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Pot Screening of Twenty Tomato Varieties (*Solanum lycopersicum L.*) in Response to Single and Interactive Effects of *Fusarium oxysporum f.sp. lycopersici* (*Schlecht*) Synder & Hansen and Root-knot Nematode (*Meloidogyne javanica*) Chitwood (*Treub*)

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Abstract:
Pot screening of twenty (20) tomato varieties was carried out with a view to determining wilting and root gall responses in terms of resistance or susceptibility to single and interactive effects of *Fusarium oxysporum f.sp. lycopersici* (Schlecht) Synder & Hansen and Root-knot Nematode (*Meloidogyne javanica*) Chitwood (*Treub*). The experiment was carried out in the screen house of the Department of Crop Protection, University of Abuja. Seeds of twenty varieties of tomato were sourced from the National Centre for Genetic Resources and Biotechnology (NACGRAB) and National Institute for Horticultural Research (NIHORT) in Ibadan. The test pathogens (*F. oxysporum* and *M. javanica*) were characterized conventionally and subsequently identified using molecular method through Polymerase Chain Reaction and gene sequencing techniques carried out carried out in the Bioscience/Molecular /Virology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The Completely Randomized Design (CRD) was used with 4 treatments and 5 replications per treatment. The disease symptomology of the interaction was documented. As a result, all the varieties recorded an average wilt severity of 15.00 ±1.58 and root gall of 37.20 ± 3.71. The red cherry tomato had the highest wilt severity (40.0%) caused by *F. oxysporum* while *Tropimech* (75.6) and Alausa (75.5) had the highest number of root galls caused by M. javanica. Simultaneous inoculation of *F. oxysporum* and *M. javanica* in pot screening experiment resulted in high Fusarium wilting and root gall formation in Beske 11 Jm4, Beske 1 Jm3 and Red cherry. However, Onitrye variety showed tendency for resistance to both pathogens. On the basis of their responses and performances, four varieties were selecte: Onityre, Zumoured, Roma savanna and Riogrande. These varieties should be subjected to further pot and field evaluations to determine the effects of the pathogens on their agronomic characteristics including growth and yield parameters, and make an informed decision to growers and breeders.

Keywords: Fusarium oxysporum, Meloidogyne javanica, Screening, Tomato, Responses

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables worldwide. Tomato has its origin in the South American Andes (Joy *et al*., 2015). It is an annual plant grown in many parts of Nigeria both as wet and dry season crops. World production of tomato was estimated at 162 million tons of which Nigeria produces about 555.630 tones (Adenuga *et al*., 2013; FAOSTAT, 2014). Tomato and tomato-based foods provide a wide variety of nutrients and many health-

related benefits to the body. In regions where it is being cultivated and consumed, it constitutes a very essential part of people's diet (FAOSTAT, 2014). It can be consumed fresh or processed into paste. It is also rich in minerals, vitamins, essential amino acids, sugars and dietary fiber (Aneta *et al.*, 2015). The fruits are consumed fresh in salads or cooked in sauces, soup and meat or fish dishes.

Tomato production is very important to the food security of Nigeria where different varieties are grown as a result of breeding efforts (Joy *et al*., 2015). However, tomato production is hampered several biotic stresses including pathogenic fungi, bacteria, viruses and nematodes (Adenuga *et al*., 2013). Interactive effects of *Fusarium* and *Meloidogyne* spp have been implicated in the poor yield of tomato (FAOSTAT, 2014; Waheed *et al*., 2014). This accounts for about 90-100% yield loss of the crop as a result of interaction of the pathogens in diseased crops which cause augmentation of wilt disease incidence and severity (FAOSTAT, 2014; Waheed *et al*., 2014). The use of resistant varieties of crops against *Meloidogyne* spp and *Fusarium* spp are cheap and also an alternate method of managing *Fusarium* wilt disease and root-knot nematodes population in the vegetable crops as compared to fungicides and nematicides which most often leave toxic residues in the soil (Muhammad *et al*., 2015). Therefore, the use of tomato varieties that are resistant to the myriads of pathogens that attack tomato plants are of significant importance (Bawa *et al*., 2014). Furthermore, identification and selection of more tomato varieties with resistant factors to *Fusarium* wilt diseases and root- knot nematode will reduce losses and increase yield as well as fruit quality. This will eventually enhance the economic power of rural peasant farmers that are engaged in the production of the crop. The emergence of these resistant varieties will no doubt, provide the much-needed alternative methods for effective management of nematode-*Fusarium wilt* disease complexes in tomato. The aim of this study was to screen twenty (20) tomato varieties and determine their responses to single and interactive effects of *Fusarium oxysporum* f.sp. *lycopersici* (Schlecht) Synder & Hansen and Root-knot Nematode (*Meloidogyne javanica*) Chitwood (Treub).

