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Soil borne *Fusarium solani* exhibited pathogenic effect on tomato cultivars in Nigeria

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ABSTRACT

The pathogenicity of soil borne fungi *Fusarium solani* on tomato cultivars was relatively unclear in Nigeria. This study thus investigates the pathogenic effect of *F. solani* on seed germination, seedling growth and fruit of two of the most commonly consumed tomato cultivars (UC82B and Ibadan Local - IL) in Nigeria. *Fusarium* species were isolated from the rhizospheric soil samples of tomato cultivars and their pathogenicity was assessed using blotter and soil inoculation techniques. Out of the 26 *Fusarium* species isolated, 6 (23%) were morphologically (with the aid of microscope) identified as *F. solani* (FS1, FS9, FS10, FS17, FS21 and FS26), among which the strains FS17, FS21 and FS26 significantly inhibited ($p < 0.05$) germination of UC82B seed, while only FS21 was responsible for poor seed germination in IL. At the same time, FS17, FS21 and FS26 significantly reduced ($p < 0.05$) plumule and radicle length more in UC82B, compared to IL. At seedling stage, only FS21 manifested visible pathogenicity. Leaves and stems of UC82B were demarcated with distinct rotting and browning; wherein such symptoms were merely visible in IL. However, the fruit of both the cultivars showed variation in their respective pathogenicity range of *F. solani*; FS21 expressing higher level of disease severity on UC82B fruit (66.50%) compared to IL fruit (55.05%). In general, *F. solani* (FS21) exhibits considerable potential, both to inhibit germination of tomato seeds as well as cause diseases on seedling and fruit of tomato cultivars in Nigeria.

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Seed; pathogenicity; cultivars; Fusarium species; germination; tomato; *Fusarium solani*; *Fusarium oxysporum*

Introduction

Among the vegetables, tomato (*Lycopersicon esculentum* Mill.) holds a cutting edge position both globally and economically (Renna et al. 2018).

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The nutritional and health benefits of tomato are numerous, and are known particularly for decreasing the risk of cancer, osteoporosis and cardiovascular diseases (Bhowmik et al. 2012; Fiedor and Burda 2014). Its low cost cultivation (Kumar et al. 2018) serves as a boon for the farmers in the market. However, tomato is detrimentally affected by many fungal pathogens around the world (Okungbowa and Shittu 2012; Shankar et al. 2014), but most extensively by *Fusarium*. They are found in both temperate and tropical soils. It is one of the most troublesome genera of Plant fungal pathogens, causing devastating diseases like *Fusarium* wilt and *Fusarium* root/stem rot in numerous economically important crops (Charoenporn et al. 2010).

Among all, *Fusarium solani* (sexual morph *Nectria haematococca*; syn. *Haematonectria haematococca*), a filamentous fungus, holds a significant place in agriculture (Šišić et al. 2018). It is an important plant pathogen associated with vascular wilts and root rots in over 100 crops (Kolattukudy and Gamble 1995), although, host-species aggressiveness of individual isolates may vary. For example, studies on the host range of *F. solani* f. sp. *pisi* (MP VI), named by its specific pathogenicity on pea (*Pisum sativum*), revealed that the species were also pathogenic on chick-pea (*Cicer arietinum*) as well as several other non-legume hosts, such as ginseng (*Panax ginseng*) and mulberry tree (*Morus alba)* (Westerlund et al. 1974). A more specific example is held from the field trials in California which showed successful establishment of infection in tomato by *Fusarium solani* f. sp. *Eumartii* isolated from infected potato fruit (Romberg and Davis 2007).

In Africa, especially Nigeria, after surveying previous reports on heavy yield losses caused by the soil borne fungal pathogens, farmers have become sceptical about growing tomato (Ogidi et al. 2012; Okungbowa and Shittu 2012). Ajilogba and Babalola (2013) have reported that the solanaceous crops which include tomato can easily become a host for *F. solani*. However, pathogenicity of *F. solani* on seed germination to seedling growth and fruit of tomato cultivars was not clearly justified. Thus, this study helps investigate the pathogenic effects of the indigenous soil borne pathogen *F. solani* on various growth stages of the tomato cultivars like seed germination, seedling growth and the fruit of selected cultivars in Nigeria.

