Nigerian Journal of Botany, Volume 30 (1), 41-50, June, 2017

MICROORGANISMS ASSOCIATED WITH RHIZOSPHERE AND PHYLLOSPHERE OF SEEDLING BLIGHT DISEASE OF TOMATO (*SOLANUM LYCOPERSICUM*) PLANTS

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Received 30th October, 2016; accepted 3rd March, 2017

ABSTRACT

Microorganisms associated with the rhizosphere and phyllosphere of seedling blight disease of tomato plant (*Solanum lycopersicum*) were investigated. Tomato seedlings that showed symptoms of seedling blight disease were collected from Malle farm (Lapai), Lambu farm, Dawaki (Suleja) and Unguwan Tukura farm in Kontagora Local Government Area of Niger State, Nigeria. The fungal and bacterial species were isolated and identified using serial dilution and agar plate methods. The percentage occurrence of the isolates were *Aspergillus niger* (89%), *Aspergillus flavus* (83%), *Fusarium oxysporum* (60%), *Microsporum gympseum* (15%), *Mucor pusillus* (44%), *Penicillium citrinium* (40%), *Rhizopus nigricans* (50%), *Trichophyton megnini* (45%), *Bacillus subtilis* (60%), *Micrococcus luteus* (33%), *Bacillus megaterium* (55%) and *Bacillus cereus* (55%). The pathogenicity test conducted showed that the positive effect of the microorganisms associated with the rhizosphere and phyllosphere was pathogenic leading to seedling blight infection on healthy tomato plants, similar to those symptoms from the various farms. The blight infections appeared on the healthy tomato plants after 14 days of inoculation with symptoms such as leaf wilting, stem blight, brownish colouration, collapse and death of seedlings of the tomato plants. This led to the reduction in quality and yields in tomato plant and loss of income for the farmers.

Keywords: Rhizosphere, Seedling blight disease, *Solanum lycopersicum*, phyllosphere

INTRODUCTION

Seedling blight is a disease that causes the seedling to rot and die (Sella *et al.*,2014). Pre-emergence seedling blight occurs as a result of infection by fungal and bacterial species that infect seedlings prior to emergence or after emergence and cause various kinds of stem lesions (Ahmad *et al.*, 2014).

The pathogens are activated by the presence of the plant and the substances released by its roots. These pathogenic organisms invade the root directly or through wounds and move into the cortical tissues or into the vascular system. They release enzymes which degrade the seedling tissue and disrupt normal plant growth processes. When conditions are favourable, the result is poor germination and poor emergence. Seedlings often die after emergence, but the most vulnerable period is from germination to emergence (Manman *et al.*, 2015). Seedlings that have already emerged are attacked at the roots and the stem at the soil line or below (Khare *et al.*, 2014).

The severity and incidence of the disease on tomato plant depend on some factors, namely, percentage of the seed infested by seed-borne fungi, soil temperature and soil moisture content. The disease is severer on vegetables that are seeded early when the soil is usually cold and damp (Clayton and Don Groth, 2012). The microorganisms associated with seedling blight diseases of crops include *Rhizoctonia* species, *Pythium*

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species, *Helminthosporium* species, *Aspergilus niger*, *Aspergillus flavus*, *Aphanomyces cichlids*, *Phoma betae*, *Curvularia* species, *Xanthomonas* species, *Fusarium oxysporum* , *Penicillium* species and *Bacillus* species. They all contribute to a disease complex causing damping-off and death as reported by Muhammad and Amusa (2003) and Muhammed (2013).

Tomato (*Solanum lycopersicum*) is an important vegetable crop world wide. Often times, its production is hindered by blight disease (Koley *et al*., 2015). Tomato seedling blight is a disease that attacks the foliage, stem and fruit of tomato plant. When it suffers from blight, the leaves and stem wither, stop growing and subsequently die (Judelson and Blanco, 2005).

According to Ivors (2008), blight disease of tomato can completely defoliate and destroy the crop within two to three weeks after planting. Due to moderate temperature, frequent rainfall during the growing season, blight also attacks tomato fruits at all stages of development. Rotten fruits are typically formed with greasy spots which eventually become leathery and chocolate in colour. These Microorganisms are known to affect tomato plants resulting in the reduction in yield. Seedling blight disease of tomato appears as wilting and browning of leaves and stem blight on the rhizosphere and phyllosphere of the tomato plants resulting in 80% reduction in yield (Hausbeck and Lamour, 2004). In view of these, this study was carried out to isolate and identify the microorganisms associated with rhizosphere and phyllosphere of tomato seedlings suffering from blight.