MATERIALS AND METHODS

Sources of Materials and Study Area

Seeds of twenty tomato varieties were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan and National Institute for Horticultural Research (NIHORT) Ibadan Oyo state. The varieties were: Uc82, Roma VF, Beske JM4, Ibadan Local Cobra F1, Alausa, Beske Jm3, Hanit, Assila, Tropimech, Plantnium, Onitrye, Tolin 1 ks, Roma Savanna, Chibli, Riogrande, Ife 1 tomato, Gamad , Zumoured and Red Cherry. Extensively galled roots of tomato were collected from an infested farm land in Bounguo-Kwali village in Kwali Area Council of the Federal Capital Territory (FCT) in Nigeria. A screening pot experiment was carried out in the screen house of the Department of Crop Protection, University of Abuja.

Sterilization of Materials

Soil samples from fertile land were collected at the back of Science Faculty block of University of Abuja. Sterilization of soil and seeds was done following the procedure outlined by Nikki (2015) and Ganpati and Judy (2014). Isolation and culturing were conducted under aseptic conditions in the laminar flow cabinet.

Morphological Identification of Nematode

Infected roots were carefully teased out in the tray using a pair of forceps and a scapel to remove the adult female nematodes for the perineal patterns (Aneta *et al*., 2015; Bello *et al*., 2015) and

confirmed with the pictorial key described by Eisenback *et al*. (1981) to determine the identity of the nematode.

Morphological Identification of Fungus

A diseased tomato stem that was suspected to be infected by the fungus *Fusarium oxysporum* (Schlecht) Snyder and Hansen f.sp. *lycopersici* was cut into bits and sterilized in 1.05% sodium hypochlorite solution for 5 minutes. They were then rinsed in 6 changes of sterile distilled water and dried in sterile blotter paper (Iheukwumere *et al*., 2009; Bonger *et al*., 2016). The cut tissues where aseptically placed on each plate containing water agar. The plates were incubated at room temperature at 27 ± 3°C for 3 days before being sub cultured on Potato Dextrose Agar plates. The plates were then incubated for 5-7 days and sub cultured repeatedly on a clean PDA until pure cultures of the isolate was obtained (Iheukwumere *et al*., 2008; Mohit *et al*., 2014). The pure culture of the fungus was observed for colony and spore characteristics.

Molecular Characterization of Pathogens

Molecular characterization was carried out in the Bioscience /Virology Molecular Laboratory of the International Institute of Tropical Agriculture (IITA). Standard methods were employed in DNA extraction (Mwangi *et al*., 2019; Zijlstra *et al*., 2021) and Polymerase Chain Reaction. Sequence Characterized Amplified Region (SCAR) markers (F-GGTGCGCGATTGAACTGAGC and R–AGGCCCTTCAGTGGAACTATAC) were used as primers for the amplification of nematode DNA. Internal transcribed spacer molecular markers (ITS4: TCCTCCGCTTATTGATATGC and ITS5: GGAAGTAAAAGTCGTAACAAGG) were used for the amplification of the fungus DNA. Amplicons were separated on the agarose gel electrophoresis chamber and viewed under UV transilluminator and photographed. The PCR products were sequenced at GATC Biotech AG, Pretoria South Africa using the Sanger's methods.

Seed Preparation and Planting

Seeds were sterilized for 5 minutes in 1.05% sodium hypochlorite and rinsed for 5 minutes in 6 changes of sterile distilled water prior to planting (Koenning and McClure, 1981; Ganpati and Judy, 2014). Four hundred perforated polythene bags of 15-centimeter diameter and 20cm height were filled with 1kg sterilized sandy-loamy soil. They were used as experimental pots. The twenty tomato varieties were with five replicates each of the experimental pots. A plant was maintained per pot at one week (seven days) after planting and I ml of the suspension containing 500 juveniles (J_2) of *M. javanica* was inoculated in the soil around the root system of the plant.