Materials and methods

Collection of rhizospheric soil samples and isolation of fusarium

Soil samples were collected from three tomato growing fields namely; National Institute of Horticultural Research in Ibadan, local farms in

Moniya (Ibadan) and Ilora (Oyo area) all from Southwestern Nigeria. In each field, rhizosphere soils adhering to tomato roots irrespective of the cultivars were collected randomly across locations, mixed together to form composite soil samples and aseptically transported to the laboratory. Serial dilution-pour plate (Reynolds 2005) and direct soil inoculation (Warcup 1950) methods were employed for isolation using peptone pentachloronitrobenzene agar. The plates were incubated at $25 \pm 2\,^{\circ}C$ for 5–7days.

Preliminary identification of fusarium isolates

Young actively growing *Fusarium* in the plates were sub cultured on Potato Dextrose Agar (PDA) and Spezieller Nährstoffarmer agar (SNA) plates, incubated at $25 \pm 2\,^{\circ}C$ for $7 - 10$ days and tentatively identified. The macroscopic identification was done by observing the colonial features on PDA and SNA plates. The observed features include texture, pigment production, rate of growth and presence or absence of mycelium. The microscopic identification was aided by observing stained slides of isolates with the use of microscope for the presence or absence of phialides, chlamydospores, microconidia and macroconidia (Leslie and Summerell 2006).

Determination of inoculum size

The mycelial growth of young cultures of each isolate on PDA was harvested using camel hair brush and the resulting suspension was filtered through sterile double-folded cheese cloth to separate the conidia from the mycelia fragments. The sieved conidia solution was however counted and readjusted using haemocytometer to 3.5×10^6 conidia/ml (Ros et al. 2005). Quantified inoculum was stored at $-60\,^{\circ}C$. Prior to greenhouse inoculation, spore germination percentage was determined and viable spores for each *Fusarium* isolate were re-diluted to concentration of 10^6 spore's mL^{-1}.

Seed sterilisation, viability and seed germination bioassay

The seeds of two commercial tomato cultivars namely; UC82B and Ibadan local were obtained from spices unit of Nigeria Institute of Horticultural Research and Training (NIHORT), Ibadan, Oyo State. The two seeds were surface-sterilised separately with 2% sodium hypochlorite for 3 minutes, rinsed severally with sterile distilled water and air dried in laminar flow (Haggag and El-Gammal 2012). After the sterilisation of

the seeds, seed viability test was carried out as described by Barbara (2006), then both cultivars were used to establish interaction with each of the *Fusarium* isolate. Briefly, seeds of each cultivar were artificially inoculated with the already quantified inoculum (10^6 spore's mL^{-1}) of each *Fusarium* isolate for 5 minutes. After the inoculation, 10 seeds of each tomato cultivar were sown in five replicates in sterilised Petri-dishes using filter paper blotter method. This was followed by incubation at 25 ± 2 °C for 7 days (Krnjaja et al. 2007). Apart from percentage seed germination, plumule and radicle length were determined with precision on metre rule.

F. solani isolates that inhibited seed germination in blotter method was selected. Their pathogenic effect on seed and seedling was re-assessed using soil inoculation method. Sandy-loam soil (< 3 mm particle size granules) were obtained from NIHORT, sterilised using hot steam and allowed to cool prior experimental set up. Soil was potted (9 by 9 by 9.5 cm) in five replicates and was separately inoculated (10^6 spore's mL^{-1}) with 4 ml each of *F. solani* isolate. After 24 hours, ten pre-sterilised seeds of each cultivar were separately sown in already inoculated potted soil while the control soil was not inoculated. Potted soil were maintained in a greenhouse with a temperature of 26 ± 2 °C, photoperiod of 16 hr light and 8 hr of darkness with average light intensity of 10,000 to 11,000 lux. Soil was held at 36.5% of water holding capacity. Watering was consistently done with tap water at two days interval and when required. The set up was completely randomised and percentage seed germination for each treatment was determined at day 7 using the formula:

$$\text{Percentage seed germination} = \frac{\text{Number of seed germinated/pot} \times 100}{\text{Total number of seed sown/pot}}$$

