UJMR, Vol. 9 No. 1, June, 2024, pp. 192 - 201 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2491.021

Received: 13 March 2024 **Accepted:** 20 June 2024

Characterization of Fungi Inducing Post-Harvest Deterioration and the Factors Predisposing Tomato Fruits to Fungal Induced Spoilage in Zaria, Nigeria.

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Abstract

This study was carried out to characterize fungi-inducing post-harvest deterioration of tomato fruits and the factors predisposing the tomato fruits to fungal-induced spoilage in Zaria, Nigeria. A total of two hundred and twenty-five spoilt tomatoes of three different cultivars were collected using guided random selection (45 each) from five markets (Dakachi, Danmangaji, Sabon-gari, Samaru, and Basawa) in Zaria, Nigeria. The fungal isolates were isolated using the standard mycological method. The cultural and microscopic characterization of fungi isolated revealed eleven genera belonging to Fusarium, Phomopsis, Phoma, Cladosporium, Al ternaria, Helminthosporium, Colletotrichum, Monilia, Curvularia, Rhizotonia, and Aspergillus. The total frequency and percentage occurrence ranged from 16(10.3%) to 42(26.%). The fungal isolate with the highest % frequency of occurrence was F. oxysporum with 23(14.7%), while the fungal isolate with the lowest frequency of occurrence was Curvularia eragrostidis with 3(1.9%). There were statistically significant differences in the mean number of occurrences among the fungal isolates (P = 0.003 < 0.05) and the markets (P = 0.0335 < 0.05). The total frequency and % occurrence of fungal isolates in tomato cultivars U T C, Ronita, and Roma VF ranged from 42(26.9) to 63(40.4%). There were statistically significant differences in the mean number of occurrences among the fungal isolates (p = 0.0000 < 0.05) and the tomato varieties (p = 0.0260 < 0.05). Tomato varieties, sorting, storage condition, packaging material, means of transportation, and nature of damage significantly affected tomato fungal-induced spoilage. Maintaining good sanitation practices in the field and during post-harvest handling of tomato fruits is crucial in minimizing the introduction and spread of fungi.

Keywords: Tomato, Fruits, Deterioration, Cultivars, Fungi and Post-harvest.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a member of the Solanaceae family, and it is one of the most popular and versatile fruits worldwide. They are commonly used in cooking, enjoyed for their taste, consumed in various cuisines, and appreciated for their vibrant colour, unique flavour, and numerous health benefits. Their rich nutrient profile, historical significance, and culinary versatility make them an important ingredient in numerous dishes (Rao *et al.*, 2019). Including tomatoes in a well-rounded diet can contribute to overall health and well-being (Rao *et al.*, 2019).

The tomato crop is susceptible to diseases, pests, and environmental factors affecting production output. Inadequate infrastructure for storage and transportation poses challenges to tomato farmers in Zaria and other regions of Nigeria. Despite these challenges, tomato farming remains an important economic activity in Zaria, contributing to local livelihoods and the agricultural sector of Nigeria as a whole. One of the major issues is the prevalence of tomato diseases, including fungal infections (Adedeji and Aduramigba 2016; Akaeze and Aduramigba 2017). These diseases can cause significant crop losses and reduce the quality and quantity of tomato production (Surechain, 2021).

Microbial spoilage of fresh tomatoes leads to waste of the produce and significant economic losses (Akinniran *et al.*, 2020). Fresh tomato fruits' quality and nutritional value are affected by their pre and post-harvest fungal colonization (Akinniran *et al.*, 2020).

Fungi are the most important plant pathogens that affect a wide range of economic fruits during storage (Akinniran *et al*., 2020). Due to the perishable nature of tomato fruits, they decay before reaching final consumers in distant parts of the country. The deteriorative nature of tomatoes cultivated locally is largely responsible for importing tomato paste into Nigeria (Sabo et al., 2017; Akinniran *et al*., 2020). The work is aimed at characterizing post-harvest deteriorating fungi and factors predispo sing tomato fruits to fungal-induced spoilage in Zaria.

MATERIALS AND METHODS

Collection of Samples

Random sampling was used in sample collection. A total of two hundred and twenty-five (225) spoiled samples of tomatoes were collected. These samples were purchased in batches of forty-five (45) from each of the five locations (Samaru, Sabogari, Basawa, Danmagaji, and Dakachi in Zaria metropolis, Kaduna State) and transported to the mycology laboratory of the Deptartment of Crop Protection, Institute of Agricultural Research, ABU, Zaria for further analysis. The samples were washed with tap water, then rinsed with distilled water, and stored in labelled containers to isolate and identify the fungi (Kenneth-Obosi, 2017).