MATERIALS AND METHODS

Collection of Diseased Tomato Plants

The blighted stem and leaves and seedlings of *Solanum lycopersicum* were collected directly from randomly selected farmers' fields of Lambu farm in Suleja Darwaki area, Malle farm in Lapai and Unguwan tukura farm in Kotangora, Niger State, Nigeria. They were put into well labeled sterilized polyethylene bags and stored at ambient temperature (28±2 ^{o}C) at the Department of Microbiology Laboratory in Ibrahim Badamasi Babangida University, Lapai, Niger State.

Isolation of Fungi and Bacterial Associated with the Rhizosphere of Blighted Plants

Samples of soil in the rhizosphere of blighted seedlings of tomato were separately collected from infected seedlings of the crop. The soil around and adhering to the roots of blighted seedlings were placed in 500 ml sterilized beaker and bulked thoroughly. One gram was taken from the bulked rhizosphere soil and mixed in 9 ml sterilized distilled water. The mixture was serially diluted from 10^{-1}-10^{-6}. One ml of 10^{-2} and 10^{-3} strength of dilution was inoculated on Potato Dextrose Agar and Nutrient Agar and incubated at room temperature (28 ± 2^{o}C) for 3- 7 days, to allow for the growth of all associated organisms. Pure cultures of the associated microorganisms (fungi and bacteria) were obtained through subculturing (Mwajita *et al*., 2013). The cultural features of each fungal isolate were carefully observed and recorded. The macro-morphological and micro-morphological features of the fungal and bacterial isolates were compared with those described in mycological guide by Barnett and Hunter (1996) and bacterial guide by Cappuccino and Sherman (2002).

Isolation of Fungal and Bacterial Species from the Phyllosphere

Diseased leaves of tomato plants that showed symptoms of seedling blight disease were collected from the selected farms as mentioned. The isolation of pathogens from the diseased leaf bits of about 1cm by 1cm cut across the leaf lesions were 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 and Nutrient Agar, respectively. The Petri- dishes were incubated at ambient temperature (28^{o} ±2^{o}C) for fungal and at 37^{o}C for bacterial growth for a period of 3-7 days. Pure cultures from the mixed growth were obtained through sub-

culturing and subsequently identified by the use of mycological atlas by Barnett and Hunter (1996) and bacterial guide by Cappuccino and Sherman, (2002). To determine the percentage relative abundance of the isolates from the blighted rhizosphere and phyllosphere, the number of times each microorganism was isolated per location was expressed as a percentage using the method of Okigbo and Ikediugwu (2000), and calculated thus:

Percentage (%) relative abundance of the isolates= $T/N \times 100$

where,

N = Total number of microorganisms isolated per blighted rhizosphere and phyllosphere in the three farms and T = Number of times of occurrence of the individual isolate in the selected farms.

Disease index (D1) = $H/N \times 100$

where,

H = Number of diseased plants sampled
N = Total number of plants sampled

Pathogenicity of the Isolates

The suspensions of bacterial and fungal isolates from blighted tomato rhizosphere and phyllosphere were maintained on V8 broth medium in 250 ml conical flasks for 6 days. The mycelia of each fungal isolate were filtered through a cheesecloth and gently pressed to remove excess liquid and blended for 3 seconds in a warring blender at the rate of 5 grams of mycelium per mililitre of sterile deionized water (Muhammad and Amusa, 2003).

Inoculation of Isolated Inoculums

The soil for raising the seedlings was sterilized in the oven at 120 °C for three hours. The sterilized soil was allowed to cool and loaded into the pots, moistened with sterilized water before the seeds were sown in three replicates. Three-week old seedlings of tomato cultivars were sown in oven-sterilized topsoil (0.5 cm) contained in 15 cm diameter pots and were inoculated with freshly prepared suspension of the isolated fungal and bacterial species. The plants were arranged on top of benches in the screen house of the Department of Biology, Ibrahim Badamasi Babangida University, Lapai, Niger State and were observed for symptoms of the disease on daily basis for four (4) weeks. The pathogens were later re-isolated from the inoculated plants and compared with the initial isolates (Muhammad *et al.*, 2001). The percentage disease incidence and severity of tomato and okra plants were evaluated by using disease index(DI), a modified method of Khanna *et al.* (1977), and calculated thus:

Disease index (DI)= $H/N \times 100$

Where,

H= number of diseased plants sampled
N= total number of plants sampled.