Post seed germination and disease assessment

After day 7, surviving seedlings were carefully maintained and thinned to one per pot. Two weeks after post inoculation, seedlings with and without disease symptoms were assessed according to the following indices. Percentage disease incidence (PDI) was calculated as the percentage of tomato seedlings showing visible signs of infection, as described by Michel et al. (1997). Disease severity (DS) was developed based on the observable symptoms, with slight modifications from Soonthornpoct et al. (2000) and Gwary et al. (2006). 0 − 0% (apparently healthy seedling); 1, (0 − 25%) of 1 leaf infected; 2, (25 − 49%) of 2-3 leaves infected/traces of stem rot; 3, (50 − 74%) all leaves infected/stunted growth/stem rot); 4, (75 − 100%) of damping off/wilting/seedling death).

Postharvest effects of soil borne Fusarium species on tomato fruit cultivars

Mature, fresh and healthy tomato fruit cultivars (UC82B and Ibadan local) were separately surface sterilised with 2% sodium hypochlorite for 3 minutes and rinsed severally in sterile distilled water. The cultivars were subsequently placed on filter paper for drying. Sand paper was used to bruise a segment on the surface of each healthy tomato. The bruised tomato cultivars (UC82B and Ibadan local) were aseptically and separately dipped into already prepared spore suspension of each *F. solani* (10^6 spore's mL^{-1}) for 15 minutes. Inoculated fruits were removed and placed in sterile container at room temperature for 7 days while the control tomato fruit cultivars were inoculated with sterile distilled water. The experiment was in five replicates. The fungal colony from disease lesion was sub cultured on PDA and re-identified. The disease symptoms were scored based on a disease scale from 0 to 4 devised by Amadi et al. (2009) with a slight modification for tomato. All scales were characterised with a particular symptom on tomato fruit rot.

Re-identification of pathogenic Fusarium isolates

Fusarium species that exhibited the highest PDI was re-isolated from diseased tomato seedlings and fruits per cultivar, using Koch's postulate method (Koch 1891; Fredricks and Relman 1996). Specifically, to ascertain pathogenicity in-line with symptoms expressed. Spore suspension of each *Fusarium* isolate that expressed disease symptoms in tomato was prepared and serially diluted from 10^{-1} to 10^{-5}. Briefly, exactly 100 µl of spore suspension was pipetted from each dilution factor into PDA using spread plate method and incubated at $25 \pm 2\,^{\circ}C$. After the 3rd day, a visible germinating spore from each isolate was removed and transferred to new PDA plates. From the pure culture obtained in the first purification, a second round of purification procedure as described above was carried out and pure culture for each *Fusarium* isolate was obtained on PDA plates. Morphological identification was aided with microscope based on shape, size of microconidia and macroconidia, phialides and the formation of chlamydospores (Rodrigues and Menezes 2005) using *Fusarium* manuals of Leslie and Summerell (2006).

