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PATHOTYPES MYCOFLORA OF TOMATO (*Lycopersicon esculentum* MILL) IN SOME AREAS OF KANO STATE, NIGERIA

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ABSTRACT
The study aimed at identifying mycoflora pathogens of tomato cultivars (*L. esculentum* Mill) varieties in selected tomato growing areas of Kano State during the rainy and dry periods of 2014/2015 was conducted. Survey was done on different farmers' fields to identify diseased plants based on signs and symptoms. Cultivar tomato infection rates were determined using disease incidence and disease index was done using disease severity scale of 1-5. Infected plant parts surveyed were collected and taken to the laboratory for pathogen culture and isolation. Identification of pathogens was done by microscopy and available literatures. Results showed the following symptoms; root rot, leaf spot, blight, wilting, fruit rot, brown stem, defoliation and leaf curl at various degrees. Incidence ranged from 24.54 % - 24.57 % in the irrigated areas, 41.90 % - 44.03 % within the rainfed tomato growing areas and disease index from 2.0 – 3.0 on disease severity scale. Disease incidence showed significant differences (P= 0.05) in the different areas and major isolates were *Fusarium oxysporum*, *Rhizoctonia solani* and *Fusarium solani*. Therefore, there is the need to proffer control strategies to assist farmers in controlling these pathogenic flora.

Keywords*:* Pathotypes, *Lycopersicon esculentum* Mill, Disease incidence and severity

INTRODUCTION

Tomato *Lycopersicon esculentum* is an herbaceous plant belonging to the solanaceae or night shade family, originating from South America, Peru and Ecuador (Smith, 1994; Suleiman, 2011).

Tomato was introduced into Europe from Mexico by the Spanish in 1523. At first it was little used for food because of its relationship with the deadly night shade family. It was referred to as object of affection and therefore known as love apple "Pomme damour" in French (Peralta and Spooner, 2001).

Tomato is a well-known ingredient in food preparation, valued for its colour, flavor and pleasant acidic taste of the fresh, canned or preserved state. It can be eaten raw, cooked and made into soup, puree or powder (Hill and Waller, 1999). Tomato is known to contain vitamin A, B, C as well as significant amounts, lycopene, sodium, iron, phosphorus, βeta-carotene, potassium and magnesium (Jones, 1999). The seeds contain about 24 % of semi-drying oil and are used for salad oil, margarine and soap production. The residual mass or press-cake is used as a stock field and fertilizers (Kiran *et al.,* 2006).

The University of California at Davis, surveyed and ranked tomato as the most important fruit or vegetable of western diets in terms of overall source of vitamins and minerals. The tomato fruits contain largely water (about 94 %), moderate quantities of soluble sugars and several organic acids (especially citric and manic acid), mineral salts and large amounts of vitamin C. It is reported that the lycopene and carotenoids pigments of tomato has potent anti-oxidants, a molecule that snuffs out cancer-causing free radicals within the mammalian systems. Thus, it was observed in Britain that people who consumed high amounts of tomato products experience marked reduction in cancer risk (Mumtaz, 2003).

Mumtaz (2003) also reported that there is a factor (Mg) which prevents platelet clot helps in cutting down deaths from heart diseases. It is also important as a weight loss diet (Peet, 2001). It produces job opportunities to thousands of people working in the tomato processing industries and local women who are in production business. It was estimated that the average daily intake of tomato in human diets is 18 % standing out at 50.6 g/person (Kateria and Mittal, 1984).

In addition to being an important vegetable crop worldwide, tomato is also used as a model plant species for genetic studies related to fruit quality, stress tolerance (biotic and abiotic) and other physiological traits. It is widely adapted to variety of climates spanning the tropics to temperate regions. In order to meet the demands of tomatoes, it is also grown in green houses (Shehu, 2012). For example, in Kano state, over 2 billion Nigerian Naira (US $ 5,585,822, US $ 1 equivalent to 357) was reported lost due to the devastating effect of *T. absoluta* in 2016 (Punch Newspaper Online 2017). Fungal infections of plants cause varying degrees of losses. The loss of yield has been estimated to be between 20 – 40 % (Bala *et al.* 2019). According to some reports, about 10 – 50 % of the world tomato harvest is lost through fungal attack and contamination (Pernezny and Roberts, 2000). These diseases are known to work with other factors such as environment and herbicide injury. In line with this development, the current study was conducted to identify mycoflora pathogens of tomato cultivars (*L. esculentum* Mill) varieties in selected tomato growing areas of Kano State during the rainy and dry periods of 2014/2015.

