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Isolation, Identification and Effect of Fungi from Rhizosphere of Diseased *Amaranthus hybridus* L., *Solanum lycopersicum* L., *Lactuca sativa L.* and *Allium fistulosum* L Vegetable Crops in selected Farms in Lagos State, Nigeria

[1]SHONDE, AT; [1] ADEKUNLE, AA; [1] SAMUEL, TO; [1]ADEOGUN, OO; [2] EBABHI, AM; [3]KANIFE, UC

*[*1] Department of Botany, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria*
[2] Biology Unit, Distance Learning Institute, University of Lagos, Akoka, Lagos, Nigeria
[2] Department of Biological Science, Yaba College of Technology, Yaba, Lagos, Nigeria.

**Corresponding Author Email: adebowaleshonde10@yahoo.com*
Co-Authors Email: aadekunle@unilag.edu.ng, tosamuel@unilag.edu.ng, oadeogun@unilag.edu.ng, ebabhi.meg@gmail.com; uchennakanife@gmail.com

ABSTRACT: Soil microorganisms play prominent roles in soil health, promotion of crop growth and incidence of disease**.** The objective of this study was to isolate, identify and understand the impact of fungi isolated from rhizosphere and diseased *Amaranthus hybridus* L., *Solanum lycopersicum* L., *Lactuca sativa L.* and *Allium fistulosum* L vegetable crops in selected farms in Lagos State of Nigeria using standard techniques. A pathogenicity test was conducted to ascertain the pathogen responsible for crop diseases. A total of 138 fungi isolates comprising of 26 fungi species were isolated from the four selected farms. Idi-Araba farm had the highest fungi isolates with 46%, Mile 12 contributed 24% fungi isolates, Iyana Iba had 18% while the least contribution was Badagry farm with 12% fungi isolates. Five were found to be pathogenic on the vegetables which include *Fusarium oxysporium, Fusarium solani, Macrophomina phaseolina, Alternaria alternata,* and *Colletotrichum gleosporoides.* Thirty-three (33%) percent of pathogenic fungi isolated were from Idi-araba farm, Iyana-Iba and Mile 12 farms contributed 25% respectively while Badagry farm contributed 17% pathogenic fungi. Our results showed that the presence of pathogenic fungi in all farms is the principal cause of crop disease and severe post-harvest losses.

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Lagos state is one of the most populous cities in West Africa, in fact, it has been proposed to be the ninth most populous city by 2030 and possibly experience a double increase in population by 2050(Badmos *et al.,* 2018). This population is partly due to high economic activity; possessing over 2000 industries as it prides itself as the economic hub of the nation (Oteri *et al.*,2016). Despite the high human capital and economic activities, the city is rather small in size for its population. Thus, agricultural activities in the state are limited and successive governments are looking to increase productivity through increasing crop productivity. Because the agricultural activity is low, Lagos typically get its agricultural produce such as rice, beans, yam, potatoes from surrounding states like Ogun, Osun, Oyo and other neighboring states while cash crops are majorly supplied from the northern part of the country. However, vegetables such as tomato,

**Corresponding Author Email: adebowaleshonde10@yahoo.com*

Lettuce, Jews mallow, *Amaranthus*, and spring onion are grown albeit on a small scale and sold in the state. Fungi disease is one of the principal causes of spoilage, loss and food shortage. Fungi is responsible for several diseases of vegetables such as Leaf spot caused by *Macrophomina phaseolina*, Fusarium wilt of A*maranthus* caused by *Fusarium spp.*, Anthracnose of Tomato caused by *Colletotrichum gleosporoides*, die-back caused by *Fusarium spp*. Earlier works by Dignam *et al.* (2021), Abawi *et al.* (2000), and Carroll *et al.* (2017) have discussed the impact of soil borne diseases on agricultural productivity. The impact of soil borne microorganisms on soils in Lagos farms is yet unknown, therefore, the objective of this study was to isolate, identify and understand the impact of fungi species isolated from rhizosphere of diseased *Amaranthus hybridus* L., *Solanum lycopersicum* L., *Lactuca sativa L.* and *Allium fistulosum* L. vegetable crops in selected farms in Lagos State, Nigeria.