Data collection and analysis

Data collected on percentage seed germination and disease incidence were subjected to statistical analysis (SAS version 9.1 of 2009, SAS Institute, Cary, NC) of variance (Student-Newman-Keuls Test). Mean of

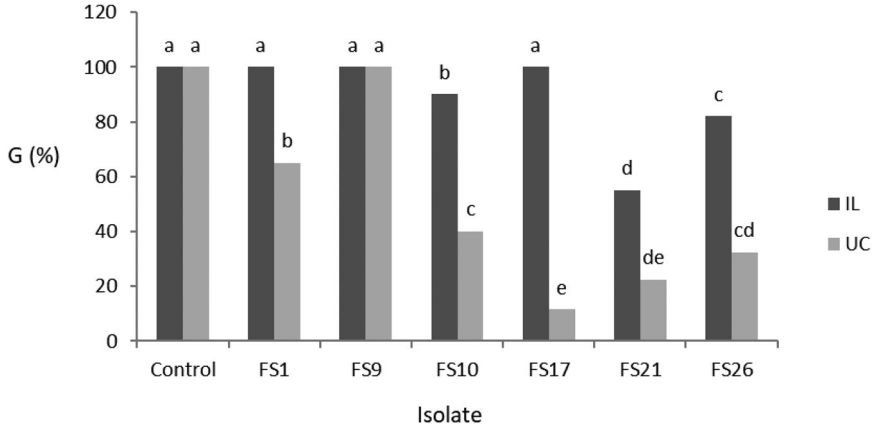

Figure 1. Effect of *F. solani* on seed germination of tomato. Means followed by the same letter (s) are not significantly different ($p \leq 0.05$) according to Student-Newman-Keuls Test. IL-Ibadan local, UC-UC82B, G (%) – Germination percentage.

seeds treated with *Fusarium* solani on the two tomato cultivars were compared and separated by least significant differences (LSD) at 95% confidence level.

Results

Rhizosphere soil samples were collected from tomato plants (two to three months old) diseased with *Fusarium* wilt from three locations in Southwestern part of Nigeria. A total of 26 *Fusarium* isolates were obtained and identified based on morphological white/cream appearance on agar plate. Under microscope, it was observed that macroconidia have 3-4septa on an average and is slightly curved with slightly blunted apical end. Microconidia are abundant, oval to kidney shaped, and are formed in false heads on a very long monophialide. Chlamydospores were formed with solitary or in chain at the middle or end of the mycelium. Further identification revealed that 6 isolates were *F. solani* (FS1, FS9, FS10, FS17, FS21 and FS26).

After Fusarium-seed interaction, only FS21 exhibited outstanding inhibitory effect on tomato seed germination. The evaluated *F. solani* delayed seed germination and $\geq$50% of the seeds were inhibited in comparison to the control. UC82B had the lowest germination due to the effects of FS10, FS17, FS21 and FS26 in comparison to IL. FS21, in particular caused delay in seed germination of UC82B and IL (Figure 1). Based on the observations during the experiment, it was found that the mycelia of *F. solani* were physically growing on the seeds after day 7 of inoculation. Interestingly, the radicle and plumule length of IL were unaffected by FS1, FS9 and FS10. Similar observation was also recorded

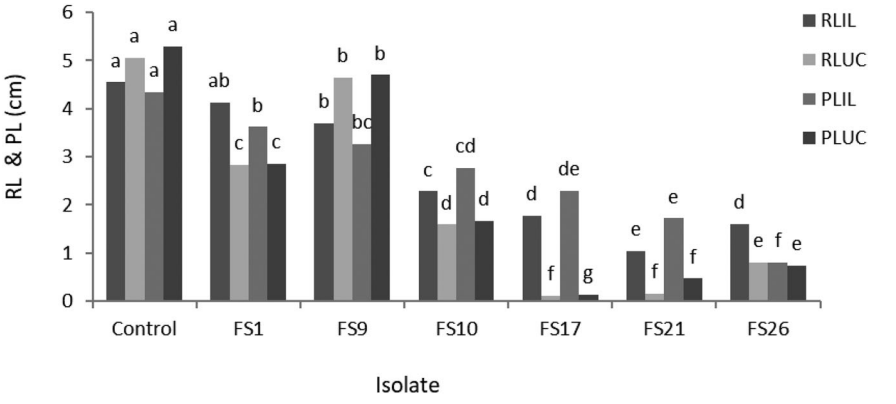

Figure 2. In-vitro effect of *F. solani* on radicle and plumule length of tomato seedling. Means followed by the same letter (s) are not significantly different ($p \leq 0.05$) according to Student-Newman-Keuls Test. IL-Ibadan local, UC-UC82B. RL – Radicle length, PL – Plumule length.

