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First Report of Die-back Disease of Onion (*Allium cepa* L.) Induced by *Fusarium equiseti* (Mart) Sacc in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author WPD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SELA and ABZ managed the analyses of the study. Author OA read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In October 2015 and 2016, onion plants of Barke Aliero exhibiting characteristic symptoms of dried leaves beginning from the tip, abnormal protrusion, root discoloration and curling of the stalk from tip was observed in Kebbi State, Nigeria. In both cases, disease was very severe on seedlings, and disease incidence was 50% or more in most farmer's fields. Diseased tissue was cut from advancing margin after sterilization and placed in agar plates. The *Fusarium* sp. isolated routinely from diseased tissue was identified at International Mycological Institute (IMI) Egham, London as *Fusarium equiseti* (Mart).Microscopic examinations showed the presence of septate (5-7) marcroconidia with tapered apical shape and aseptate microconidia. Pathogenicity test using *Fusarium equiseti* (IMI No. 604243) isolated from the roots and leaves of diseased plants showed typical symptoms. Three inoculation methods used confirmed multiple soil borne inoculum transmission after the Koch postulate.

Keywords: Onion; farmers; kebbi state; Fusarium equiseti; danzzazalau.

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1. INTRODUCTION

Onion, a monocotyledon in the Alliaceae family [1] which is cultivated mainly for its bulb varies in size, shape and colour from which are derived phytochemicals that have important usage as human and animal medicines [2] Used almost daily in every home it is an essential ingredient in Nigerian diet [3]. Commercial production of onions is domiciled in the northern regions of Nigeria especially in Borno, Sokoto, Kaduna, Jigawa, Zamfara, Kano and Kebbi States [3] Hussaini et al. [4] showed the largest area of cultivation and consequently highest production to be in Kebbi State. Onion cultivation in Nigeria is curtailed by nutrient deficient soils, poorly developed secondary irrigation facilities, low farming technology adoption rates and numerous insect pests and diseases [5,4]. Emechebe et al. [6] listed the following as very important economic fungal diseases affecting onion production in northern Nigeria: purple blotch (*Alternaria porri*) black mold diseases (*Aspergillus niger)*, neck and bulb rot (*Botrytis allii),* Onion twister (*Colletotrichum cingulata)*, downy mildew (*Peronosporadestructor*) pink rot (*Pyrenochaeta terrestris)* and bulb rot induced (*Fusarium oxysporium*).

One major constraint to profitable production of onions in Kebbi State is the incidence of a disease locally called 'Danzazzalau' as reported by Kebbi Agricultural and Rural Development Authority (KARDA) to the Institute for Agricultural Research (IAR) in 2009 [7] at Aliero and Maiyama L.G.A. By 2015 Cropping Scheme meeting, the increased devastation and spread of the disease was reported in four Local Government Areas: Maiyama, Aliero, Jega and Yauri by the (KARDA) staff representative. In response to the onion farmer's plight, the devastating effect of the disease on onion productivity necessitates the studies.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Pathogen

Samples of infected plants from farmer's field were collected in labeled paper bags and taken to the Vegetable Pathology Laboratory of the Department of Crop Protection, Ahmadu Bello University, Zaria. Roots and leaves of infected plants were surface sterilized and plate in PDAs for the isolation and identification of fungi.

Seeds of "Barke Aliero" were dressed with Dress force (20% Metalaxyl –m, 20% Imidacloprid, 20% hexaconazole) at 0.6g/kg and raised on heat sterilized soil in nursery tray in the screen house of Crop Protection Department, IAR/ A.B.U Zaria. The soil in nursery tray was mulched with dry grass, watered regularly and kept weed free through hand weeding. Six-week old seedlings were transplanted to plastic pots and inoculated with isolated fungi namely *Fusarium* sp., *Aspergillus* sp. and *Rhizopus* sp. by mycelia paste method as described by Ebenebe [6].

Ten milliliters of spore suspension was pipette into each hole in potted sterilized soils and onion seedling with washed roots was transplanted in the pots (12 cm diameter).

At 28 days after inoculation (DAI), the roots and leaves of plants showing typical wilt symptoms and curling were collected, taken to laboratory for re-isolation of the organism by plating on PDAs. Fungi growths were subultured for identification and compared with original culture to confirm Koch's postulate. Isolated *Fusarium* sp. was sent to IMI for identification.