MATERIALS AND METHODS

Description of study area: The study area is part of Lagos State Nigeria where four farms were selected for sampling. They include Idi-Araba Farm, Iyana Iba farm, Mile 12 farm, and Badagry farm (Figure 1).

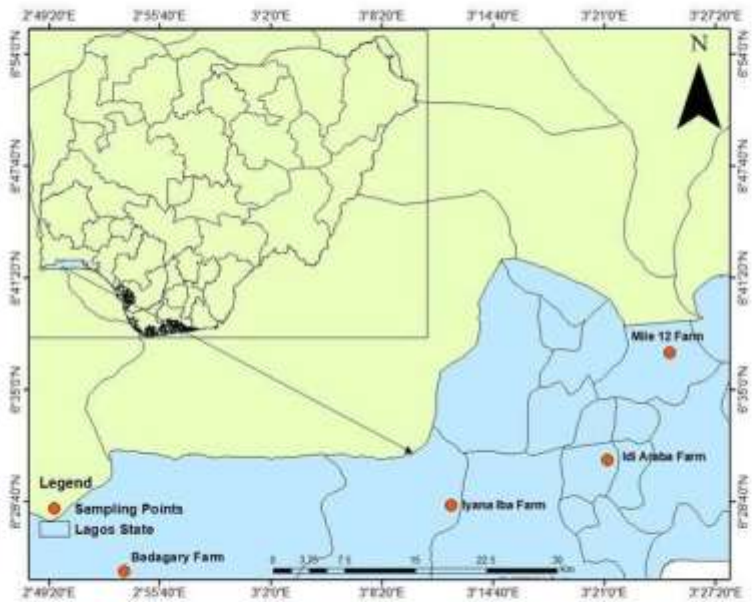


Fig 1: Map showing the four selected farms in Lagos, Nigeria

Collection of diseased crops: Four farms situated in Lagos, Nigeria namely; Idi araba, Iyana Iba, Mile 12, and Badagry where *Amaranthus hybridus* L., *Solanum lycopersicon* L., *Lactuca sativa* L. and *Allium fistulosum* L. were primarily cultivated were identified and diseased crops were collected from the farms in sterile Ziploc bag before being transported to the laboratory for isolation of the fungi on the same day of collection.*Preparation of agar:* Thirty-nine grams (39.0g) of Potato dextrose agar was dissolved in a 1000ml conical flask of distilled water. It was placed in a water bath and shook gently until there was homogenous solution of the P.D.A. It was then autoclaved for 30 minutes at 121℃, an antibiotic (Chloramphenicol 250mg) was added to it. It was transferred into a water bath at 40℃ for 20 minutes before it was poured into Petri dishes in aseptic conditions.

Isolation of fungi from rhizosphere: Soil sample were collected from the farmland (on the allocated portion dedicated to planting of the crops). Triplicate samples were analysed (10^{-1}, 10^{-2}, 10^{-3}) to estimate the fungi population of the soil before planting and after planting. The fungi entities were estimated using serial dilution.

Pathogenicity test: Pathogenicity test was carried out on *Lactuca sativa L.*, *Amaranthus hybridus L.*, *Solanum lycopersicon L.*, *Allium fistulosum L.*, in strict conformity with Koch's postulate (Adekunle, (2008).

Sources of plant pathogens: Fungi isolated from crops in the selected farms (*Amaranthus hybridus* L., *Solanum lycopersicon L.*, *Lactuca sativa L. Allium fistulosum* L.) were all used to determine the causal organisms of the diseases in a screen house.