Disease severity was determined as reported by Bem *et al.* (2010) using a numerical scale of 0-5 as follows:

0-No infection (o %)
1- Mild infection (1-20%)
2-Moderate infection (21-40%)
3- High infection (41-50%)
4- Severe infection (51-60%)
5- Highly severe infection (61 % and above).

RESULTS

Table I: Percentage Relative Abundance of Fungal Species Isolated From Blighted Tomato Plants from Three Selected Farms

Locations	*Aspergillus niger*	*Aspergillus flavus*	*Tricophyton megnini*	*Mucor pusillus*	*Microsporum gympseum*	*Fusarium oxysporum*	*Rhizopus nigricans*	*Penecillium citrinium*
Bida	89.3±1.15^{a}	22.2±0.50^{c}	45.5±1.50^{a}	44.4±1.00^{a}	11.11±0.33^{b}	60.0±1.10^{a}	50.00±1.00^{a}	40.0 ± 1.10^{a}
Suleja	50.0±1.10^{b}	50.5±1.11^{b}	44.4±0.50^{b}	44.4±1.00^{a}	14.00±0.50^{a}	50.0 ± 1.10^{a}	30.00 ± 0.10^{b}	12.00±0.50^{b}
Kontagora	00.00±0.0^{c}	83.0±1.21^{a}	22.20±0.10^{b}	22.2±0.00^{b}	15.30±1.00^{a}	43.00±0.40^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}

Mean values ± S.E percentage bacterial species isolated. Values with the same superscript letter(s) down the column are not statistically significant at ($p \geq 0.05$) different as tested by Duncan's Multiple-Range Test

The results in Table I show that there was a high significant difference (P 0.05) in percentage relative abundance of fungal species isolated from blighted tomato plants between farms I, II and III. *Aspergillus* niger had the highest percentage occurrence (89.3 %.) in farm I. which was significantly different ($P \leq 0.05$) from farm II; but it was not isolated from farm III. A Similar trend of occurrence was observed in *Fusarium oxysporum, Rhizopus nigricans and Penecillium citrinium* isolated from the three selected farms. *Aspergillus* flavus had the highest percentage (83 %) in farm III which was significantly different ($P \leq 0.05$) from farms I (50.5 %) and II (22.2 %), respectively (Table I). *Tricophyton megnini* had the highest percentage relative abundance (45.5%) in farm I and was significantly different from farms II and III (22%). *Mucor pusillus* had the highest percentage of 44% in farm I but this value was similar to farm II. The results show that *Microsporum gympseum* percentage was statistically similar in farms II and III but were significantly different from farm I.

Table 2: Percentage Relative Abundance of Bacterial Species Isolated From Blighted Tomato Plants from Three Selected Farms

Locations	*Bacillus megaterium*	*Bacillus cereus*	*Bacillus subtilis*	*Micrococcus luteus*	*Streptococcus faecalis*	*Staphylococcus aureus*
Bida	55.50±2.50^{a}	55.00±2.50^{a}	45.5±1.50^{b}	33.10±1.10^{a}	0.00±0.0^{c}	50.50±1.50^{a}
Suleja	33.00±0.00^{c}	45.50±1.21^{b}	33.30±0.50^{c}	22.22±0.21^{b}	55.50±2.05^{a}	0.00± 0.00^{c}
Kontagora	46.50±1.08^{b}	34.50±0.41^{c}	60.00±1.95^{a}	22.00±0.10^{b}	50.50±1.07^{b}	22.22± 0.10^{b}

Mean values ± S.E percentage bacterial species isolated. Values with the same superscript letter(s) down the column are not statistically significant at ($p \geq 0.05$) different as tested by Duncan's Multiple-Range Test

The results revealed (Table II) that there was high significant difference ($P \leq 0.05$) in percentage relative abundance of bacterial species isolated from blighted tomato plants between Farms I, II and III . *Bacillus megaterium*, *B. cereus* and *Staphylococcus aureus* had the highest percentage of occurrence of (45.5-55.5 %) in farm I. *B. subtillis* had the highest relative abundance of (60 %) from farm III. However, this value was significantly different ($P \leq 0.05$) from farm II (33.3 %) and farm I (45.5 %). *Streptococcus faecalis* and *Staphylococcus aureus* were not isolated from farms I and II.