MATERIALS AND METHODS

Survey Areas

The survey was conducted in small and large tomato fields in major tomato cultivation areas of Kano State, Nigeria, for both rainy and dry seasons farming. This was done from July – October, 2014 and November – April, 2015 respectively, in the following selected tomato growing areas, Dawakin Tofa, Kumbotso, Kura and Dambatta Local Government Areas of Kano State.

These were considered as representatives of Kano agro-ecological zones in terms of bio-physicalcharacters and tomato production (Shu'aibu *et al.,* 1997; Shehu, 2012).

These were selected and surveyed with the help of extension farm officials from Kano Agricultural Research Development Agency (KNARDA). Kano is located in Sudan savanna Agro-ecological zone, latitude 120°03N longitude 08°31E and altitude of 1500 m above sea level (Kowal and Knabe, 1972; Kutama *et al.,* 2009).

Five locations/angles were sampled and surveyed from each selected Local Government Area. At every location, four tomato farms/farmers fields of sole tomato and tomato inter-cropped with other crops (mention them) were surveyed for fungal diseases (Asiama and Yeboah, 2003; Zarafi and Emechebe, 2005; Bem *et al.,* 2010; Kutama, 2012).

In each selected farm tomato plants were randomly sampled and assessed for fungal diseases based on disease signs and symptoms such as spots, wilts, damping off, leaf sclerosis, blights/necrosis, rots etc. The varying characteristic disease symptoms were compared and identified using field hand books of Wyenandt and Nitzsche (2005), Gleason and Edmunds (2006) and Dry Season Crop Protection Guide (2008). Incidence of disease on each field was determined by disease index (DI) as used by Khanna *et al.* (1977) and modified by Bem *et al.* (2010).

Disease Index (DI) = h/n × 100/1
Where h =number of diseased plants sampled.
n = total number of plants sampled
Disease severity was also determined by using a numerical scale of 1-5 based on that of Khanna *et al*. (1977) and Kutama *et al*. (2010) as shown below:

1 = No infection
2 = 1- 25 % = mild infection
3 = 26- 50 % = moderate infection
4 = 51-75 % = high infection
5 = 76 – 100 % = severe infection

Collection of soil and Diseased Plant Samples

Diseased plants were randomly uprooted from each field and placed in a labeled sterile polythene bags/envelopes to prevent dehydration. The diseased plants were taken to the laboratory for etiological studies. Soil from the Rhizosphere was collected from around each diseased plant root zones using soil auger, placed in sterile zipper bags/labeled envelopes for laboratory culture, isolation and possible identification of pathogens. Other parameters like geographical location of each field/ farm using GPS 12(GARMIN, 198 model), estimated size of the farm (hectares), type of cultivar grown, agronomic status of the farm(weeded / not weeded, fertilized or not), farming system (Sole, Intercropped), type of manure and fertilizer applied, presence or absence of pest/diseases, soil type and age of plant (not less than physiological/green maturity/ 6 weeks) were determined during the survey (Baraka *et al.,* 2006; Arogundade *et al.,* 2007).

Culture, Isolation and Identification of Pathogens from Diseased Plant Parts and Soil

Isolation from Rhizosphere soil, roots and shoot samples to confirm presence of associated fungi were done using standard procedures:

Plant parts

Plant samples were washed thoroughly under running tap water to remove soil debris. These were chopped up into 2-5 mm segments using flame-sterilized scalpel. The materials were surface sterilized with 5 % NaOCl (Sodium Hypochlorite Solution) for 1 minute, rinsed in 3 changes of sterile distilled water and blotted dry between sterile filter papers.

The materials were plated separately and aseptically on Potato dextrose Agar (PDA), prepared according to manufacturer's instructions amended with streptomycin, at the rate of 3-5 pieces per plate. All plates were incubated at 27±2 ^{o}C for 5-7 days placed upside down on a laboratory bench, and observed daily for fungal growth (Arogundade *et al.,* 2007).

