCTION

Bacteria are regarded to be the common microorganism inhabitants of the phyllosphere. These microbes can be found both as plant that grows on other plant surface and as endophytes within plant tissues (Arnold *et a*l. 2000; Inacio*et al.,* 2002; Lindow and Brandl 2003; Stapleton and Simmons 2006). There are three habibats of micrroorganisms which include the phyllosphere, the rhizosphere and endosphere).,microorganisms which inhabit such habitats are called epiphytes, rhizophytes and endophytes respectively (Montesino, 2003). Phyllosphere therefore is a microhabitat on the surface of plant's leaf where diffferent group of microorganisms colonize and carry out their various but definite ecological function. The difference of the microbial composition of phyllosphere includes algae, bacteria, filamentous fungi, yeast and in rare cases nematodes and protozoans (Morris *et al.,* 2002; Lindow and Brandl, 2003). The majority of phyllosphere fungi are commensal. Some provide specific ecosystem services such as phytoremediation of toxic pollutants (Ali *et al*., 2012) and biogeochemicalcycling of important elements (Feurnkranz*et al*., 2008).

II. MATERIALS AND METHODS

The leaves used to carry out this research were harvested from matured vegetables. All the leaves were collected from two different locations, which are FADAMA, Federal University of Agriculture, Abeokuta, (FUNAAB) and Student Union Building (SUB), FUNAAB, Abeokuta. The leaf samples were put separately into sterile bags and transported to Microbiology Laboratory, Federal University of Agriculture, Abeokuta (FUNAAB**)**.

The plant used for the research was identified by DR. AKINTOKUN from the Department of plant science and seed technology, Federal University of Agriculture, Abeokuta (FUNAAB**)**, Nigeria. The research was carried out at the department of Microbiology Laboratory, College of Biosciences, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, NIGERIA.

➢ *Isolation of fungi*

Ten discs each of 10 mm in diameter was cut in a sterile environment from each of the leaf samples using a 10 mm cork borer. Each leaf samples was put in asample bottlecontsining 10 ml sterile distilled water in and hand shaken for 10 minutes. Serial dilution up to the eight diluent was done using 1 ml from the stock culture. This was repeated for other leaves samples each time shaking for uniform distribution of the cells (conidia). One mililitre of the aliquots from 10^{-2} diluent of each leaf sample, was transferred to sterile microbiological plates, pour plate method was employed. Two replicates for each dilution were made for each of fungi growth to ascertain accuracy of result. Potato Dextrose agar (PDA) was poured into microbiological dishes, (pour plate method) was employed. Colony forming units per millilitres (cfu/ml) were counted as described by Mukhtar*et al.,* (2010). The medium PDA used was weighed following the manufacturer's specification and dispensed into separate clean conical flask; the distilled water was poured into the conical flasks and allowed to dissolve. The conical flasks were corked immediately and transferred to the autoclave for sterilization at 121^{o}c for 15 minutes. Serial dilution was done, thus, Ten

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mls of distilled water was measured and dispensed after sterilization into the first test tube which was the stock solution and a tenfold serial dilutions was done .1ml from the stock solution was pipetted aseptically into the test tube labeled 10^{-1} and mixed. 1ml from 10^{-1} was then transferred to the next test tube (10^{-2}) and mixed and repeated up to the last test tube (10^{-2}). This was done for the ten vegetable leaves samples. Then 1ml each of the diluents 10^{-2} was inoculated on the plates followed by the agar and gently rotated. It was then allowed to cool and gel. Afterwards, the plates were inverted for the vapour to leave and kept at room temperature for 4-7 days for fungal isolates.

➢ *Microbial Count*

Microbial count was carried out to determine the microbial concentration in a given sample from each sample and to also compare the amount of growth of microorganisms under various conditions (Onyeagba, 2004). Examination of the fungi was performedby modified needle mount preparation. The fungi were examined by their colonial characteristic as follows; Colour of the colony, Fluffiness of the colony, Consistency i.e moist or dry, Reverse side pigmentation, Colour of the sporulation.