Percentage Disease Incidence of Tomato Seedlings Inoculated with Fungal species

Table 3 shows percentage disease incidence of the fungal isolates.

The percentage disease incidence of *Aspergillus niger,A flavus and Microsporum gympseum* inoculated on Replicates 1, 2 and 3 all produced high and moderate infections.

Table 3: Percentage Disease Incidence of Fungal Species Inoculated on Healthy Tomato Seedlings

	Replicates		
Fungal species	1	2	3
Aspergillus flavus	49	35	35
A niger	..	36	49
Fusarium oxysporum	47	33	45
Mucor pusillus	43	35	36
Penecillium citrinium	45	34	45
Tricophyton megnini	46	35	50
Rhizopus nigricans	47	37	50

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

Percentage Disease Incidence of Bacterial Species Inoculated on Healthy Tomato Seedlings

The results obtained (Table 4) on percentage disease incidence of the bacterial isolates on Replicates 1, 2 and 3 inoculated with *Bacillus cereus, B. subtillis, B. megaterium, Micrococcus luteus* and *Staphylococcus aureus* produced severe infections on the tomato seedlings.

Table 4: Percentage Disease Incidence of Bacterial Species Inoculated on Healthy Tomato Seedlings

	Replicates		
Bacterial species	1	2	3
Bacillus cereus	74	65	80
Bacillus subtillis	73	66	79
Bacillus megaterium	80	63	75
Micrococcus luteus	79	64	63
Staphylococcus aureus	85	70	64

DISCUSSIONS

Fungal species isolated from the rhizosphere and phyllosphere of blighted tomato plants were *Aspergillus niger, A. flavus, Tricophyton megnini, Mucor pusillus* and *Microsporum gypseum* from the three farms. Janisiewicz and Korsten (2002) reported the isolation of *Tricophyton megnini* from tomato plants observed that it attacked the plants at all stages of growth. In young seedlings, cotyledons become to brown; young leaves become chlorotic along veins and finally seedlings die. *Microsporum gypseum*, *Aspergillus niger* and *Mucor pusillus* were isolated from the leaves and roots as has also been reported Hull (2002). In adult tomato plants, leaves become flaccid resulting in drooping and wilting of the leaves infected by the pathogens. Shakir *et al.* (2000) reported similar results. *Aspergillus* species affect seedlings plants at all stages of growth from seedling to fruit formation causing damping-off of seedlings which results in the infection of stem just above and below the soil surface (Hausbeck and Lamour, 2004).

Bacterial species isolated from the rhizosphere and phyllosphere were from the genus *Bacillus* (*Bacillus subtilis, Bacillus cereus, Bacillus megaterium*) and *Micrococcus luteus*. They were all predominant in farms II and III. This corroborated Obradovic *et al.*(2004), who observed that *Bacillus subtilis* and *Micrococcus luteus* were usually associated with seed rots,collapse of stem and death of seedlings of tomato plants in fields and gardens causing seedling blight disease. Bartz *et al.*(2004) reported similar results.

The bacterial and fungal isolates when inoculated on three-week old healthy tomato seedlings caused moderate, severe and high infection in the three triplicates. This agrees with the results of Keith *et al.*(2006) that late blight disease of potato showed symptoms on affected plants with severe yellowing and browning of leaves on the seedlings and finally caused death of the plants under high humidity conditions. Similarly, Abada *et al.* (2000) reported severe infection of tomato plants, such as leaf lesions enlargement that caused blighting of leaves.This caused great reduction in the quantity and quality of tomato yield.

Seedling blight diseases of agricultural crops have remained a very serious constraint to vegetable production in Nigeria. Apart from having a wide host range, some of the fungal pathogens produce sclerotia that remain viable in soils for many years (Muhammad and Amusa, 2003).

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