Soil sample

Soil samples from Rhizosphere obtained from the fields were labeled appropriately in sterile envelops for isolation of soil mycoflora following standard procedure of soil serial dilution technique (Taylor, 1997).

Preparation of Pure Cultures

Fungal growth/mixed growths were sub-cultured onto fresh PDA plates to obtain pure/axenic cultures by using sterile needle to remove small portions of the spore masses and transferring them to the fresh streptomycin amended PDA plates. Subsequent sub-culturing was done until pure cultures were obtained (Bem *et al*., 2010).

Identification of Fungal Isolates

Slides of each isolate of purified soil and foliar pathogens were prepared using lactophenol in cotton blue staining agent. These were examined and identified up to generic and/or species level under the microscope, based on macroscopic and microscopic appearance which comprises growth pattern, pigmentation/color of hyphae, septation, shape and type of asexual spores (Nelson *et al*., 1983; Bernett and Hunter, 1992; Artsor, 2002; Leslie and Summerell, 2006). The frequency of isolated fungi from all samples was calculated using the formula:

$$\text{Fungal frequency (\%)} = \frac{Noofisolatesforeachfungus}{Totalnoofallisolates} \text{ X } \frac{100}{1}$$

(Titus, 2011; Ukeh and Chiejina, 2012). The pure isolates were maintained as stocks in PDA slants at 4 ^{o}C for further experimental purposes.

STATISTICAL ANALYSIS

Descriptive statistical methods such as percentage and frequencies were employed to summarize and analyze data on disease incidence and frequencies of isolated fungi. Standard Analysis of Variance (ANOVA) procedure using Statistical Analysis System (SAS) was employed to analyze the data while to test the significant differences between means, Fisher's protected least significant differences (LSD) at P=0.05 was used (Statistical Analytical System (SAS, 2000).

RESULTS AND DISCUSSION

The investigation revealed that tomato plants disease symptoms in the rainy and dry seasons were observed in all the areas under study. Wilting, blight and defoliation were observed to be more prevalent in the study areas during the rainy season than the dry season. Fruit rot was more severe in the rainy season but was completely absent during the dry season on the farms studied (Table 1and 2). This shows the higher rate of disease symptoms in the tomato plants grown in the survey areas during the rainy season. The organisms associated with the diseased tomatoes include *F. oxysporum, F. Solani, F. acuminatum, R. solani, Exerohilium sp* , T. *harzanum, T. viridea, Curvularia sp.*, *Alternaria solani*, *Circinella sp*, *Pythium* sp and *Cladosporium* sp. The mycoflora were isolated from tomato stem, leaves, roots and soil samples at varying degrees of occurrences. *Fusarium oxysporum* had the highest occurrence, with (34.91 %). This was next to *Rhizoctonia solani*, present with (32.39 %) while *Fusarium solani* was isolated with (18.37 %) occurrence. The fungi with the lowest occurrence were *Circinella* sp (01.11 %) and, *Pythium*sp (02.22 %), from all the studied areas (Table 3 and 4).

Disease incidence was significant at P<0.05 in the rain fed tomato farming areas studied. The highest incidence of the fungal diseases was found in Kumbotso (44.03 %) and the lowest in rainy season tomato (42.97 %) than in the dry season (24.56 %). Disease severity ranged from mild (2) infection in the dry season to moderate (3) in the rainy season (Table 7).

Table 1: Disease Symptoms of Tomatoes in Rainfed Survey areas of Kano State, 2014

		PLANT DISEASES							
Areas	**Locations/ fields**	**Root rot**	**Leaf spot**	**Blight**	**Wilting**	**Leaf curl**	**Fruit rot**	**Brown Stem**	**Defoliation**
Kumbotso	Gaidar makada	+	++	++	++	+	-	+	++
	Tudun Karsa	+	+	++	++	-	-	+	++
	Gadama	+	+	++	++	-	-	++	++
	Rinji	+	++	++	++	-	-	++	++
	Tsamawa	+	+	++	++	-	-	++	++
Dawakin Tofa	Hayin hago	+	-	++	++	-	++	-	++
	Bagadawa	+	+	++	++	-	++	-	+
	Zangon dawanau	+	++	++	++	-	++	++	++
	Kwa	+	-	+	+	-	-	-	-
	Rigar kofa	+	+	+	+	-	++	-	-