RESULTS AND DISCUSSION

Fungi species were isolated from the four selected Lagos farms: Idi Araba, Mile 12, Iyana Iba, and Badagry. 64 fungal isolates were recorded from Idi araba, 24 from Iyana-Iba, 33 from Mile 12 and 17 from Badagry farm. Idi-Araba farm had the highest occurring fungal species, accounting for 46% of the total fungi isolated, as shown in Fig 8. Although one hundred and thirty-eight (138) fungi isolates were recorded, only five were pathogenic on crops and identified as *Fusarium oxysporium, Fusarium solanii, Macrophomina phaseolina, Alternaria alternata, Colletotricum gleosporoides*. *Aspergillus flavus, Fusarium oxysporium* had the highest percentage contribution of fungi isolated from the four farms, making up 14.5%. *Macrophomina phaseolina* had the second highest percentage, with 8.69%. *Rhizopus* spp contributed 7.97%, and *Trichoderma spp* made up 6.52% of the total fungi isolated. *Mucor* spp had a percentage of 5.07%, *Aspergillus acealatus* and *Geotrichum spp* had a joint contribution of 4.34%, *Aspergillus niger* made up 2.17% of the total fungi isolated from the four farms, *Penicillium nigritatum* and *Fusarium solanii* made up 1.44%, the least isolated fungi were *Colletotrichum gleosporoides, Alternaria alternata* and they contributed only 0.72 % of the total fungi isolated from the four farms. In Idi-Araba farm, *Aspergillus flavus* and *Rhizopus* sp. were the most frequently isolated fungi with seven isolates each, *Aspergillus acealatus* and *Geotrichum sp.* were next with four isolates each, *Mucor* sp., *Fusarium oxysporium, Trichoderma sp*. all had three isolates each. In Iyana-Iba farm, *Aspergillus flavus* was the most frequently isolated with five different isolates, *Rhizopus* spp. and *Trichoderma* spp. had two isolates each, *Aspergillus niger, Aspergillus acealatus, Fusarium oxysporium, Macrophomina phaseolina* and *Geotrichum* spp. had the lowest frequency distribution. *Macrophomina phaseolina* had the highest frequency distribution of isolates with nine, *Aspergillus flavus* had a frequency of four isolates, *Mucor* spp was next with four isolates, *Trichoderma* spp. had two isolates while *Fusarium oxysporium, Fusarium* spp, and *Rhizopus spp* have just one isolate each to round up the frequency of fungi in Mile 12 farm. Badagry farm had the lowest frequency distribution of fungi isolates; *Aspergillus flavus* the highest frequency distribution with a total of three isolates, *Trichoderma* spp had two isolates while *Aspergillus acealatus, Fusarium oxysporium, Rhizopus spp, Macrophomina phaseoli* and *Coletotrichum* spp had the lowest isolate with one isolate each. These results infer that *Aspergillus flavus, Fusarium oxysporium, Rhizopus spp, Macrophomina phaseolina*, and *Trichoderma spp* were common fungi isolated from all the soils. Total number *of Aspergillus niger* isolated from the four farms was 3, *Aspergillus flavus* had the highest frequency distribution of isolates with 20 isolates, *Aspergillus acealatus* had six isolates overall, *Neurospora* spp had just one isolate, *Alternaria alternata* had just one isolate, *Fusarium oxysporium* had six isolates, *Fusarium spp* had two isolated species, *Mucor spp* had 7 isolates, *Rhizopus* spp was isolated 11 times, *Colletotrichum* spp had 1 isolate, *Macrophomina phaseolina* had 12 isolates, *Trichoderma spp* had 9 isolates, *Geotrichum spp* had 6 isolates, while *Penicillium sp* has just 2 isolates. The unknown species in Idi-Araba farm was 25 isolates, Iyana-Iba farm was 10 isolates, Mile 12 farm was 10 isolates while Badagry farm had 6 unknown fungi isolates as described in table 1 below. Based on the above findings, Idi-Araba farm comprises the most contaminated with pathogenic fungi at least 4 different species of pathogenic fungi namely; *Fusarium oxysporium, Fusarium spp, Alternaria alternata,* and *Macrophomina phaseolina.* Contributing 33% of pathogenic fungi isolated from this work. Mile 12 and Iyana-Iba farmland had 3 pathogenic fungi isolates namely; *Fusarium oxysporium, Fusarium spp,* and *Macrophomina phaseolina* contributing 25% of pathogenic fungi isolated from this study. In Iyana-Iba, two pathogenic fungi, namely *Fusarium oxysporium* and *Macrophomina phaseolina*, were reported. On the other hand, Badagry had the lowest number of isolated pathogenic species, accounting for only 17% of the recorded cases as described in Fig 4 below.