2.2 Effect of Inoculation Methods on Plant Growth parameters

The inoculum of isolated *Fusarium equiseti* (Identified by IMI) was adjusted (1 x 10^6 conidia/ml) and used to inoculate six-week old seedlings using soil drench, root dip and mycelia paste methods.

Mycelia paste of isolated fungus was also used to inoculate the seedlings. For soil drench, 10 ml spore suspension was sprayed at base of each seedling, for root dip, seedling roots were dipped and left in 10 ml suspension for 3 mins before transplanting. Mycelia paste involves pouring paste of harvested mycelia directly into holes before seedlings were transplanted in and covered with soil. Seedlings with sterile distilled water (SDW) served as controls. For each treatment, two seedlings were transplanted on heat sterilized soilswith 12 cmdiameter in plastic pots (2seedling/pot) each and covered with transparent polythene bags 24 h after treatment.

Labeled pots were arranged in a Completely Randomized Design (CRD) on the screen house bench with daily observations made for symptom development. Each treatment was replicated four times. Symptom development on the seedlings

was monitored daily from day 1 (D1) up to day 28 (D28) as described by Yates et al. [8].

Data collected included, root length (RL), leaf length (LL) and stem diameter (SD) using vernier caliper were measured and used to calculate the Absolute Growth Rates (AGR) using the formula described by Radford [9].

$$AGR = \frac{h_2 - h_1}{t_2 - t_1}$$

Where,

h_1 and h_2 = leaf length(LL_1-LL_2), root length (RL_1-RL_2) and stem diameter (SD_1-SD_{28}) at D1 and D28 respectively.

t_1 and t_2 = Time at D1 and D28 respectively.

2.3 Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the SAS (2002 version) and means were separated using Student Newman Keuls (SNK) at 5% level of significance.

3. RESULTS

3.1 Isolation, Identification and Pathogenicity of *Fusarium equiseti*

Fungi isolated from diseased onion plants from farmer's field at Alerio were *Aspergillus* sp. (Plate 1), *Rhizopus* sp. (Plate 2) and *Fusarium* sp. (Plate 3). The observed *Aspergillus* conidiophores were upright with clavate swelling at the apex (Plate 1). Sporangiospores from root-like rhizoids and sporangia with no cross-walls were observed on the *Rhizopus* species (Plate 2). Microscopically the *Fusarium* sp. had septate (5-7) marcroconidia with tapered apical shape and aseptate microconidia in the *Fusarium* species (Plate 3). From diseased onion roots, *Fusarium* sp. isolated was pathogenic on onion based on the symptoms observed on the leaves and roots. Seedlings inoculated with *Aspergillus* sp. and *Rhizopus* sp. did not show any symptom typical to Danzzazalau disease (Table 1).

3.2 Effect of Inoculation Methods on Absolute Growth Rates of Seedlings

Symptoms observed on inoculated plants using different inoculation methods were drying of leaves beginning from the tip, curved and abnormal seedlings (Plates 5, 6 and 7) compared with control plants (Plate 4). Uprooted roots 28 days after inoculation showed dark brown discolouration. The *Fusarium* sp isolated from the root tissues was identified at International Mycological Institute (IMI) Egham, London as *Fusarium equiseti* (Mart) Sacc. (IMI 604243).

There were no significant difference (P≤0.05) between the daily rates of increase in root number and stem diameter among the inoculation methods used (Table 2), Although highest number of roots/day(0.606/day) was recorded from plants inoculated by root dip, followed by mycelia paste (0.463) and soil drench (0.363) inoculation methods respectively. The daily stem diameter increase was highest on plants inoculated with mycelia paste (0.010 mm/day) and least (0.006 mm/day) on soil drench method.