for FS1 and FS9 on UC82B radicle and plumule length. Apart from the control, at day 7, UC82B treated with FS9 had the highest radicle (4.65) and plumule (4.70) length, though with little or no difference from that of IL. There was no significant difference ($p \leq 0.05$) in IL treated with the FS1 and FS9. With respect to FS17, FS21 and FS26, plumule and radicle length of UC82B was adversely affected in comparison to IL (Figure 2).

F. solani were re-evaluated in soil to ascertain their significant effects on tomato seed cultivars. At day 7 of sowing, there was significant ($p \leq 0.05$) effect of germination in both cultivars with respect to each *F. solani* isolate. Germination percentage of UC82B seeds inoculated with FS21 was lower (42.5%) in comparison to that of IL (54.0%). The germination of IL seeds treated with FS26 was delayed (53.0%) in comparison to the control (Figure 3).

Pathogenicity was spotted to be more on UC82B and comparatively less on IL seedlings. Distinctively on the cultivar UC82B, the strains FS21 (DI = 75%; DS = 4.0), followed by FS26 (DI = 65%; DS = 3.0), FS10 (DI = 50%; DS = 2.0) and FS17 (DI = 45%; DS = 3.0) expressed the highest level of aggressiveness and virulence while FS9 (DI = 0.0%; DS = 0.0) showed the lowest (Figures 4 and 5). Only FS21 (DI = 35%; DS = 3.5) exhibited remarkable disease incidence and severity on IL. There was more browning and rotting in the lower stem of UC82B and less on IL tomato seedlings. Thus UC82B cultivar proved to be more susceptible than IL, although IL too displayed susceptibility to FS21, but to a minimal range of pathogenicity. Only FS9 did not show any sign of disease symptoms.

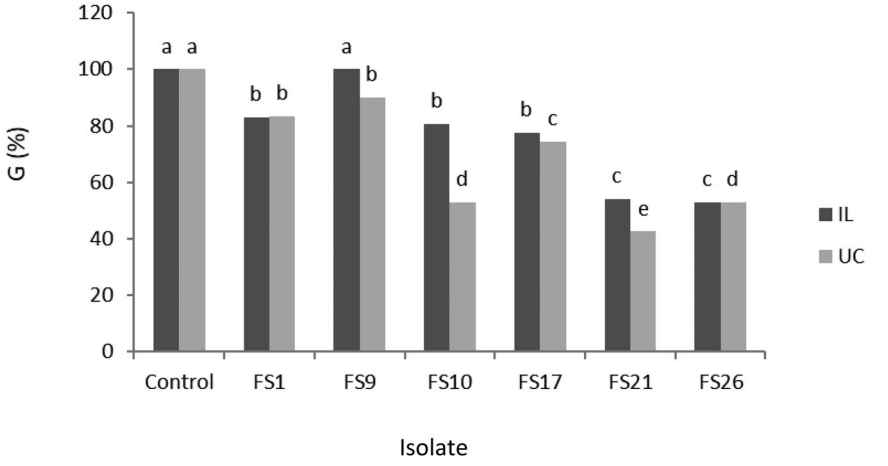

Figure 3. Pathogenic effect of *F. solani* on seed germination of tomato. Means followed by the same letter (s) are not significantly different ($p \leq 0.05$) according to Student-Newman-Keuls Test. IL-Ibadan local, UC-UC82B, G (%) – Germination percentage.