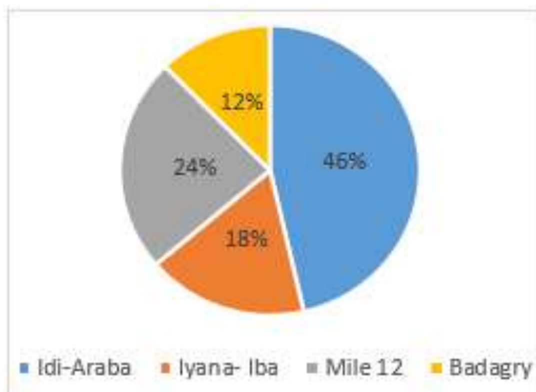


Fig 2: Percentage fungi contribution per farm

*Pathogenicity Studies of the Crops***:** *Fusarium oxysporium and Fusarium solanii,* were pathogenic on Spring onion (*Allium fistulosum* L.) causing white rot of spring onion. There was a characteristic chlorosis beginning from the tip

Table 1: Frequency of Fungi Isolated From Four Selected Farms in Lagos, Nigeria

	Agricultural Farmlands	**Total Colonies**	Average number of fungi colonies														
			Aspergillu			***Fusarium***											
			A. Niger	***A. flavus***	***A. wentii***	***Neurospora spp***	***Alternaria spp***	***F.O***	***F.SPP***	***Mucor spp***	***Rhizopus spp***	***C.O***	***M.P***	***T.M***	***G.O***	**P.N**	***Unknown***
1	Idi-Araba	64	2	7	4	1	1	3	1	3	7	-	1	3	4	2	25
2	Iyana- Iba	24	1	5	1	-	-	1	-	-	2	-	1	2	1	-	10
3	Mile 12	33	-	5	-	-	-	1	1	4	1	-	9	2	-	-	10
4	Badagry	17	-	3	1	-	-	1	-	-	1	1	1	2	1	-	6
	TOTAL	138	3	20	6	1	1	6	2	7	11	1	12	9	6	2	51
	% Contribution		2.17	14.5	4.37	0,72	0.72	4.43	1.44	5.07	7.97.	0.72	8.69	6.52	4.34	1.44	36.96

KEY: C.O- *Colletotrichum gleosporoides,* **M.P**- *Macrophomina phaseolina,* **T.M**- *Trichoderma harizanum* **G.O-** *Geotrichum sp,* **P.N-** *Penicillium sp*

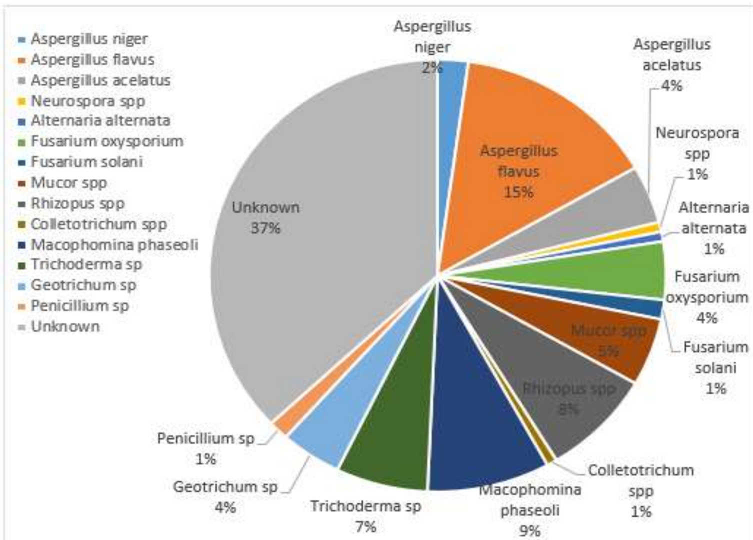


Fig 3: Percentage contribution of fungi isolates from four farms in Lagos, Nigeria

The results from this study showed Lagos soil has high amount of soil microbial population with pathogens occurring more frequently in all the four selected farms. According to Abawi et al. (2000) and Dignam et al. (2021), of the leaf then subsequent die-back of the crops. *Fusarium oxysporium*, *Macrophomina phaseolina* caused leaf spot disease of Lettuce (*Lactuca sativa* L.). Pathogenicity test equally revealed that leaf spot disease on *Amaranthus hybridus* was caused by *Macrophomina phaseolina*. Brown necrotic spots were visible on leaves of the plant.