Microscopic Examination: Direct Microscopic Mounts was carried out using the following techniques;using sterile technique, a small portion of the colony was removed with an inoculation sterile needle into a drop of 70% ethanol**.** It was mixed gently to tease the colonies**;** a drop of Lactophenol Cotton Blue stain was added on a clean cover slip was gently placed on the preparation**.** It was examined with X40 light objective microscope lensSize, shape and arrangement of hypha, conidia and sporangiophore were examined.

➢ *Fungi Isolates From the Phyllosphere Samples*

Culture–dependent techniques was employed in this research to study the different fungidwellingon the phyllopshere of the five selected plants which included: okra (*Abelmoschusesculentus),* pumpkin (*Telfariaoccidentalis),* African spinach (*Amaranthuscruentus),* Lagos spinach (*Corchorusolitorius)* and Jew's mallow (Celosia*argentea).* A total of 18 fungi species covering 5 genera respectively was isolated and characterized as *Fusarium, Penicillium, Aspergillus, Acremonium* and*Geotrichium.*

A total number of 18 fungi was isolated from both sampled sites. Isolation from Lagos spinach had a total of 5 fungi isolates (Fig1) representing 27.8% while African spinach and pumpkin had the lowest of isolates having 2 and 3 (Fig1) respectively representing 11.1% and 16.7% respectively.*Fusariumoxysporum* had the highest frequency of 44.4% (Fig 2), while the remaining fungal species had 1 isolate each representing (11.1%) except *Aspergillus specie* having 2 isolates each representing 22.2% (fig 2)

TABLE 1: Microscopic and Macroscopic Characterizations of Fungi Isolated From the Phyllosphere Samples Obtained From the sampled sites.

S/N	MACROSCOPY	MICROSCOPY	IDENTITY
AS1	White aerial cotton mycelium	Conidiophores are short, single cell. Macroconidia appearing fusiform, slightly curved with pointed tip. Microconidia are abundant, not in chain, non-septate	*Fusariumoxysporum*
OS1	White aerial cotton mycelium	Conidiophores are short, single cell. Macroconidia appearing fusiform, slightly curved with pointed tip. Microconidia are abundant, not in chain, non-septate	*Fusariumoxysporum*
OS2	Green dense, fluffy surface. Dark brown reverse side	Conidia appear single cell, chin phialides and flask shapedfrom single metula. Conidiophores smooth, rough walled	*Penicilliumnotatum*
OS3	Flat granular with yellowish green fluffy colonies.	Conidia is radial in loose column, biseriateborned from phliades on vesicle and globose. Conidiophores are coarsely rough, close to vesicle.	*Aspergilusflavus*
LS1	White aerial cotton mycelium	Conidiophores are short, single cell. Macroconidia appearing fusiform, slightly curved with pointed tip. Microconidia are abundant, not in chain, non-septate	*Fusariumoxysporum*
LS2	Green dense, fluffy surface. Dark brown reverse side	Conidia appear single cell, chin phialides and flask shapedfrom single metula. Conidiophores smooth, rough walled	*Penicilliumnotatum*
LS3	White folded suede-like white surface	Hypha are erect phialides, conidia is single-celled, gloose, cylinderica	*Acremonium specie*
LS4	brown-green filamentous	Large globose conidiophores. Loose columna with serated hypha	*Aspergilus fumigates*
JS1	White aerial cotton mycelium	Conidiophores are short, single cell. Macroconidia appearing fusiform, slightly curved with pointed tip. Microconidia are abundant, not in chain, non-septate	*Fusariumoxysporum*
JS2	White folded suede-like white surface	Hypha are erect phialides, conidia is single-celled, gloose, cylinderica	*Acremonium specie*
JS3	Creamy white folded suede-like surface with no reverse pigment	Hypha is hyaline, smooth conidia, sub-globoseseptate	*Geotrichum specie*