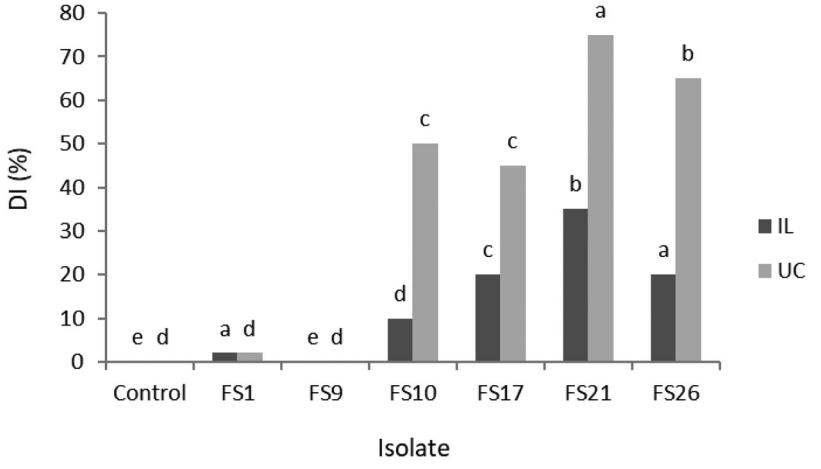

Figure 4. Disease incidence of each *F. solani* on tomato seedling. Means followed by the same letter (s) are not significantly different ($p \leq 0.05$) according to Student-Newman-Keuls Test. IL-Ibadan local, UC-UC82B, DI – Disease incidence.

FS21 expressed the highest rot (DS = 66.50%) in UC82B tomato fruits, followed by FS17 (DS = 38.5) and FS26 (DS = 30.0%). Excluding the control plant, least pathogenicity was recorded for FS9 on UC82B and FS10 on IL. FS21 outrightly produced rot, not only on UC82B but also on IL fruit (Figure 6). Mycelia growth of FS21 was visible after day 3 of inoculation on both cultivars. In addition, there were visible patches of tissue necrosis on UC82B fruits. The FS21 have penetrated deep into the epicarp and lived within the fleshy parts of the mesocarp. The necrosis of the epicarp enlarges outwards and the fruit becomes water-soaked as the infestation of *F. solani* continued.

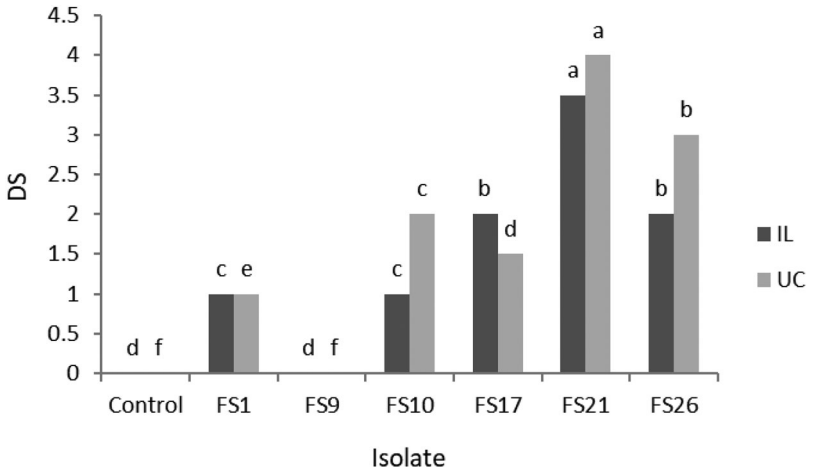

Figure 5. Disease severity of each *F. solani* on tomato seedling. Means followed by the same letter (s) are not significantly different ($p \leq 0.05$) according to Student-Newman-Keuls Test. IL-Ibadan local, UC-UC82B, DS – Disease severity.

