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OCCURRENCE AND IDENTIFICATION OF FUNGAL ROTS IN TOMATO (LYCOPERSICUM ESCULENTUM MILL) FRUIT

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ABSTRACT

Tomato fruits have high nutritional qualities, but their spoilage by soil-borne pathogens results in food poisoning. It is important to isolate and identify the strains of fungi associated with tomato rot, to understand their pathogenic state. Seeds of 3 genotypes of tomato were grown in both hydroponics and soil systems. It is a factorial experiment laid in a completely Randomized Design with four replicates. The number and weight of fruits produced, number of infected and uninfected fruits by rots were recorded. Data collected were analyzed using ANOVA and means were separated using LSD at 5 % significance level. At harvest, the infected and uninfected fruits were harvested and 10 g each were sliced, dissolved in sterile distilled water and were serially diluted before plating on sterile Potato Dextrose Agar-PDA and Sabouraud Dextrose Agar-SDA media with 2 % streptomycin and incubated at 28^{o}C for 3 – 7 days. Genotypes and substrates were significant in the rate of fruit infection, with the Roma tomato having 78.1 % fruit infection rate, while the identified fungi from the infected tomato fruits are Aspergillus flavus and Mucor racemosu, as no organism grew in the uninfected fruits cultured in both PDA and SDA culture media.

Keywords: Infection, Genotypes, Tomato, Fungi, Hydroponics, Pathogenic.

JEL Classification Codes: Q10

INTRODUCTION

Tomato (Lycopersicum esculentum) is a yearly, dicotic, angiospermic short lived perennial plant belonging to solanaceae family. The fruit is used globally and it is consumed as both raw and processed forms (Moneruzzaman et al., 2008). The fruit contains vitamins (A, B, C and E), carbohydrates (fructose and glucose), Minerals (phosphorus, sodium, potassium, calcium, magnesium), trace elements (iron, copper, zinc and dietary fibres for humans (Smith, 1994; Andrew, 1994) and it is also used as raw materials for agro allied Industries.

This important staple vegetable is highly perishable and deteriorates easily few days after harvest, which could result in loss of needed qualities or total waste of these vegetables. The deterioration is mainly caused by microbial contamination, natural ripening processes and environmental conditions such as heat and drought (Idah et al., 2007). Over the years, bacteria and especially fungi have been reported as causing spoilage and deterioration of nutrients in different fruits (Willey et al., 2008).

Plants are susceptible at all stages of development, but symptoms are most obvious at or soon after flowering (Rowe et al., 1995). Fungi are the most significant and ubiquitous pathogens, infecting variety of fruit and causing economically important losses of fruits. Different fungal species such as Aspergillus, Fusarium, Penicillium, Mucor, Rhizopus, Geotrichum, Phytophthora and Botrytis have been reported as spoilage organisms, and Aspergillus niger, Rhizopus stolonifer and Mucor sp. have all been isolated from spoilt tomatoes (Sharma, 2012).

Thousands of spores are produced in spots of infected leaves and are capable of causing more infections. When conditions are favorable, fungal fruiting bodies appear as tiny black specks in the middle of the spots (Sanoubar & Barbanti, 2017). The colonization process involves the ability of the microorganism to establish itself within the produce (Besser et al., 1993). Pathogenic microorganism in tomato is recognized as a source of potential health hazard to man and animals following ingestion, this is due to their production of toxins which are capable of causing diseases like respiratory infection, meningitis, gastroenteritis, diarrhoea in man (Beuchat, 2006; E, O. E, 2018).

The contamination of tomato by microorganism could be as a result of poor handling practices in the tomato production environment, predominantly due to soil borne disease infection (Beuchat, 1996). The above reason coupled with the prolonged cultivation period and little yield in the soil system have attracted researchers attention in the hydroponic system (Andriolo et al., 1999). This system provides a good platform to identify if the deterioration encountered in tomato variety is as a result of environmental infection or a generic disease state, coupled with the high crop quality yield as documented (Logendra et al. 2001).