Keys: - = Absent, + = present, ++ = Abundant/Severe

Table 2: Disease Symptoms of Tomatoes in Irrigated Surveyed areas of Kano State, 2014/2015 Dry Season

		PLANT DISEASES							
Areas	**Locations**	**Root rot**	**Leaf spot**	**Blight**	**Wilting**	**Leaf curl**	**Fruit rot**	**Brown Stem**	**Defoliation**
Kura	Butalawa	+	+	++	++	-	-	-	-
	Kadani	+	-	++	++	+	-	-	+
	Dalili	+	+	++	+	+	-	-	-
	Gilmo	+	-	++	++	-	-	-	+
	Rigad duka	+	+	++	++	-	-	-	++
Dambatta	Garin kwalba	+	-	++	++	+	-	-	++
	Fadamar mantau	+	-	++	++	+	-	-	-
	Fagwalo	+	-	++	++	++	-	-	-
	Shiddar	+	-	-	++	+	-	-	-
	Thomas	+	-	+	++	-	-	+	++

Keys: - = Absent, + = present, ++ = Abundant/Severe

Table 3: Major Fungi Isolated from Disease Plant parts and soil of surveyed Tomato Growing Areasof Kano State, 2014/15.

	PLANTS PARTS AND SOILS			
Fungi	**Leaf**	**Stem**	**Root**	**Soil**
F. oxysporum	+	+	+	+
F. solani	+	+	+	+
F. acuminatum	+	+	+	+
R. solani	+	+	+	+
T. harzianum	+	-	+	+
T. viridae	+	-	+	+
Curvularia sp	+	-	+	+
Alternaria solani	-	-	+	-
Ciranella sp	-	-	+	+
Exserohilium sp	+	-	+	+
Pythium sp	-	+	+	+
Cladosporium sp	+	-	+	-

Key: + = Present, - = Absent

Table 4: Frequency of Occurrence of Pathogens Isolated from rain-fed Study Areas, 2014.

Pathogens	**KUMBOTSO**		**DAWAKIN TOFA**		
	Freq of Occur.	**% Occurrence**	**Freq. of Occur.**	**% Occurrence**	**Total (%)**
Fusarium oxysporum	26	28.89	08	6.02	34.91
Fusarium solani	03	3.33	20	15.04	18.37
F. acuminatum	-	-	09	6.77	06.77
Rhizoctonia solani	19	21.11	15	11.28	32.39
Rhizotonia sp	04	4.44	-	-	04.44
Curvularia sp	11	12.22	-	-	12.22
Alternaria solani	01	1.11	08	6.02	07.13
T. harzianum	08	8.89	-	-	08.89
T. viridae	09	10.00	-	-	10.00
Trichoderma sp	-	-	03	2.26	02.26
Circinella sp	01	1.11	-	-	01.11
Exserohilium sp	03	3.33	-	-	03.33
Pythium sp	02	2.22	-	-	02.22
Cladosporium sp	0.3	3.33	01	0.75	04.08

Dawakin tofa (41.90 %) (Table 5). There was no significant difference (T=0.05) between the irrigated tomato growing areas studied within the seasons (Table 6). The effect of season on disease incidence and severity was highly significant at P<0.05. Disease incidence was higher in

Table 5: Mean Disease Incidence (DI) of Rainfed Tomato Growing Areas Studied.

Area	D.I (%)	Severity Scale
Dawakin Tofa	41.90[b]	3.00[a]
Kumbotso	44.03[a]	3.00[a]

Table 6: Mean Disease Incidence (DI) of Irrigated Tomato Growing Areas Studied.

Areas	D.I%	Severity Scale
Dambatta	24.54a	2.00[a]
Kura	24.57[a]	2.00[a]

Table 7: Comparative Mean Disease Incidence and Severity of Rainfed and Irrigated Tomato Growing Areas Studied 2014-2015.

Growing season	DI%	Severity Scale
Rainfed/Rainy	42.97[a]	3.00[a]
Irrigated/Dry	24.56[b]	2.00[b]

DISCUSSION

The presence of various fungal disease symptoms in all the study areas showed that plants were actually diseased. The symptoms observed in this study, such as root rot, leaf spots blight, wilting, fruit rot, brown stem and defoliation are conform with the work of Bem *et al*., (2010) who observed the presence of plant disease symptoms as discolorations, blights, rots, wilting and necrosis on tomato plants in some tomato growing areas in Benue State during rainy season. Pernezny and Roberts (2000) also reported that such varying symptoms indicated the presence of plant disease caused by infectious and non – infectious plant disease causal agents.