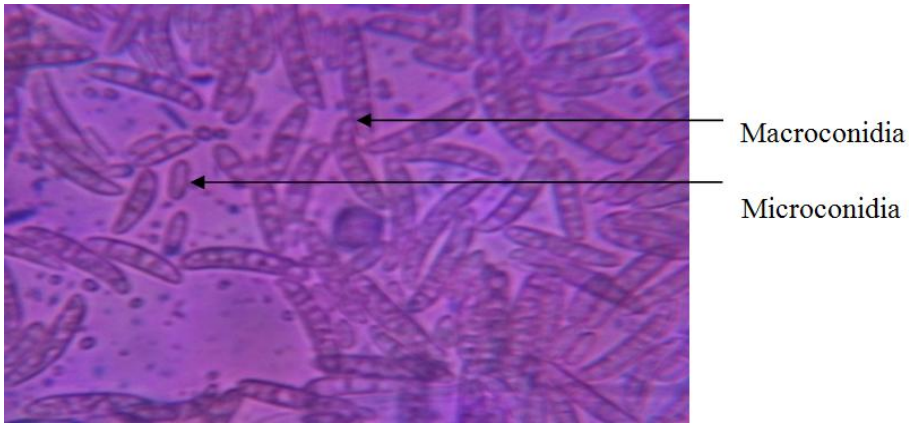

Plate 1. Aseptate microconidia and septate macroconidia of *Fusarium equiseti* (x40)

Table 1. Results from test of pathogenicity

Fungus	Reaction	Description
Aspergillus spp.	_	No wilt of above ground parts, uprooted roots appeared normal after 21 days
Rhizopus spp.	_	No wilt of above ground parts, uprooted roots appeared normal after 21 days
Fusarium spp.	+	Leaf curled, tip drying noticed from 10 DAI, roots discoloured and plants frail.

Keys:--; No lesions/symptoms after inoculation with fungus; + Lesions/symptoms were observed after inoculation.

Table 2. Absolute Growth Rates of seedlings under three methods of inoculation with *Fusarium equiseti*

	Absolute Growth Rate (AGR)			
Methods of Inoculation	**Roots (No/day)**	**Root Length (mm/day)**	**Stem Diameter (mm/day)**	**Leaf Length (mm/day)**
Root dip	0.606	0.067^{ab}	0.009	4.078^{c}
Soil drench	0.363	0.043^{b}	0.006	7.828^{a}
Mycelia paste	0.463	0.037^{b}	0.010	4.103^{c}
Control	0.286	0.092^{a}	0.007	4.630^{b}
LSD	0.957	0.041	0.008	0.302

Means with the same superscript in each column are not significantly different at 5% level of significance (P≤0.05) on the Least Significant Difference (LSD).

