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FUNGAL SPECIES ASSOCIATED WITH THE PHYLLOSHERE AND RHIZOSPHERE OF BLIGHTED OKRA (*Abelmoschus esculentus*) PLANTS IN SELECTED FARM LANDS IN NIGER STATE, NIGERIA

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ABSTRACT

Blight is a disease cause by many fungi species that infects seedlings prior to or after germination resulting into stunted growth, blighted stem and seedling death. Infected okra plants were collected from three selected farms in Lavun, Chanchaga and Wushishi Local Governments Areas of Niger State. Fungal species were isolated using serial dilution and pour plates methods. Morphological characteristics of the isolates was confirmed using mycological atlas and microscopically using lactophenol cotton blue. Fungal species identified with their percentage relative abundance are *Trichophyton megnini* (8.40-14.37%), *Microsporium gympeum* (5.65-7.18%), *Aspergillus niger* (41.6-56.63%), *Aspergillus flavus* (6.29-33.28%), *Mucor pusilus* (0.0-8.32%), *Mucor* species(8.40-31.43%). Pathogenicity and Percentage disease incidence revealed the presence of the pathogens using healthy okra seedlings. Severe, high and highly severe infections symptoms of blight disease were observed after 12 days of inoculation. These study revealed the identity fungal species associated with blight disease of okra plants which lead to poor yield and in turn affected farmer's productivity.

Keywords: Okra; phyllosphere; rhizosphere; blight.

INTRODUCTION

Seedling blight is a disease that occur as a result of infection caused by fungi species which infects seedlings prior to emergence or after emergence and cause various kinds of stem lesions (Ahmad *et al.*, 2015). Infections of the stem occur near the soil surface and result in some shade of brown, red to orange discolouration. Older lesions may appear sunken with less red colour and eventually rot the entire outer portion of the stem, thus causing the plant death (Kucharek, 2000). Fungal species enter the young seedlings to kill or injure them. Blighted plants that emerge from the soil die soon after emergence. Those that survive are generally lack vigour, turn yellow to



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pale green and do not compete well with the healthy seedlings. When infection occur prior to emergence of the seedling, the resulting disease is called pre-emergence seedling blight which is often lethal (Muhammad, 2016).

According to (Don Groth and Clayton, 2010) many fungal species caused seedling blights disease of vegetables these include *Pythium* species *Rhizoctonia solani*, *Fusarium solani* and *Pseudomonas* species attacked vegetable causing infection on all plant parts such as seed decay, malformation of leaves, seedling death and pod blight. (Baker *et al.*, 2010; Muhammed and Muhammad, 2013) reported that pathogens causing blight diseases of crops include *Rhizoctonia* species, *Pythium* species, *Helminthosporium* species, *Aspergillus niger*, *Aspergillus flavus*, *Aphanomyces cochloides*, *Phoma betae*, *Curvularia* species, *Xanthosomonas* species, *Fusarium oxysporum* and *Bacillus* species.

Blight disease of okra plant caused by *Sclerotium rolfsii* attacked plant parts such as roots, stems, leaves and fruits that are in contact with the soil surface (Kone *et al.*, 2012). Visible symptoms are yellowing, wilting of the leaves and finally death of the plant. According to Catarino *et al.*, 2016 *Pseudocercospora* species produced an olive grey to brown coloured fungal growth on the leaf surfaces of okra. The disease produced symptoms as leaf wilt, curl, drop and collapse of the plant.

Okras are affected by seedling blight disease at young and mature stage (Muhammed and Muhammad, 2013). Okra seeds contain about 20% protein similar to the amino acid composition of soybean protein (Muhammad, 2011). Therefore this study aimed to isolate and identify the fungal pathogens associated with rhizosphere and phyllosphere of blighted okra plants.

MATERIALS AND METHODS

Collection of Soil and infected Rhizosphere and Phyllosphere Okra Seedlings

Samples were collected from soil (rhizosphere) and plant part (phyllosphere) of blighted okra seedlings from six selected farms. The infected plants display blighted stem, wilting of the leaves and death of the seedlings. Soil auger was used to collect soil adhering to the roots of the infected plant at 5 m depth. The samples were collected into dry sterilized polythene bags and



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transported to the laboratory for isolation of the microorganisms. The sample areas are Rogota in Lavun Local Government area (Farm I), Chanchaga Local Government area (Farm II) and Zungeru, Wushishi Local Government area (Farm III) of Niger State (Dongmo and Oyeyiola, 2006).