Hence, due to the serious food safety risk posed by the consumption of pathogenic contaminated tomato fruits coupled with the economic damages, it is then important to identify and isolate the fungal microorganisms associated with tomato spoilage while using a controlled hydroponics system.

MATERIALS AND METHODS

Planting tomato seeds: Seeds of here tomato genotypes (Roma, San marzano and Red Cherry Large) were sourced from the Soilless Farmlab, Abeokuta, Ogun State, Nigeria and raised in hydroponics troughs containing dissolved cocopeat for 2 weeks before transplanting. More cocopeat blocks were dissolved in water and poured into the 12 hydroponics troughs, while 12 troughs were filled with topsoil. The raised tomato seedlings were sown directly on the troughs

in a completely randomized design with 4 replicates. The plants were watered every 3 days and fertigated with dissolved poultry manure (100 mg of poultry manure was dissolved in 100 ml of water to give a concentration of 1 mg/ml) every week till harvest. At 2 and 4 weeks after transplanting, plant height and number of leaves were taken. The number of days to 50 % flowering and days to fruiting, number of fruits produced, average fruit weight, number of infected fruits and uninfected fruits were also recorded. The data collected were subjected to statistical analysis using ANOVA and the differences in treatment means were separated using the least significant differences at 5 % level of significance.

Collection of rotten tomato fruits: Infected tomato fruits and few uninfected fresh tomato fruits were harvested from the plant into a sterilised polythene bag and were taken to the laboratory for further analysis.

Materials Sterilization: All the glass wares were appropriately washed, dried and sterilized in the oven at 1600C for one hour. The entire working surfaces were also disinfected with ethanol to decrease contaminants.

Samples Processing: 10 g of the spoilt and uninfected tomatoes was carefully cut with the aid of a sterile scalpel and enriched in sterile sabouraud dextrose broth and Potato Detrose broth for twenty four hours. Ten fold serial dilutions of the samples were thereafter carried out.

Isolation of associated fungi: Infected and uninfected Roma tomatoes samples were first washed separately with running tap water. 10 g size of spoilt tomatoes were carefully cut with the aid of sterile blade then sterilized with 70% ethanol and rinsed in sterile distilled water. 10 g of sliced portion was dissolved in sterile distilled water and was serially diluted then plated on sterile PDA and SDA medium with 2% streptomycin to inhibit bacterial growth and then incubated at 28oC. Incubation was carried out in inverted positions of petriplates for 4-7 days. The colonies that developed were subcultured repeatedly on sabouraud dextrose agar and Potato Dextrose Agar plates to obtain pure cultures. They were later stored on SDA and PDA slants for characterization and identification (Burnett, 1992).

Characterization and Identification of the Isolates: The pure cultures of the fungi were identified on the basis of their colony growth pattern, conidial morphology and pigmentation using the slide culture technique and microscopic examination.

RESULTS AND DISCUSSIONS

Result obtained on the agronomic parameters showed that the height of the Red Cherry Large tomato genotype (7.88±0.33) was significantly taller than both Roma (6.74±0.33) and San Marzano (5.86±0.33) genotypes at 2 weeks after transplanting. However, at 4 weeks after, the height of the genotypes were statistically same. Also, the number of leaves produced by the Red Cherry Large (24.63±1.86) at 4 weeks after transplanting was significantly higher than the San Marzano (17.63±1.86), however, both San Marzano and the Roma (22.75±1.86) was statistically same (Table 1).

It took the Roma genotype a period of 47.00±0.77 number of days for 50 % of the plants to produce flower, which was significantly higher than the period taken by both San Marzano (42.50±0.77) and Red Cherry Large (37.25±0.77) genotypes respectively. Also, it took the Red Cherry Large genotype 52.13±1.15 days to produce fruit, and it was significantly quicker than the 62.13±1.15 and 61.38±1.15 taken by both Roma and San Marzano respectively to produce

fruits. The number of fruits produced by the Red Cherry Large genotype (2.38±0.69) was significantly higher than the number produced by both Roma (8.00±0.69) and San Marzano (6.75±0.69) (Table 2).