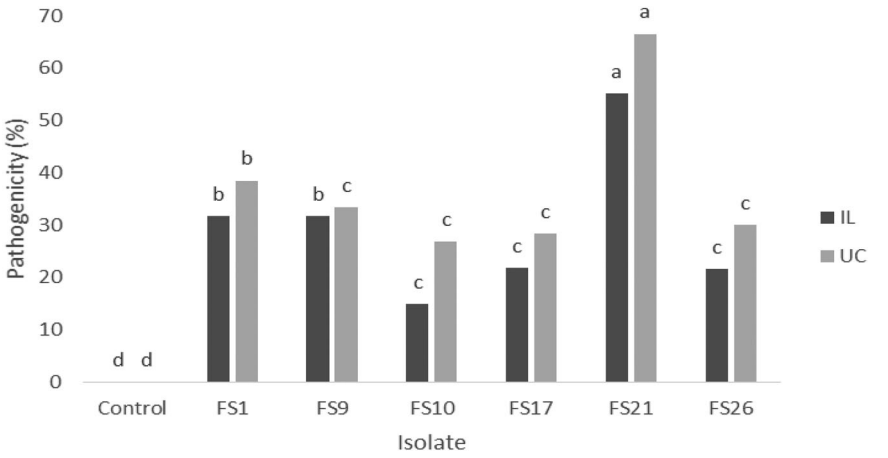

Figure 6. Pathogenicity of *F. solani* on tomato fruit. Means followed by the same letter (s) are not significantly different ($p \leq 0.05$) according to Student-Newman-Keuls Test. IL-Ibadan local, UC-UC82B.

Discussion

Globally, *Fusarium* has been pathogenically associated and characterised with tomato (Ignjatov et al. 2012; Shankar et al. 2014). Just like in other countries, the pathogenic effect of *Fusarium* species on tomato cannot be overemphasised in Nigeria. Previous studies in Nigeria (Bankole 1996; Ogidi et al. 2012; Okungbowa and Shittu 2012) have strategically established the pathogenicity of *F. oxysporum* on tomato (Ignjatov et al. 2012; Isaac et al. 2018) while that of other *Fusarium* species especially *F. solani* remains undocumented. Thus, this study

provided additional information on magnitudes of pathogenicity of *F. solani* on tomato cultivars.

The presence of *F. solani* in soil (Ignjatov et al. 2012) is an important criterion. *F. solani* is commonly found in soil, where it thrives as dormant propagules and grows saprophytically or pathogenically (Ajilogba and Babalola 2013) with other host specific crops such as millet, maize, and sorghum. The isolated *Fusarium* species were characterised morphologically (with distinct mycelia, conidia, philiades, chlamydospores and colony growth), aided by microscope (Smith et al. 1988; Burgess et al. 1994; Raghu et al. 2016). The aerial mycelium appears white/cream and gradually changes to gray/light yellow to justify identity of *F. solani* (Smith et al. 1988; Raghu et al. 2016). The occurrence of *F. solani* was evidenced (Kosiak et al. 2004; Schollenberger et al. 2005) in the collected composite soil samples for this study as 6 out of 26 isolates were identified as *F. solani*. This could ascertain the presence of *F. solani* in subtropical region of the world (McGovern et al. 2001) which includes Nigeria.

The isolated *F. solani* significantly retarded seed germination of both tomato cultivars tested, though the effect was more on UC82B and less on IL. FS21 had the lowest seed germination rate. This suggests that FS21 has the potential to inhibit germination of tomato seed from growth to the seedling stage. This is similar to the observation of Raghu et al. (2016) on Chilli seed. The effects of other *F. solani* (FS1, FS9, FS10, FS17 and FS26) were not significant as compared to that of FS21. As the observation on seed germination progresses, the plumule and radicle length were qualitatively and quantitatively observed. FS17, FS21 and FS26 affected the plumule and radicle length more in comparison to FS1 and FS9. During the experiment, mycelia growth of *F. solani* was obvious on the germinated seeds. This may have actually contributed to the poor development of radicle and plumule length (Krnjaja et al. 2007). One can assume that the isolates did not cause instant death of the seed but weakened the plumule and radicle with time.