Isolation of Fungal Isolates from Spoilt Tomatoes

The spoilt tomato samples were washed under a running tap, dipped in 1% sodium hypochlorite solution for 3 minutes, and rinsed in three changes of sterile distilled water before blotting dry with sterile filter papers. The affected area of the fruits was cut using a sterile scalpel and plated on Potato Dextrose Agar supplemented with streptomycin sulphate solution (Udoh *et al*., 2015). The culture plates were incubated at 25°C for 72h and observed daily for fungal growth, which was sub-cultured to obtain pure cultures.

Characterization of Fungal Isolates

The pure cultures of the fungal colonies were subjected to macroscopic and microscopic examination for their identification based on growth patterns, the colour of mycelia, and vegetative and reproductive structures using fungal atlases. References were made to existing stock cultures for ease of identification, (Mbajiuka & Emmanuel, 2014). SPSS version 27 using ANOVA was adopted for statistical analysis. To determine the factors predisposing tomatoes to fungi-induced spoilage in Zaria, Kaduna State, 225 tomato marketers were interviewed using a random sampling method. However, the responses of only 196 marketers were used in the analysis. The data collected include tomato variety, grade, sorting, storage condition, packaging materials, means of transportation, storing before selling, and nature of damage. Regression analysis was employed to determine the factors predisposing tomatoes to fungal-induced spoilage.

RESULTS

The fungi isolated consist of eleven genera, namely *Fusarium spp, Phomopsis spp, Phoma spp, Cladosporium spp, Alternaria spp, Helminthoporum spp, Colletotrichum spp, Monilia spp, Curvularia spp, Rhizoctonia spp* and *Aspergillus spp* with *Fusarium spp* as the predominant genus.

Table1. Fungal Isolates Growth Showed Various Cultural and Morphological Features of the Fungal Isolates Observed at 7 days

Fungal Isolates	Color	Texture	Edge Shape	Reverse Color	Diameter	Feature Revealed Under Microscope
Fusarium solani	Light Pink	Cottony	Irregular	Orange	40.0 mm	Small Oval Microconidia with one septum
Fusarium fujikuroi	White	Cottony	Irregular	Light Orange	63.4 mm	Abundant Crescent Shaped Macroconidia, Singly
Fusarium equiseti	Whit Pinkish	Cottony	Circular	Pinkish Brown	57.0mm	Branched Mycelia with Variable Sizes
Fusarium subglutinans	White	Cottony	Circular	Dark Orange	46.8 mm	Microconidia in Abundance with Macrocondia
Fusarium oxysporum	White	Cottony	White	Orange	48.3 mm	Fusiform Pointed at Edge With 4 Septa
Curvularia eragrostidis	White	Suede	Irregular	Dark Brown	58.6 mm	Abundant Mycelial
Phomopsis vexans	Dark Ash	Suede	Circular	Brown White	48.0mm	Pycnidia Phomopsis Revealed
Phoma destructiva	Dark Brown	Granular	Irregular	Black	62.5 mm	Pycnidia of Phoma
Cladosporium cladosporioides	Pale Green	Floccose	Irregular	Brown Black	25.0 mm	Branching Chains of Conidia
Alternaria alternata	Dark Brown	Cottony	Irregular	Black	41.0 mm	Branched Chain of Conidia with Terminal Beak at Ends
Helminthosporium sativum	Dark Brown	Velvety	Irregular	Black	58.0 mm	Cylindrical Conidia, 8 Cells with Fragmented Hyphea
Colletotrichum lindemuthiamum	Brown/Peach	Granular	Irregular	Black	33.4 mm	Conidia and Spine from one of the Conidia
Monila fructicola	Light Brown	Creamy Center	Floccose	Peach and Brown Center	52.0 mm	Conidiophore with rounded Conidia Acropetally
Curvularia lunata	Dark Brown	Velvet	Circular	Black	61.5 mm	Conidiophore Bearing Conidia
Rhizoctonia solani	White Patches	Velvet	Circular	Black	54.0 mm	Sclerotia and Mycelium
Aspergillus niger	Brownish Black	Powdery	Irregular	Cream	78.5 mm	Conidiosphore with Large Black Sporing Heads, Black round Conidia covering surface of Vesticles.