However, in Roma, 6.25±0.31 of the fruits produced were infected by rot, which was significantly higher than the 0.00±0.31 recorded in Red Cherry Large and San Marzano genotypes respectively. While the 2.00±0.35 number of uninfected fruits recorded in the Roma tomato was significantly lower than the Red Cherry Large (9.63±0.35) and San Marzano (6.63±0.35) respectively. Then the weight of the fruits produced by the San Marzano (20.13±0.93) and Roma (18.28±0.93) was significantly larger than the 8.80±0.93 recorded in the Red Cherry Large genotype (Table 2).

Table 1. The height and number of leaves of three genotypes of tomato plants after 2 and 4 weeks of transplanting

Genotypes	**PH2**	**PH4**	**NOL2**	**NOL4**
RCL	7.88a	15.60a	4.63a	24.63a
Roma	6.74b	14.95a	4.38a	22.75ab
San Marzano	5.86b	14.96a	4.38a	17.63b
$LSD_{(0.05)}$	0.98	3.09	0.51	5.53
SE	0.33	0.17	1.04	1.86

Means with the same alphabet down the group are not significantly different from each other at 5 % level of significance, SE: Standard error, LSD: Least significant differences, PH2 and PH4: Plant height at 2 and 4 weeks after transplanting respectively, NOL2 and NOL4: Number of leaves at 2 and 4 weeks after transplanting respectively.

Table 2. Yield performance and occurrence of fungal rot in three genotypes of tomato

Genotypes	**DT50%F**	**DTF**	**NOF**	**NoIF**	**NoUF**	**FW**
RCL	37.25c	52.13b	12.38a	0.00b	9.63a	8.80b
Roma	47.00a	62.13a	8.00b	6.25a	2.00c	18.28a
San Marzano	42.50b	61.38a	6.75b	0.00b	6.63b	20.13a
LSD(0.05)	2.28	3.41	2.04	0.91	1.04	2.75
SE	0.77	1.15	0.69	0.31	0.35	0.93

Means with the same alphabet down the group are not significantly different from each other at 5 % level of significance, SE: Standard error, LSD: Least significant differences, DTF: Days to fruiting, NoIF: Number of infected fruits, NoUF: Number of uninfected fruits and FW: Fruit weight DT50%F: Days to 50 % flowering.

Table 3 showed that at 2 and 4 weeks after transplanting, the height of the plants and numbers of leaves produced were insignificant in terms of the substrate they were grown. Also, the number of days taken by 50 % of the plants to produce flowers, to produce fruits, number of infected fruits and the average fruit weight were significantly same in both topsoil and cocopeat substrates respectively. However, the number of uninfected tomato fruits in the cocopeat

substrate (7.00±0.28) was significantly higher than the fruits produced in the soil (5.17±0.28) (Table 4).

Table 3. Effect of cocopeat and topsoil substrates on the plant height, number of leaves of different genotypes of tomato

Substrate	**PH2**	**PH4**	**NOL2**	**NOL4**
Soil	6.83a	15.83a	4.58a	21.42a
Cocopeat	6.82a	14.52a	4.33a	21.92a
LSD(0.05)	0.8	2.52	0.42	4.52
SE	0.27	0.14	0.85	1.52
Source of variation				
Genotypes	8.15*	1.11ns	0.17ns	105.04*
Substrates	0.02ns	10.27ns	0.38ns	0.82ns
Genotypes*Substrates	0.02ns	1.31ns	0.50ns	0.15ns

Means with the same alphabet down the group are not significantly different from each other at 5 % level of significance, SE: Standard error, LSD: Least significant differences, PH2 and PH4: Plant height at 2 and 4 weeks after transplanting respectively, NOL2 and NOL4: Number of leaves at 2 and 4 weeks after transplanting respectively, DT50%F: Days to 50 % flowering, DT50%F: Days to 50 % flowering, *: 5 % level of significance and **: 1 % level of significance.