Isolation of Fungal Species from the Rhizosphere of infected Okra Seedlings

Soil sample of the rhizosphere of blighted seedlings of okra collected from infected the plants. The sample was placed in 500ml capacity sterilized beaker and mixed thoroughly. One gram was taken from the sample and mixed in 9 ml sterilized distilled water in a test tube. The mixture was serially diluted from 10^{-1} - 10^{-6} . One ml of tube 10^{-2} and 10^{-3} strengths of dilution was inoculated on Potato dextrose agar plate, and incubated at room temperature for 48 hours. Pure cultures were obtained by sub-culturing into another freshly prepared PDA plates. Identification of the isolates was done by the use of mycological atlas and microscopically by use of Lactophenol cotton blue (Mwajita *et al.*, 2013). Percentage relative abundance calculated using the method of Okigbo and Ikediugwu (2000). Percentage (%) relative abundance of the isolates= $T/N \times 100$; Where N= Total number of microorganisms isolated per blighted rhizosphere in the locations; T= Number of times of occurrence of the individual isolate in the locations.

Isolation of Fungal Species from the Phyllosphere of Infected Okra Seedlings

Diseased leaves of okra plants that have symptoms of seedling blight disease was collected. The isolation of the pathogens on the leaf disc was done using cork borer bits of about 1cm by 1cm. The portion cut across the leaf lesions was surface sterilized by submerging in 1% alcohol for 30 seconds and was rinsed in five changes of sterilized distilled water and placed separately on Potato Dextrose Agar plates. The Petri-plates was incubated at ambient temperature ($28^{\circ} \pm 2^{\circ}\text{C}$) for fungi growth. Pure cultures from the mixed growth was obtained by sub culturing and subsequently identified by the use of mycological atlas of (Barnett and Hunter, 1996). Percentage relative abundance was calculated using the method of Okigbo and Ikediugwu (2000).Percentage (%) relative abundance of the isolates= $T/N \times 100$

Where N= Total number of microorganisms isolated per blighted phyllosphere in the locations:
T= Number of times of occurrence of the individual isolate in the locations.



Preparation of Fungal Inocula

The inoculums were produced on rice according to the method of Adebola and Amadi (2014). Five mycelia plugs of 3 days old culture, were transferred to a potato broth medium (200g/l) and autoclaved for 60 minutes at 1 atmosphere pressure. The suspension were left inside the shaker for five days at $28 \pm 2^\circ \text{C}$ at 95rpm. Two polythene bags were separately filled with 300grams of rice and 70ml distilled water. They were then autoclaved and allow to cool at room temperature. Thirty millitre of each of the liquid inoculum was poured into the bags containing the rice, they were opened after 48 hours and the contents sprayed on trays and incubated for another 3 days at room temperature for the production of spores. The suspension of each inoculum was prepared by blending solid rice substrates with distilled water, 20% pectin and ammonium diphosphate at pH5.5 for ten seconds in a warring blender and then filtered through a sterilized cheese cloth. The suspensions were stored in sterile containers and kept inside refrigerator at 4°C for further usage.

Inoculation

The suspension were applied on okra seedlings using a hand operated sprayer at 500 -1000ml per pot. Two applications were made routinely for a period of six weeks (Adebola and Amadi, 2014).

Disease incidence tests of the Fungal isolates

Disease incidence of the fungal isolates was evaluated using the method of Bem *et al.* (2010). The percentage disease incidence of tomato and okra plants using disease symptoms of blight was evaluated using disease index (DI) a modified method of Khanna *et al.* (1977). Disease index (DI) = $H/N \times 100$. Disease severity was determined using modified method of Bem *et al.* (2010) using a numerical scale of 0-5 as follows:

0-No infection; 1-1-20%= mild infection; 2-21-40%= moderate infection; 3-41-60%= high infection; 4-61 and above= severe infection; 5-71 and above= high severe infection

RESULTS

The results revealed that there was high significant difference ($P \leq 0.05$) in relative abundance of fungal species isolated from blighted okra plants in the sample areas (Farms I, II and III) (Table 1). The percentage relative abundance of isolated *Aspergillus niger* in farm II and III were (42.72%) and (41.60%) respectively. These values were not significant difference ($P \geq 0.05$) but



significantly different from farm I having (56.63%). The percentage relative abundance of *Aspergillus flavus* isolated from farm I (6.29%) and farm II (14.31%) varied significantly compared to farm III having (33.28%). The result of relative abundance of *Mucor species* showed that farm I had the highest relative abundance of 31.43% and farm II (21.56%) and farm III (8.40%) which showed significantly difference ($P \leq 0.05$). The result of relative abundance of *Tricophyton megnini* showed (14.37%) and (8.40%) in farm II and farm III with non isolated from farm I. Similarly, *Mucor pusillus* was isolated only in farm III but showed no growth in farm I and farm II. The relative abundance of *Microsporium gympseum* was 5.65% and 7.18% with non isolated in farms III.