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PS1	Numerous greenish black spore, reverse brownish grey	Large conidia, globose with loose colum. Conidiophores are smooth-walled biseriated with septatephiliades. Conidia are globose and rough walled.	*Aspergilusniger*
AF1	White aerial cotton mycelium	Conidiophores are short, single cell. Macroconidia appearing fusiform, slightly curved with pointed tip. Microconidia are abundant, not in chain, non-septate	*Fusariumoxysporum*
OF1	Creamy white folded suede-like surface with no reverse pigment	Hypha is hyaline, smooth conidia, sub-globoseseptate	*Geotrichum specie*
LF1	White aerial cotton mycelium	Conidiophores are short, single cell. Macroconidia appearing fusiform, slightly curved with pointed tip. Microconidia are abundant, not in chain, non-septate	*Fusariumoxysporum*
JF1	Numerous greenish black spore, reverse brownish grey	Large conidia, globose with loose colum. Conidiophores are smooth-walled biseriated with septatephiliades. Conidia are globose and rough walled.	*Aspergilusniger*
PF1	Pinkish brown cotton aerial mycelium appearing white, reverse is pink	Macroconidia are multi-celled, fusiform with elongated cell. Chlamydoconidia are present	*Fusariumsolani*
PF2	White aerial cotton mycelium	Conidiophores are short, single cell. Macroconidia appearing fusiform, slightly curved with pointed tip. Microconidia are abundant, not in chain, non-septate	*Fusariumoxysporum*

KEY NOTE:

Isolate representation: the first letter indicate the name of the vegetable samples, African Spinach (A), Okra (O), Lagos Spinach (L), Jew's Mallow (J) and Pumpkin (P), the second letter represent the sample sites, Student Union Building (S) and Fadama (F), while the Arabic Numerals represent the isolate number.

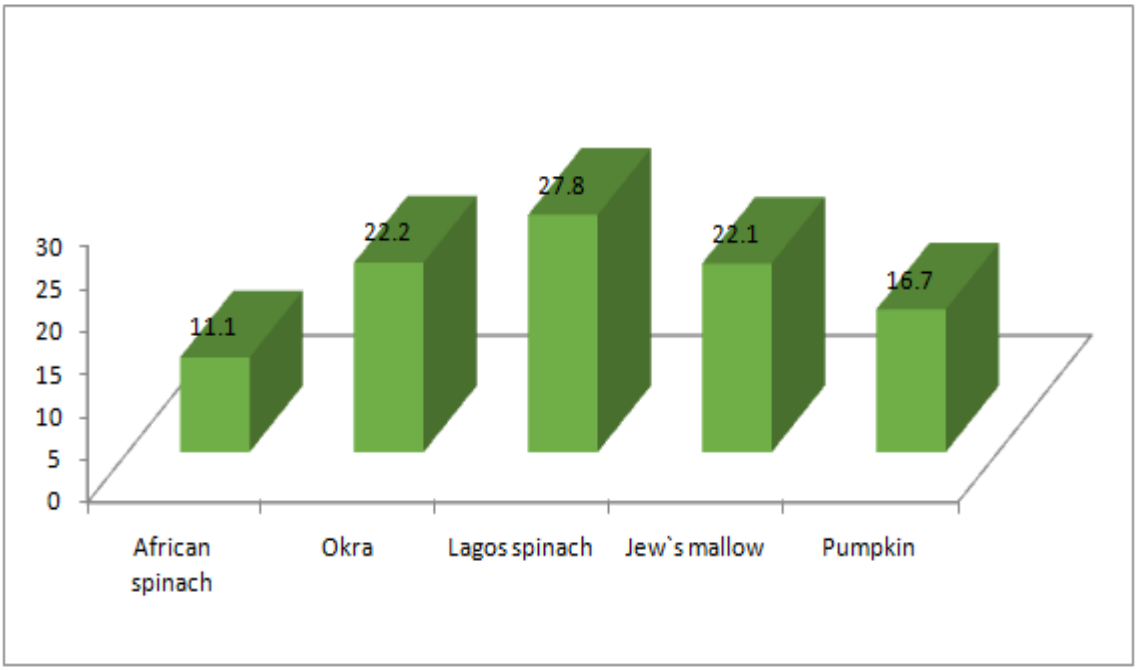

Fig 1. Rate of occurrence of fungi isolates from the phyllosphere samples.