Table 4. Effect of cocopeat and topsoil substrates on the yield and occurrence of fungal rot in tomato plant

Substrate	**DT50%F**	**DTF**	**NOF**	**NoIF**	**NoUF**	**FW**
Soil	41.58a	58.25a	8.75a	1.75a	5.17b	14.94a
Cocopeat	42.92a	58.83a	9.33a	2.42a	7.00a	16.53a
LSD(0.05)	1.86	2.78	1.66	0.74	0.85	2.25
SE	0.63	0.94	0.56	0.25	0.28	0.76
Source of variation						
Genotypes	190.50**	248.17**	69.79**	104.17**	118.04**	295.27**
Substrates	10.67ns	2.04ns	2.04*	2.67ns	20.17**	15.05ns
Genotypes*Substrates	0.17ns	2.17ns	1.79ns	2.67*	12.04**	38.76*

Means with the same alphabet down the group are not significantly different from each other at 5 % level of significance, SE: Standard error, LSD: Least significant differences, DT50%F: Days to 50 % flowering, DTF: Days to fruiting, NoIF: Number of infected fruits, NoUF: Number of uninfected fruits and FW: Fruit weight, *: 5 % level of significance and **: 1 % level of significance.

Figure 1 a and b: Roma tomato plants with infected fruits

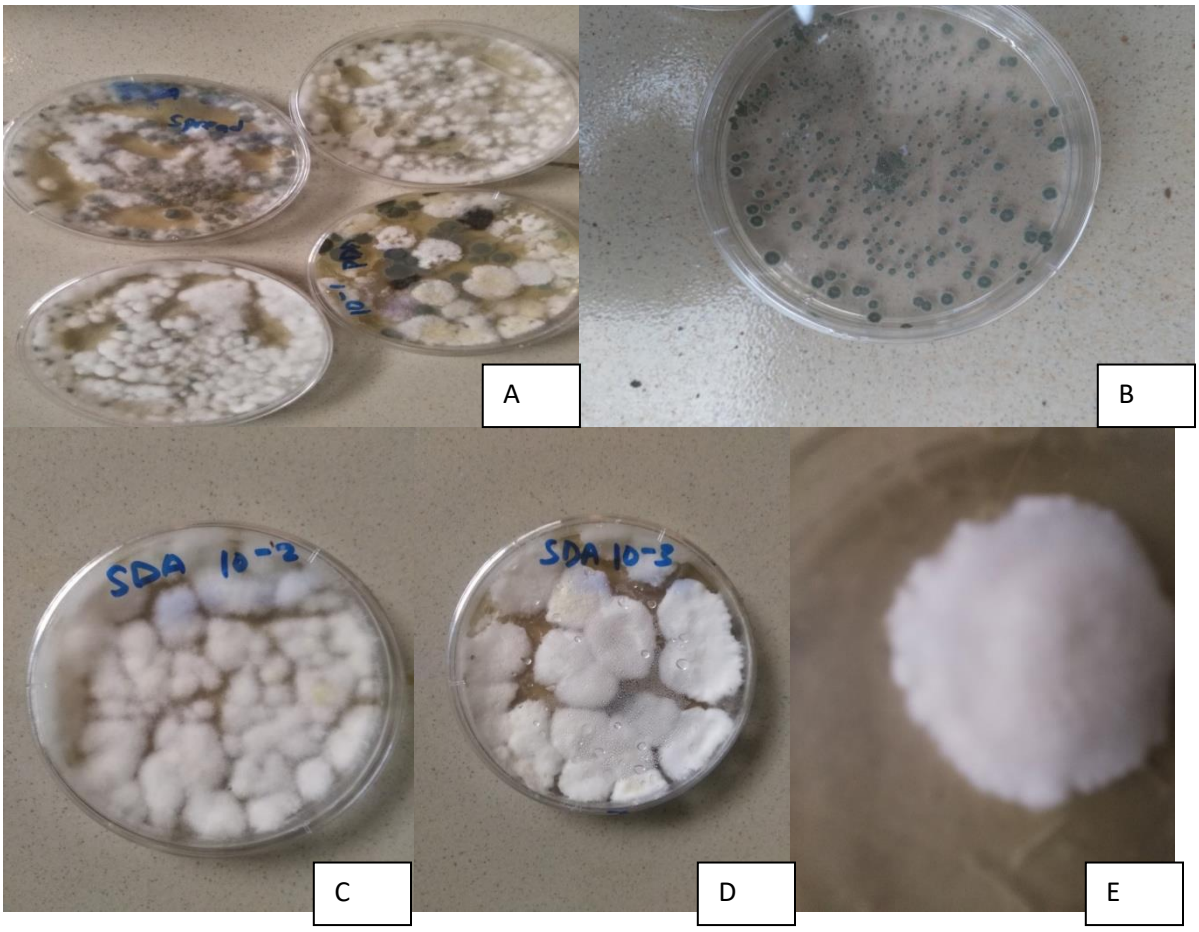

Figure 2. (A): Pour plate of Serially diluted infected tomato (B): Aspergillus flavus on PDA plate (C, D and E): Mucor racemosus on SPA plate.

The growth of the three genotypes of tomato used in this study developed at same rate; irrespective of the substrate they were grown. This shows that growing tomato in a hydroponics

system that is fertigated optimally is as effective as tomato soil farming and this supports the work of Flávio et al. (2018) and Ossai et al. (2020). The higher number of fruits produced by the Red Cherry Large tomato could be the reason behind the small fruit weight comparable to Roma and San Marzano fruits which produced larger fruit sizes but with a small number of fruits per plant, either grown in a hydroponics system or in soil. However, the different tomato genotypes were developed for different purposes (Baisden, 1994).

Despite the large fruit size produced by the Roma tomato, about 78.1 % of the produced fruit developed block rots at the base, while only 21.9 % of the fruit were without rot. Further investigation of the causal organism of the rots indicated that the tomato fruits were contaminated by fungal pathogens. Fungi such as Aspergillus flavus, Mucor racemosus were isolated from the infected fruits. This is in agreement with the findings of Kutama et al. (2007), who also reported numerous fungal pathogens linked with tomato.