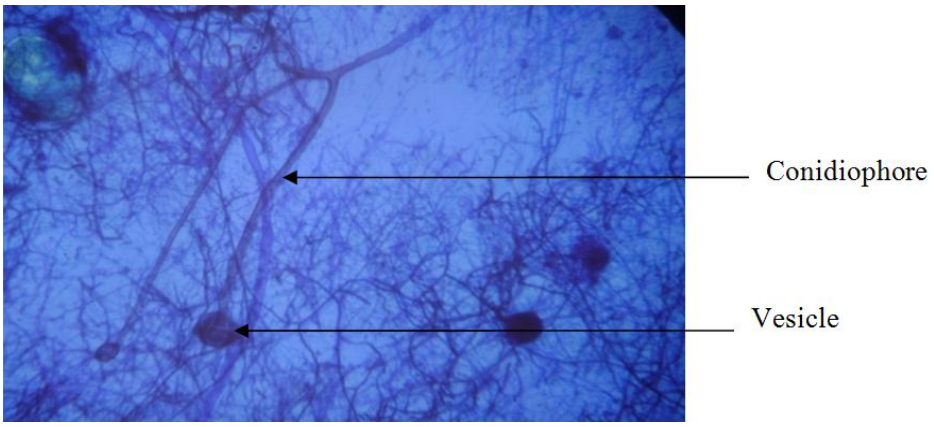

Plate 2. Photomicrograph of *Aspergillus* sp. x40

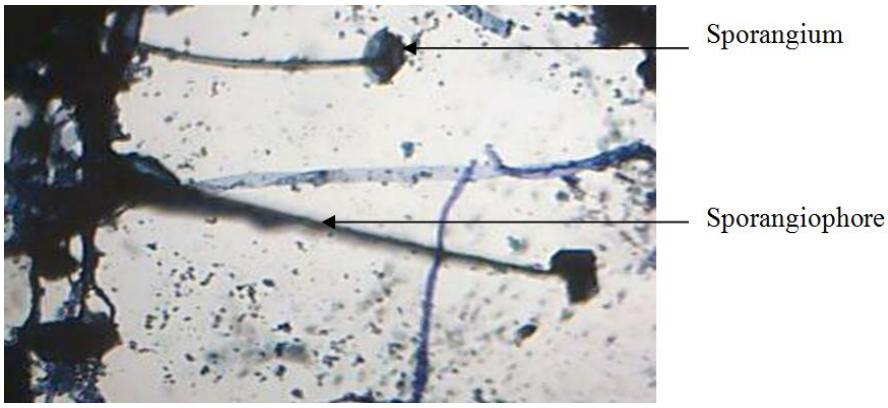

Plate 3. Photomicrograph of *Rhizopus* sp. x40

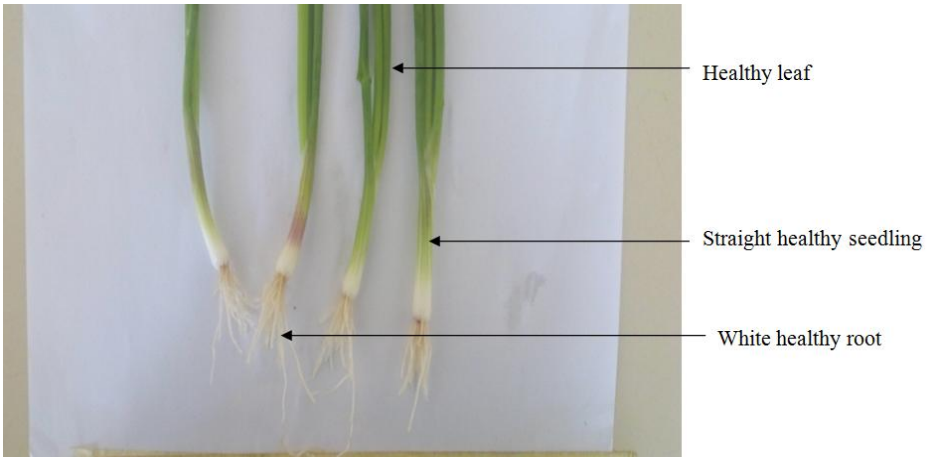

Plate 4. Healthy onion plants (uninoculated control plants)