The characteristics plant disease symptoms were more prominent in the rain-fed areas than the irrigated tomato growing areas. Pathogenic as well as environmental factors such as moisture and high relative humidity may be also be responsible for the high infection rate favored during the wet season and agree with earlier findings of Pernezny and Roberts (2000) stating that infection of crop plants by pathogens is highly favoured during the wet season.

The occurrence of different pathogens in association with tomato plants in the studied areas suggests their prevalence in the soils in the areas. Jiskani *et al.* (2007) isolated *Alternaria solani, Fusarium oxysporum, Rhizoctonia solani* and *Verticillium albo-atrum* from diseased tomato plants and soils in Hydrabad. The most predominantly isolated pathogens were *F. oxysporum, F. solani* and *R. solani*. This suggests that apart from the ubiquitous nature of *Fusarium,* the prevailing environmental conditions and continuous cultivation of tomato in the studied areas might have enhanced their predominance. This is because the areas studied are known for their extensive tomato cultivation. Earlier findings of Mahesh and Satish (2008) indicated the isolation of *F. oxysporum, F. solani, F. semitectum, F. equiseti* and *F. chlamydosporum,* with *F. oxysporum* as the most prevalent. Jiskani *et al.* (2007) also reported that *R. solani* was the predominant pathogen isolated from various tomato fields in Hydrabad. The infestation of tomato plants by these pathogens usually brings about chlorophyll destruction, leading to necrosis (symptoms complex), wilting and rot, retarding the plant productivity (Jones and Woltz, 1991; Bem *et al*., 2010).

Comparing the disease incidence and severity for the surveyed areas in the two indicated seasons showed significant variation in percentage disease incidence of the areas. Higher disease incidence was recorded in the rainy season than in the dry season. This may be attributed to the conducive weather conditions prevailing in the areas during the rainy season which favoured the growth and multiplication of pathogens. It is also possible that continuous cultivation of tomato in the areas may have caused the high incidence of the pathogens and the diseases they caused. Agrios (1988) found variations association with soil borne fungal diseases at different times and locations. This result is also confirmed by the findings of Asiama and Yeboah, (2003) who recorded a high incidence of tomato wilt diseases associated with *F. solani* and root-knot nematodes in the rainy season in central region of Ghana. Jiskani *et al*. (2007) also recorded variations in disease incidence of tomato associated with *Rhizoctonia solani* and *Verticillium albo-atrum* in tomato fields in Hydrabad.

High disease incidence or rating that was observed varied from one area to another. This may be attributed to the environmental factors that favour pathogen growth, especially during wet season. Also, continuous cultivation of tomato on the same piece of land might have enhanced the rapid multiplication and spread of the pathogens in the respective studied areas. Disease incidence of up to 44.03 % was recorded in Kumbotso, which is in agreement with the findings of Asiama and Yeboah (2003), who reported 58.3 % in central region of Ghana. Erinle (1979), in Bem (2009) reported similar cases in Zaria and Jos, Plateau State. Agrios (1988) found variations in association fungi with soil borne diseases at different places. The percentage incidence of the diseases in the studied areas ranged from 22.54 – 44.03 %. These values could lead to epidemic levels in the absence of timely application of fungicides and appropriate cultural control measures which are hereby suggested.

CONCLUSION

Observations and data collected in the two seasons of study indicated that *Fusarium* and *Rhzoctonia* wilt disease are the most important diseases on tomato in the surveyed areas, these co-existed with other diseases such as early and late blight, fruit rot, stem/root rot defoliation and stunting on the tomato varieties across the fields. The predominant species identified to be associated with the diseased symptoms were *Fusarium oxysporum, Fusarium solani and Rhizoctonia solani* and are therefore suspected to be the causal agents of the fungal diseases observed on the tomato plants in the study areas.

RECOMMENDATION

Based on the findings of the present research, tomatoes should be more stringently guarded against fungal diseases in the wet seasons than in the dry seasons, through the use of appropriate fungicides as well as cultural practice such as crop rotation.

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