The results also showed that tomatoes can be contaminated by fungal pathogens such as Aspergillus flavus and Mucor racemosus even before harvesting. Although Mbajiuka and Enya (2014) also isolated Aspergillius spp, Penicillum spp and Saccharomyces cerevisiae from spoilt tomatoes. Wogu and Ofuase (2014) also isolated Aspegillius spp, Penicillum spp, Fusarium spp and Saccharomyces spp from spoilt tomato fruits. Mbajiuka and Enya (2014) also isolated Aspergillius spp, Penicillum spp and Saccharomyces cerevisiae from spoilt tomato.

Fungal contamination of tomatoes is mainly as result of high water content, environmental surroundings, method of handling, type of storage facilities and the quality of the tomatoes (Logendra et al., 2001). Being that three genotypes were evaluated and only Roma tomato was infected could point the infection to be genotypic prone. However, it was more prevalent in the soil farming system than the hydroponics system which means the prevalence can be reduced below economic damage level by proper sterilization protocols as Beuchat (1996) reported that the fungal rots can be soil borne.

CONCLUSIONS AND RECOMMENDATIONS

Tomato fruits have high nutritional qualities, but their spoilage by fungi results in loss of economic resources as well as food poisoning. These fungi obtained in this study causes intoxication of mycotoxins which are harmful to human health. Aspergillus spp. is a source of Ochratoxin which is a potent Carcinogen; as a result substandard tomatoes must not be eaten but disposed off, because consumption of spoilt tomatoes could be detrimental to human health. Farmers are advised to ensure that the tomatoes seeds are healthy before planting and only tomatoes in good condition should be harvested and dispatched to the end users to reduce the danger of these toxins that are detrimental to health. However, proper soil sterilization techniques is essential in reducing the spread of the fungal rot in tomato to the lowest minimum, and it is also recommended that more evaluation should be carried out to ascertain a host-plant relationship between the fungal rots and Roma tomato as other genotypes evaluated were not infected by the rots.

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