Plate 5. Soil drench inoculated seedlings with symptoms

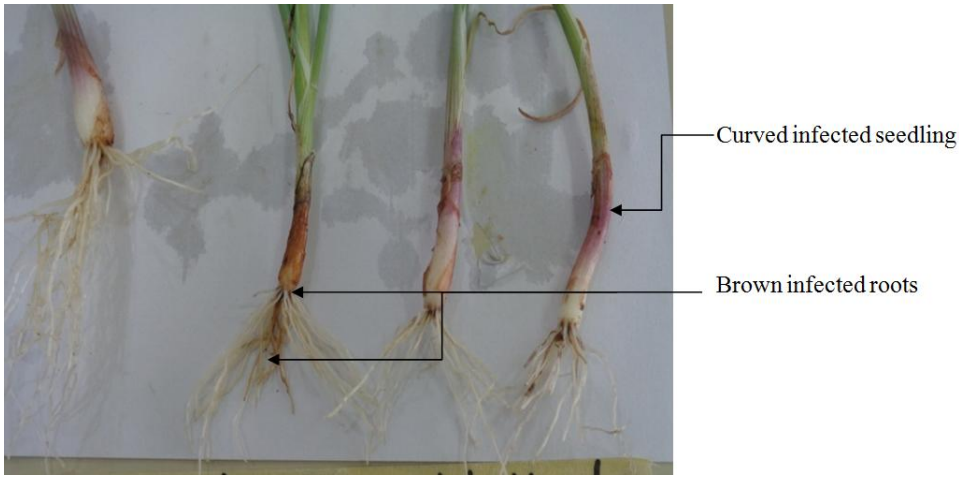

Plate 6. Root dip inoculated seedlings with symptoms

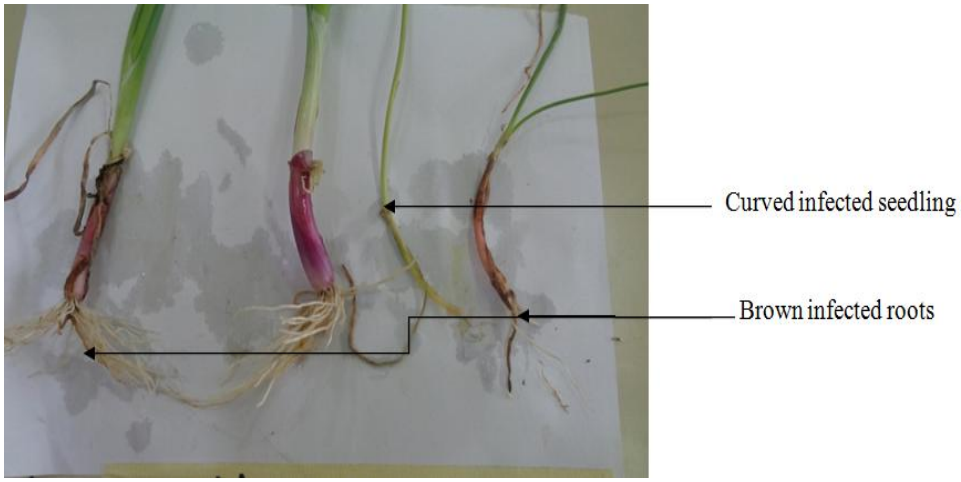

Plate 7. Mycelia paste inoculated seedlings with symptoms

There was significant difference at (P=0.05) in root and leaf length daily increase rate using the different inoculation methods. Root length/day was fastest on control (0.092 mm/day) followed by root dip 0.067mm/day, which were at per (Table 2). Least root length/day was recorded with mycelia paste (0.037 mm/day). The rate of increase in longest leaf was fastest (7.828 mm/day) with soil drench.

4. DISCUSSION

This result to my knowledge is the first report of *F. equiseti* causing disease on onion in Nigeria. Most commonly isolated fungi from onion in Nigeria are *Glomerella cingulata* by Ebenebe [6] and *Fusarium oxysporium* by Ibrahim [10] Goswami et al. [11] observed reddish brown discoloration due to *F. equiseti* infection on ginseng roots, while brownish discolouration and water-soaking symptoms developed on roots of alfalfa (*Medicago sativa*), canola (*Brassica napus*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and oat (*Avena sativa*) seedlings. According to Bayraktaret al [12] *F. equiseti* caused 37.8% mortality of onion plants in Turkey under controlled environment. *Fusarium equiseti* have also been reported to cause *Fusarium* fruit rot on watermelon [13] tomato rot on tomato [14] and pepper [15] and other hosts which includes cowpea in Nigeria [16] and cereals [17,18].

Other *Fusarium* species have also been associated with root rots of onion or other alliums, including *F. proliferatum, F. redolens* and *F. avenaceum ,* but are generally less common than *Fusarium oxysporum* [19-22]

The disease transmission by the soil inoculation with *F. equiseti* inoculum suggest the pathogen residence in soil and ability to cause the disease via soil infection. This agrees with the work of Goswami et al. [11], who reported *F. equiseti* as a soil inhabitant and ability to infect seeds, roots, tubers, and fruit of several crop plants.

Fusarium equiseti, from the study had significant effect on the rate of increase in leaf, using the soil drench method of inoculation (7.83 mm/day). This observation of a plant pathogenic fungus acting as a plant growth promoter, was first observed in 1926, when Japanese scientists observed rice plants infected with *Gibberella fujikuro* had abnormally long stems due to its gibberellin production [23,24] similar report was made by Vinale et al. [25] on seedlings etiolation in pea (*Pisum sativum*) tomato (*Lycopersicum esculentum*) and canola (*Brassica napus*) due to the effect of *Trichoderma harzianum*. This explains why there was a general increase in all growth parameters where plants were inoculated with *F. equiseti*. Horinouchi et al. [23] defined *Fusarium equiseti* as a growth hormone promoting fungus.

All the three methods of inoculation showed infection; suggest that plants could be infected by *Fusarium equiseti* through multiple ways. Previous reports indicated that the *Fusarium equiseti* residence in the soil from where it infects the roots, stem plate and fleshy leaf bases of the onion plant, in that sequence [19,13].

5. CONCLUSION

The fungus *Fusarium equiseti*, whose identity was confirmed at the International Mycological Institute (IMI) Egham, London induced typical symptoms of the "Danzzazalau" disease observed on the farmers field in Kebbi State, Nigeria. This work is the first report implicating *F. equiseti* as a fungal pathogen of onion in Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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