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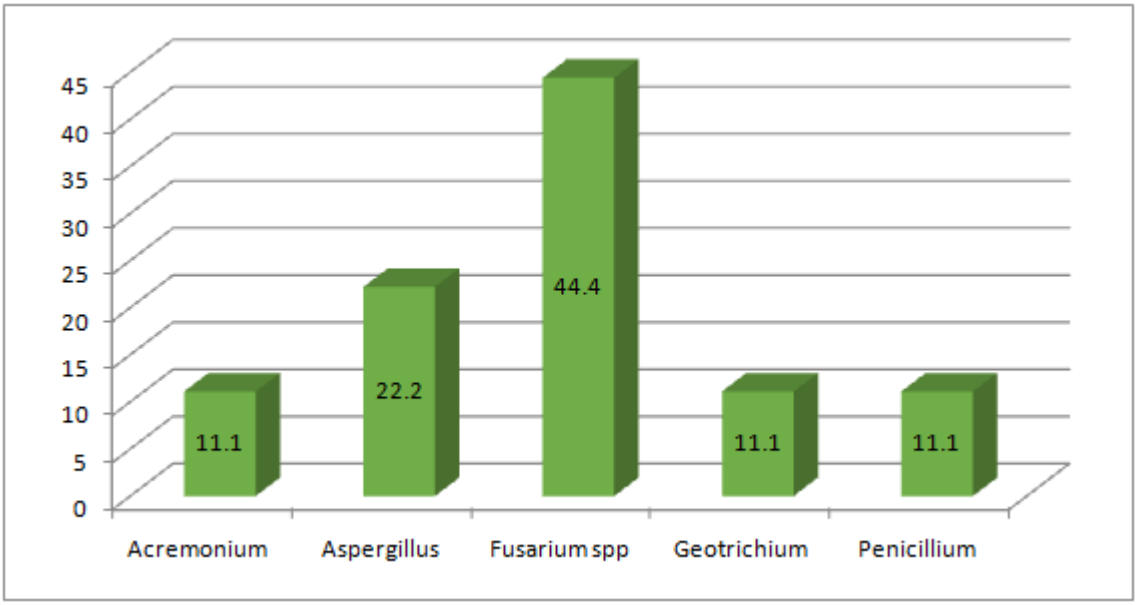

Fig 2 :Rate of occurrence of fungigenera isolated from the two sampled sites.

III. DISCUSSION

The genera isolated and identified include, *Acremonium, Fusariumspp,, Aspergillusspp, Geotrichiumspp* and *Penicilliumspp. Fusariumspp*representing (44.4%) of the total isolated fungi specie was found to be more frequent. The proportion and quantity of nutrients, that aid the growth of phyllosphere microorganisms, are affected by the plant species, leaf age, leaf physiological status, and the presence of tissue damage (Hallmann*et al.*, 1997, Annapurna and Rao, 1982). Similarly, host plants, leaf age, leaf position, physical environmental condition, and availability of immigrant inoculum have also been suggested to be involved in determining population size and difference of microorganism in the phyllosphere (Andrews *et al*., 1980; Cabral, 1985; Wilson and Lindow, 1994; Hata*et al.*, 1998; Yadav*et al*., 2011). Phyllosphere microorganisms have also been implicated as bio control agents in plants (Shahjahan*et al.*, 2001, Kawamata*et al.*, 2004). Application of fertilizers containing substantial amounts of nitrogen have also been found to affect colonization of certain phyllosphere microorganism (Giorgio *et al.*, 1997) likewise treatment with cement dust during pre and post inoculation process (Singh and Rai, 1997).

The variation in microbial load from the different samples site was attributed to the natural and human activities in the various sites. Sample sites Fadama have more trees, crops and plants planted in the site well compared to sample site Student's union building which is dominated by cooking and selling food-related activities.

IV. CONCLUSION

The findings from the research showed that phyllosphere are microhabitats which support the growth of various groups of microorganisms including microorganism that causes food borne illness. Most of the edible leafy vegetables which are consumed by human have less waxy phyllosphere, which allows microbial growth. It is necessary that they are washed and cooked properly before eating to ensure healthy living. It is recommended that they are washed and cooked properly before eating to avoid food borne illness and food poisoning.

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