Inoculation of Potted Test Plants

Plants were not watered the day before inoculation. Seven–day old seedling was inoculated with 500 juveniles of the nematode and 1.0×10^6 / ml spore suspension of the fungus per plant (Waheed *et al*., 2014; Nasrin *et al*., 2015). The experiment was arranged in a completely randomized design with 4 treatments and 5 replications per treatment on a cemented platform in a screen house at temperature of 32 ± 3°C. Treatments given to the 7-day-old seedlings are gigen as follows:

N= Inoculation of test plant with 500 juveniles (J_2) of nematodes only
F= Inoculation of test plant with 1.0×10^6/ ml spore suspension of the fungus only
N+F= Simultaneous inoculation of test plants with 500 juveniles of nematodes and 1.0×10^6/ ml spore suspension of the fungus)
C= the uninoculated control plants

Harvesting of Tomato Plants

The test plants were harvested eight weeks after inoculation. To ensure easy removal of the plants from the soil, the sides of the polythene sleeves were pressed to loosen the soil. The soil was then removed from the roots by gently shaking the plants. The roots of the harvested tomato were each washed separately and dabbed dry with tissue paper (Kankam and Adomako, 2014).

Disease Assessment

Plants were observed weekly for the appearance of symptoms such as wilts and leaf yellowing. The plants were also watered on alternate days till the end of the experiment at eight weeks after inoculation.

Root gall index were measured on a scale as 0 = No galls (Highly Resistant, HR); 1 =1-2 galls (Resistant, R); 2 =3-10 galls (Moderately Resistant, MR); 3 =11-30 galls (Moderately Susceptible, MS); 4 =31-100 galls (Susceptible, S); 5 = More than 100 galls (Highly Susceptible, HS (Muhammed *et al.*, 2014). Disease severity by the fungus were scored on a scale of 0-5 as follows: 0 = No wilt (Highly Resistant), 1 = 1-10% wilted (R: Resistance); 2 = 11-20% wilted (Moderately Resistant, MR); 3 = 21-30% wilted (MS: Moderately Susceptible); 4 = 31-50% wilted (S: Susceptible) and 5 = 51-100% wilted (HS: Highly Susceptible) (Muhammed *et al.*, 2014). Wilt severity was assessed by visually counting the number of infected plants (wilted plants) after inoculation and dividing it by the total number of both healthy and infected plant in each variety and then multiplying by 100 (Mohit *et al.*, 2014). Plant showing wilting and dropping was then quantified on the basis of scale, of percentage wilt severity and was calculated with this formula:

$$\text{Percentage wilt severity} = \frac{\text{Number of plants wilted}}{\text{Total plant population}} \times 100$$

Data Analysis

The Genstat 17 application package was used to analyse the data set using the one-way ANOVA (Analysis of variance) tools. Mean separation was done using the LSD method at P≤0.05.

RESULTS

Table 1 showed that there were significant differences at (P ≤ 0.05) in percentage wilt severity among the tomato varieties when *Fusarium oxysporum* was inoculated singly. Red cherry tomato plant had the highest percentage wilt severity of (40.0%) and it was susceptible to the fungus. Tropimech (20.0%), Riogrrande (20.0%) and Ife (20.0%) varieties were moderately resistant to the fungus. Some varieties including Gamad, Ibadan local, Chibili, Plantinum, Assilia, Alausa, Haint, Zumoured and Roma Savanna varieties were resistant to the fungus. Four varieties (Roma Savanna, Cobra, Onitrye and Roma VF had no cases of wilting (0.0%) and were highly resistant to the fungus. All the varieties had an average wilt severity of 15.00 ±1.58.

Table 2 showed the effects of single inoculation of *M. javanica* on the number of root galls of twenty tomato plant varieties. There were significant differences in the number of galls among the tomato varieties (P ≤ 0.05). Tropimech (75.6) and Alausa (75.5) had the highest number of galls and were susceptible to the root-knot nematode, followed by Gamad and Regrade (60.4). The lowest number of galls were observed among Plantimum (1.0), Onityre (0 .90) and Haint (0.80^{k} varieties resistance to the nematode. There was no variety found to be highly resistant or highly susceptible to the nematode. All the varieties produced an average of 37.20 ± 3.71 galls

Table 3 shows the effects of simultaneous inoculation of *F. oxysporum* and *M. javanica* on the responses of twenty tomato varieties in pot screening experiment. There were significant differences among the tomato varieties (P ≤ 0.05). The highest wilt severity recorded was 50.0% as observed in Beske 11 Jm4, Beske 1 Jm3 and Red cherry varieties. This made susceptible to the fungus *F. oxysporum*. Tropimech and Ife 1 recorded 30.0% wilt severity and they were moderately susceptible to the fungus. Chibili (10.0%), Roma Savanna (10.0%), Cobra F1 (10. 0%), Onitrye (10.0%) and Roma VF (10.0%) had the lowest wilt severity and were resistant to the fungus. No variety was found to be highly resistant or highly susceptible to the nematode. Variety mean wilt severity was 27.00 ±2.38. Significant differences (P ≤ 0.05) were recorded in the number of root galls among the tomato varieties. Beske 11 Jm4, (10.30) and Beske 1 Jm3 (29.70) had the highest number of root galls being susceptible to nematode while Plantimum (0.80), Onitrye (0.60) and Haint (0.50) had the lowest value being resistant to the nematode. None of the varieties was found to be highly resistant or highly susceptible to the nematode. Variety mean number of galls was 24.63 ± 2.60.