Few *Fusarium* species are generally destructive to tomato (Haggag and El-Gammal 2012). In our study, only FS21 expressed high level of disease incidence and disease severity. Based on our findings, the symptoms of disease begins on tomato seedlings as slight vein clearing on outer leaflets and later drooping of leaf petioles, followed by wilting of the lower leaves and loss of colour, from green to yellow and ultimately death of the entire plants even before reaching maturity. Estimation of disease incidence revealed UC82B as the most susceptible in comparison to IL. Stem rot with a dark colouration of vascular system in seedling was also observed particularly in the initial stage of infection. This corroborated

with the submission of Burgess et al. (2008) that brown vascular discolouration can be observed in stem tissue cross sections near the soil line, even though these stems remain firm and green on the outside. In addition, seedlings damped off as they emerge from the soil (Ajilogba and Babalola 2013). Damping off of diseased seedlings is in agreement with Ivic (2014) that *F. solani* is among the *Fusarium* species that encouraged seedling diseases of soybean. In addition, *F. solani* have been established in California causing foot rot and folia symptoms on tomato based on field and greenhouse trials (Romberg and Davis 2007).

Susceptibility of tomato cultivars to causal organisms varied in this study. This is supported by Haggag and El-Gammal (2012) who also reported similar observation on pathogenicity of *F. solani* on some tomato cultivars. By the time tomato shows any outward sign of infection, it is already too late, the plant wilts and dies (Ajilogba and Babalola 2013). Death of tomato plant may result due to failure of the infected xylem to meet the water requirements of the plant (Burgess et al. 2008; Okungbowa and Shittu 2012). In addition, the *F. solani* may vary in their virulence status and host-specific pathogenic aggressiveness. Thus, continuous exposure of tomato to soil borne pathogenic *F. solani* can lead to tomato wilting. Our understanding suggest that other internal factors such as resistance genes, enzymes, growth – regulating compounds, environmental and growth conditions may have been altered (Isaac et al. 2018).

One of the factors affecting the quality and shelf life of tomato fruits in Nigeria is biotic constrain and this account for 10-30% of losses (Etebu et al. 2013). One of the common and important biotic constraints is fungal pathogens which include *Fusarium*. In Nigeria, Opadokun (1987) reported that 21% of harvested tomato was lost to rot which include *Fusarium* rot. *F. solani* (FS21) isolated in this study exhibited rot on tomato fruit. The disease symptoms include rots and extend into the centre of the fruit. The rotted tissue is often water-soaked and becomes covered by white and greyish mycelium externally while the infected tissue is discoloured and appears pale brown (Denis 1983). This is in-line with the report of Yoltas (1985) that *Fusarium* rot is one of the most common diseases among fresh tomato fruits in storage. This could be attributed to low pH, high moisture content and nutrient composition, which makes the environment fragile for *Fusarium* attack. The succulent epicarp could also enable the fungal hyphae to penetrate deeply into the fruit (Tournas and Katsoudas 2005). As a result, the yield of this economically important farm product is affected, hence lowering the production rate (Salleh and Mushitah 1991) and diminishing its market value (Nurulhuda et al. 2009).

Based on our continuous observation, isolate FS9 did not exhibit any pathogenic effect on tomato. That is, FS9 did not inhibit seed germination and the radicle and plumule lengths. Similarly, no symptoms of any tomato diseases were expressed on either seedling or fruit of both tomato cultivars. This suggests that some *F. solani* are asymptomatic, and can act as saprophytes on tomato.

Conclusion

Ultimately, the soil borne *F. solani* stands the chance to change from moderate to highly pathogenic strain. Their effect on seed germination significantly reduced plumule and radicle length. UC82B cultivar was more susceptible in comparison to IL cultivar. Thus, tomato has the potential to host both soil borne asymptomatic and pathogenic *F. solani* species. To mitigate this challenge, sustainable control measures should be put in place to manage pathogenic effect of *F. solani* on tomato cultivars in Nigeria.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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