Table 1: Relative Abundance of Fungal Species Isolated From Blighted Okra Plants From Three Selected Farms

Relative abundance (%)						
Farms	<i>A. niger</i>	<i>A. flavus</i>	<i>M. species</i>	<i>T. megnini</i>	<i>M. pusillus</i>	<i>M. gympseum</i>
I	56.63±2.05 ^a	6.29±0.00 ^c	31.43±1.05 ^a	0.00 ±0.00 ^c	0.00±0.00 ^b	5.65±0.05 ^a
II	42.72±0.03 ^b	14.37±0.01 ^b	21.56±0.02 ^b	14.37±0.05 ^a	0.0±0.00 ^b	7.18±0.04 ^a
III	41.60±0.01 ^b	33.28±0.05 ^a	8.40±0.00 ^c	8.40±0.02 ^b	8.32±0.01 ^a	0.00±0.0 ^b

Mean values ± S.E percentage fungal species isolated. Values with the same superscript letter(s) down the column are not statistically significant at ($p \geq 0.05$) different.

Percentage Disease incidence Results for Fungal isolates on Healthy Okra Plants

The results of analysis of variance (ANOVA) revealed (Table 2) that there was high significant difference ($P \leq 0.05$) in the percentage disease incidence of the fungal isolated from healthy okra seedlings. *Aspergillus niger*, *A.flavus*, *Microsporium gympseum* and *Mucor pusillus* inoculated on Replicates (1, 2, 3 and 4) produced high percentages of 80%,76%,75% 63%, 62%,61%, and 56% respectively. Thus all produced highly severe, severe and high infection on the seedlings. These values were highly significant with the seedlings inoculated with *Mucor* species that revealed moderate and mild infections on the replicates.



Table 2: Percentage Disease Incidence on Okra Seedlings Inoculated With Fungal Isolates

Okra Seedlings Replicates	Isolates					
	<i>Aspergillus nige</i>	<i>A.flavus</i>	<i>Mucor</i> species	<i>Microsporium</i> <i>gympseum</i>	<i>Mucor pusillu</i>	<i>Tricophyton</i> <i>megnini</i>
Rep 1	63.0±4.05 ^a	55.3±3.06 ^b	30.1±2.00 ^c	76.0±5.00 ^a	65.7±4.03 ^a	55.9±4.00 ^a
Rep 2	62.5±4.00 ^a	39.0±2.50 ^c	20.0±1.00 ^d	40.1±2.00 ^c	61.0±4.00 ^a	62.5±4.05 ^a
Rep 3	40.1±2.05 ^c	56.5±3.00 ^b	19.5±1.05 ^d	75.5±5.02 ^a	61.5±4.00 ^a	35.5±2.01 ^c
Rep 4	50.5±4.02 ^a	50.0±3.02 ^b	40.0±2.02 ^c	80.3±5.05 ^a	70.0±4.05 ^a	32.0±2.00 ^c

Mean values ± S.E percentage fungal species isolated. Values with the same superscript letter(s) down the column are not statistically significant at ($p \geq 0.05$) different.

0%-No infection; 1-20%= mild infection; 21-40%= moderate infection; 41-60%= high infection; 61-70%= severe infection; 71% and above= highly severe infection

DISCUSSION

Fungal species isolated from blighted okra are *Aspergillus niger*, *A. flavus*, *Mucor* species, *Tricophyton megnini*, *Mucor pusillus*, and *Microsporium gympseum*. These organisms are similar to those isolated by Nartey (2011); Kone *et al.* (2012); Lamichhane *et al.* (2015) and Catarino *et al.* (2016) who reported the isolation of fungal species from blighted vegetables. Similar fungal species have been isolated from blighted tomato plants by Muhammed and Muhammad (2013). *A. niger*, *Fusarium oxysporum* and *Rhizopus nigricans* were isolated from blighted tomato, okra and cabbage seedlings as reported by Muhammed *et al.* (2016). The isolation of these fungal species from blighted seedlings showed that they are ubiquitous in nature. The disease severity test of the fungal species isolated from blighted okra further confirmed that they are causative organisms of blight disease of these plants as earlier reported by Mair *et al.* (1992); Krebs *et al.*, (2006); Abada *et al.*, (2008); Kleemann *et al.*, (2008); Koley *et al.*, (2015); Bushman *et al.* (2015) and Rashid *et al.* (2016). Out of all the fungi isolated, *Aspergillus niger*, *Microsporium gympseum*, *Mucor* species and *Tricophyton megnini* were found to be high in disease incidence, probably because they are fast growing and produce enzymes which enhance the colonization of their host plants. This assertion was earlier reported by Rai *et al.* (2008); Baker *et al.* (2010); Jarvis (2014) and Gousous *et al.* (2010) respectively.



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Aspergillus niger, *Aspergillus flavus*, *Trichophyton megnini*, *Mucor* species, *Microsporium gymipseum* and *Mucor pusillus* were found were to be associated with both rhizosphere and phyllosphere of okra plants. These fungal species lead to loss of yield and quality of the crop (Muhammed and Muhammad 2013).

Proper sterilization of the soil to deactivate the effects of soil and surface borne fungi species associated with the rhizosphere and phyllosphere causing blight disease.

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