Table 1: Effect of single infection of *F. oxysporum* on percentage wilt severity and responses on twenty tomato varieties in pot screening experiment

Tomato Varieties	% Wilt Severity	Wilt responses
Red Cherry	40.00 [a]	S
Beske Jm3	30.00 [b]	MS
UC82B	30.00 [b]	MS
Tolin I ks	30.0 0[b]	MS
Beske Jm4	30.00 [b]	MS
Tromipech	20.00 [c]	MR
Riogrande	20.00 [c]	MR
Ife 1	20.00[c]	MR
Gamad	10.00[d]	R
Ibadan Local	10.00[d]	R
Chibili	10.00[d]	R
Plantimum F1	10.00[d]	R
Assisla	10.00[d]	R
Alausa	10.00[d]	R
Haint	10.00[d]	R
Zumoured	10.00[d]	R
Roma Savanna	0.00[e]	HR
Cobra F1	0.00[e]	HR
Onitrye	0 00[e]	HR
Roma VF	0.00[e]	HR
Variety means	15.00 ±1.58	
LSD at P ≤ 0.05	0.42	

Each value is a mean of five replicates. Means with different superscripts on the same vertical column differ Significantly y (P ≤ 0.05) according to Duncan's multiple range test. Gall indices Percentage wilt severity on a scale of 0-5 where 0= No wilt (Highly resistant), 1 = 1-10% wilted (Resistant) 2 = 11-20% wilted (Moderately resistant), 3 = 21-30% (Moderately susceptible), 4 = 31-50% wilted (Susceptible) and 5 = 51-100% (Highly susceptible

Table 2: Effect of single infection of *M. javanica* on number of root galls and responses on twenty tomato varieties in pot experiment

Tomato varieties	Number of galls	Responses
Tropimech	75.60 [a]	S
Alausa	75.50 [a]	S
Gamad	60.40[b]	S
Riogrande	60.40[b]	S
Tolin I Ks	59.70[b]	S
Roma Savanna	56.10[b]	S
Ife 1	53.50[c]	S
Ibadan local	51.10[d]	S
Beske Jm4	50.80[d]	S
Red cherry	44.10[d]	S
Roma VF	40.70[f]	S
Cobra F1	38.80[h]	S
UC82B	36.90[h]	S
Assila	15.00[i]	MS
Beske Jm3	13.80[i]	MS
Zumoured	5.00[j]	MR
Chibili	3.90[j]	MR
Plantimum	1.00[k]	R
Onityre	0.90[k]	R
Haint	0.80[k]	R
Variety means	37.20 ± 3.71	
LSD at P ≤ 0.05	1.53	

Each value is a mean of five replicates. Means with different superscripts on the same vertical column differ significantly (P ≤ 0.05) according to Duncan's multiple range test. Gall indices on the scale of 0-5 where 0= No galls (Highly resistant), 1 = 1- 2galls (Resistant), 2 = 3-10 galls (Moderately resistant), 4= 11-30 galls (Moderately susceptible), 5= More than 100 galls (Highly susceptible)

Table 3: Effects of simultaneous inoculation of *F. oxysporum* and *M. javanica* on the responses of twenty tomato varieties in pot screening experiment

Tomato varieties	(%) Wilt severity	Responses	Number of galls	Responses
Beske 11 Jm4	50.00[a]	S	10.30[f]	MS
Beske 1 Jm3	50.00[a]	S	29.70[d]	MS
Red cherry	50.00[a]	S	26.60[ed]	MS
Riogrande	40.00[b]	S	43.60[bc]	S
UC82B	40.00[b]	S	24.60[ed]	MS
Zunoured	40.00[b]	S	0.90[g]	R
Tolin 1 KS	40.00$_b$	S	28.60[ed]	MS
Tropimech	30.00[c]	MS	43.30[c]	S
Ife 1	30.00[c]	MS	51.10[a]	S
Ibadan local	20.00[d]	MR	43.70[bc]	S
Assilia	20.00[d]	MR	2.90[g]	R
Gamad	20.00[d]	MR	50.00[ba]	S
Haint	20.00[d]	MR	0.50[g]	R
Alausa	20.00[d]	MR	50.20[ba]	S
Plantimum	20.00[d]	MR	0.80[g]	R
Chibili	10.0[e]	R	2.80[g]	MR
Roma Savanna	10.0[e]	R	49.80[ba]	S