Fig 4: Pathogenic fungi contribution in four selected farms in Lagos, Nigeria

The existence of soil-borne pathogens on the farm leads to significant crop productivity reduction due to the occurrence of diseases in the cultivated crops. The findings from this study confirm the presence of soil-borne pathogens causing diseases in vegetable crops in Lagos state. The identification of pathogenic soil-borne organisms, including *Fusarium oxysporium*, *Fusarium solanii*, *Colletotrichum gleosporoides*, *Macrophomina phaseolina*, and *Alternaria alternata*, aligns with previous investigations conducted by scientists studying soil microorganisms. The outcomes of this study support the earlier findings of Dauda et al. (2018), which identified *Fusarium equiseti* as the causal agent of dieback in *Allium cepa* (onion). Furthermore, our results reports the first case of *Fusarium oxysporium* causing dieback in *Allium fistulosum* in Nigeria

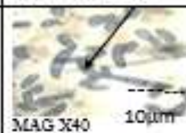
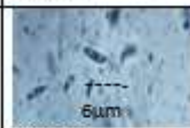
Code	Front View	Backview	Photomicrograph	Fungus Name
S.O IDI Araba				*Fusarium oxysporium*
A.M Iyanaba				*Fusarium solani*
A.M MILE 12				*Alternaria alternata*
S.O IDI Araba				*Macrophomina phaseolina*
T.M BAD				*Colletotrichum gleosporoides*

Fig 5: Pathogenic fungi isolated from four selected farms in Lagos, Nigeria

Our results report anthracnose disease on tomato fruit caused by *Colletotrichum gleosporoides*, this corroborates the earlier studies of Huang *et al.* (2020), Kim *et al.* (2014), Mahto *et al.* (2020), Martinez-Blay. (2020). This work reports Fusarium wilt disease of *Amaranthus hybridus* caused by *Fusarium oxysporium* in Lagos, Nigeria, this corroborate with earlier results from earlier research on Fusarium wilt caused by *Fusarium oxysporium* Chen *et al.,* (2002), Chen *at al.,* (2000) also reported the disease on *Amaranthus hybridus* in South Africa., Fusarium wilts are among the most important phytopathogenic and toxigenic diseases Okungbowa *et al.* (2012). *Fusarium oxysporium* have been reported to cause severe post-harvest losses in vegetables of up to 80% wilting. *Fusarium solani* and *F. Oxysporium* have also been reported to causes serious crop losses in India, Boozobani *et al.,* (2020). Results from this study implies farmers are experiencing severe losses due to the presence of these pathogens. *Colletotrichum gleosporoides* have been described as the primary causal of anthracnose in tomato (*Solanum lycopersicum*, L.) fruits (Ahmad *et al.,* 2021, Etebu *et al.,* 2013 Ciofini *et al.,* 2022), this research shows that the only tomato farm in Lagos (Badagry) is experiencing severe losses caused by *Colletotrichum gleosporoides.* This implies that the soil is severely contaminated and requires urgent attention. Although results from this study revealed that *Trichoderma sp* was isolated which have been described in previous study as a growth promotor and has protective properties against pathogens in soil. Kareem *et al.,* (2016) described the biocontrol and growth promoting abilities of *Trichoderma longibachiatum*. Akram *et al.,* (2021) also gave his insight on the antagonistic abilities of *Trichoderma sp* against Fusarium wilt of tomato (*Solanum lycopersicum* L.). Although, *Trichoderma sp* is present, it is not effective against expression of diseases on vegetables.

Conclusion: The presence of fungi plant pathogens of economic importance in Lagos soils for crop production is baffling. The country already suffers from a serious level of hunger as well as an unprecedented level of food insecurity in recent years. Excellent initiatives towards improving soil health, reducing or eradicating pathogenic fungi on crops should be implemented quickly to improve food security.

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