Cobra F1	10.0^{e}	R	10.2^{f}	MS
Onitrye	10.0^{e}	R	0.60^{g}	R
Roma VF	10.0^{e}	R	22.30^{e}	MS
Variety means	27.00 ±2.38		24.63 ± 2.60	
LSD at P≤ 0.05	2.35		3.02	

Each value is a mean of five replicates. Means with different superscripts on the same vertical column differ significantly (P ≤ 0.05) according to Duncan's multiple range test. Gall indices Percentage wilt severity on a scale of 0-5 where 0= No wilt (Highly resistant), 1 = 1-10% wilted (Resistant) 2 = 11-20% wilted (Moderately resistant), 3 = 21-30% (Moderately susceptible), 4 = 31-50% wilted (Susceptible) and 5 = 51-100% (Highly susceptible

DISCUSSION

In single infection with pathogens during pot screening experiment, it was found that red cherry tomato had the highest wilt severity and high susceptibility to *F. oxysporum* whereas Tropimech and Alausa varieties were predisposed to gall formation as induced by *M. javanica*. Siddiques *et al*. (2022) recently reported that the disease wilt severity on tomato was an indication of the virulence of the pathogens on the crop tested. Bonger *et al*. (2016) reported that resistant /moderately resistant varieties have gene resistance to *M. javanica* and that root of resistant varieties reacted to root-knot nematodes attack by reducing its catalase activity, thus conferring resistance to the host plant. Resistant plants may possess active principles that inhibit candidate enzymes involved in pathogenicity. This mechanism might explain the resistance pattern to nematode and fungus as observed in few tomato varieties tested. The above observation is in agreement with Zahid *et al.* (2021) who reported that resistance and susceptibility to root-knot nematode reflects the effect of the plant product on the nematode's ability to reproduce in the host plant. The comparable responses of all the twenty tomato varieties tested for resistance to nematode infection showed that some of the tomato varieties had resistance genes that directed the synthesis of inhibitory proteins which in turn conferred to the host plant the ability to stop the penetration, development and reproduction of nematode juveniles.

Simultaneous inoculation of *F. oxysporum* and *M. javanica* on the responses of twenty tomato varieties in pot screening experiment resulted in high *Fusarium* wilting and root gall formation in Beske 11 Jm4, Beske 1 Jm3 and Red cherry as a result of their susceptibility to *F. oxysporum* and *M. javanica* respectively. In spite of this, some varieties were screened and selected for their resistance to the pathogens. For instance, Roma Savanna, Cobra, Onitrye and Roma VF had no cases of wilting and were highly resistant to *F. oxysporum*. Onitrye tomato variety showed tendency for resistance to both *F. oxysporum* and *M. javanica* while other varieties showed tendency for resistance to only one of the two pathogens.

The differential responses of the twenty screened varieties to fungus and nematode infection could possibly be due to genetic factors, depending on the depending on the susceptibility or resistance pattern of the varieties to infections. The above observation was supported by Kumar *et al.* (2017) who found that all genotypes of the tested crops showed variation in responses to root-knot nematode from resistance to susceptibility. This implies that some varieties were more tolerant/resistance to disease than others. This view agrees with the findings of Aslam *et al*. (2016) who similarly noted the influence of genetic makeup of different cultivars on the *Fusarium* wilt severity. The present study agreed with the views of Karssen and Moenens (2021) who reported that susceptible tomato plant varieties supported greatest/ maximum number of juveniles penetrated and completed their development to maturity as shown by highest number of galls present in the roots. On the other hand, resistant and moderately resistant tomato varieties

allowed only a limited number of juveniles of *M. javanica* to penetrate the roots, leading to maturity as an evident by number of galls on their roots. This was the position of other authors who reported that resistant cultivars contained fewer developed nematodes than susceptible plants (Ansari *et al.,* 2014). Tomato crop, being an important vegetable food crop has received attention among breeders to meet set objectives. Some varieties have been improved for disease resistance and yield among other agronomic qualities.

CONCLUSION

Simultaneous inoculation of *F. oxysporum* and *M. javanica* in pot screening experiment resulted in high *Fusarium* wilting and root gall formation in Beske 11 Jm4, Beske 1 Jm3 and Red cherry. However, Onitrye variety showed tendency for resistance to both pathogens. On the basis of their responses and performances, four varieties were selecte: Onityre, Zumoured, Roma savanna and Riogrande. These varieties should be subjected to further pot and field evaluations to determine the effects of the pathogens on their agronomic characteristics including growth and yield parameters, and make an informed decision to growers and breeders.

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