KEY: *Color, Texture, Edge Shape, Diameter, Microconidia, Conidiosphore, Macroconidia*

Table 2. Frequency of Occurrence of Fungal Isolates associated with Tomato samples from the five market locations in Zaria Metropolis

	MARKET											
	DAK		DMG		SG		BSW		SAM		Total	
Fungal isolate	N	%	N	%	N	%	N	%	N	%	N	%
Fusarium solani	2	12.5	4	14.3	4	11.1	2	5.9	3	7.1	15	9.6
Fusarium fujikuroi	3	18.8	3	10.7	6	16.7	6	17.6	4	9.5	22	14.1
Fusarium equiseti	1	6.3	1	3.6	2	5.6	1	2.9	2	4.8	7	4.5
Fusarium subglutinans	0	0.0	4	14.3	3	8.3	0	0.0	2	4.8	9	5.8
Fusarium oxysporum	3	18.8	5	17.9	4	11.1	4	11.8	7	16.7	23	14.7
Curvularia eragrostidis	2	12.5	0	0.0	1	2.8	0	0.0	0	0.0	3	1.9
Phomopsis vexans	1	6.3	1	3.6	0	0.0	7	20.6	1	2.4	10	6.4
Phoma destructiva	1	6.3	2	7.1	1	2.8	2	5.9	2	4.8	8	5.1
Cladosporum cladosporioides	1	6.3	0	0.0	0	0.0	2	5.9	1	2.4	4	2.6
Alternaria alternata	0	0.0	0	0.0	2	5.6	2	5.9	4	9.5	8	5.1
Helminthosporum sativum	0	0.0	0	0.0	0	0.0	2	5.9	2	4.8	4	2.6
Colletotrichum lindemuthiamum	0	0.0	1	3.6	0	0.0	0	0.0	4	9.5	5	3.2
Monilia fructicola	0	0.0	2	7.1	1	2.8	1	2.9	3	7.1	7	4.5
Curvularia lunata	0	0.0	2	7.1	3	8.3	1	2.9	1	2.4	7	4.5
Rhizoctonia solani	0	0.0	1	3.6	7	19.4	2	5.9	3	7.1	13	8.3
Aspergillus niger	2	12.5	2	7.1	2	5.6	2	5.9	3	7.1	11	7.1
Total	16	10.3	28	17.9	36	23.1	34	21.8	42	26.9	156	100.0

KEY: *DAK -Dakachi, DMG -Danmagaji, SG-Sabon Gari, BSW -Basawa, SAM -Samaru; N=number of occurrence of organism, %=Percentage of frequency of occurrences*

The total frequency and percentage occurrence of fungal Isolates from Dakachi, Danmangaji, Sabo-Gari, Basawa, and Samaru were 16(10.3%), 28(17.9%), 36(23.1%), 34(21.8%) and 42(26.9%) respectively. The market with the highest frequency of occurrence was Samaru at 42 (26.9%), while the market with the lowest frequency of occurrence was Dakachi at 16 (10.3%). The fungal isolate with the highest frequency of occurrence was *Fusarium Oxysporum* with 23 (14.7%), while the fungal isolate with the lowest frequency of occurrence was *Curvularia eragrostidis* with 3 (1.9%). The ANOVA results indicate that there are statistically significant differences in the mean number of occurrences among the fungal isolates ($p=0.0003<0.05$) and the markets ($p = 0.0335 < 0.05$). The fungal isolates can be grouped into 4 homogenous groups based on the multiple comparison results, indicating significant differences between some isolates but not within each group. The ANOVA results indicate that there are statistically significant differences in the mean number of occurrences among the fungal isolates ($p=0.0003<0.05$) and the markets ($p=0.0335<0.05$). The fungal isolates can be grouped into 4 homogeneous groups based on the multiple comparison results, indicating significant differences between some isolates but not within each group:
Group 1: *Curvularia eragrostidis, Phomopsis vexans, Phoma destructiva, Cladosporum cladosporiodes, Helminthosporum sativum* - These isolates had the lowest mean number of samples (0.6 to 0.8).
Group 2: *Alternaria alternata, Fusarium equiseti* – Had a moderate mean number of occurrences (10 to 1.6).
Group 3: *Fusarium solani, Fusarium subglutinans, Aspergillus niger, Monilia fructicola, Curvularia lunata, Rhizoctonia solani* – Had a moderately high mean number of occurrences (2.0 to 2.6). Group 4: *Fusarium fujikuroi, Fusarium oxysporum* vii – Had the highest mean occurrence (3.0 and 4.6).

The markets were also grouped into 2 homogeneous groups: Group 1: DAK -Had the lowest mean number of isolates (1.0). Group 2: DMG, SG, BSW, SAM - Had moderately higher mean numbers of isolates (1.8 to 2.6), with no significant differences among them. The multiple comparisons show that *Fusarium fujikuroi* had significantly more occurrence than all other isolates except *Fusarium oxysporum,* which had significantly more occurrence than all isolates except *Fusarium fujikuroi* and *Fusarium solani*. DAK market had significantly fewer samples than the SG and SAM markets. The number of occurrences varied significantly between fungal isolates and markets. *Fusarium oxysporum* and *Fusarium fujikuroi* isolates had the most occurrence overall, while *Curvularia eragrostidis* had the least. The DAK market had significantly fewer isolates than some other markets.

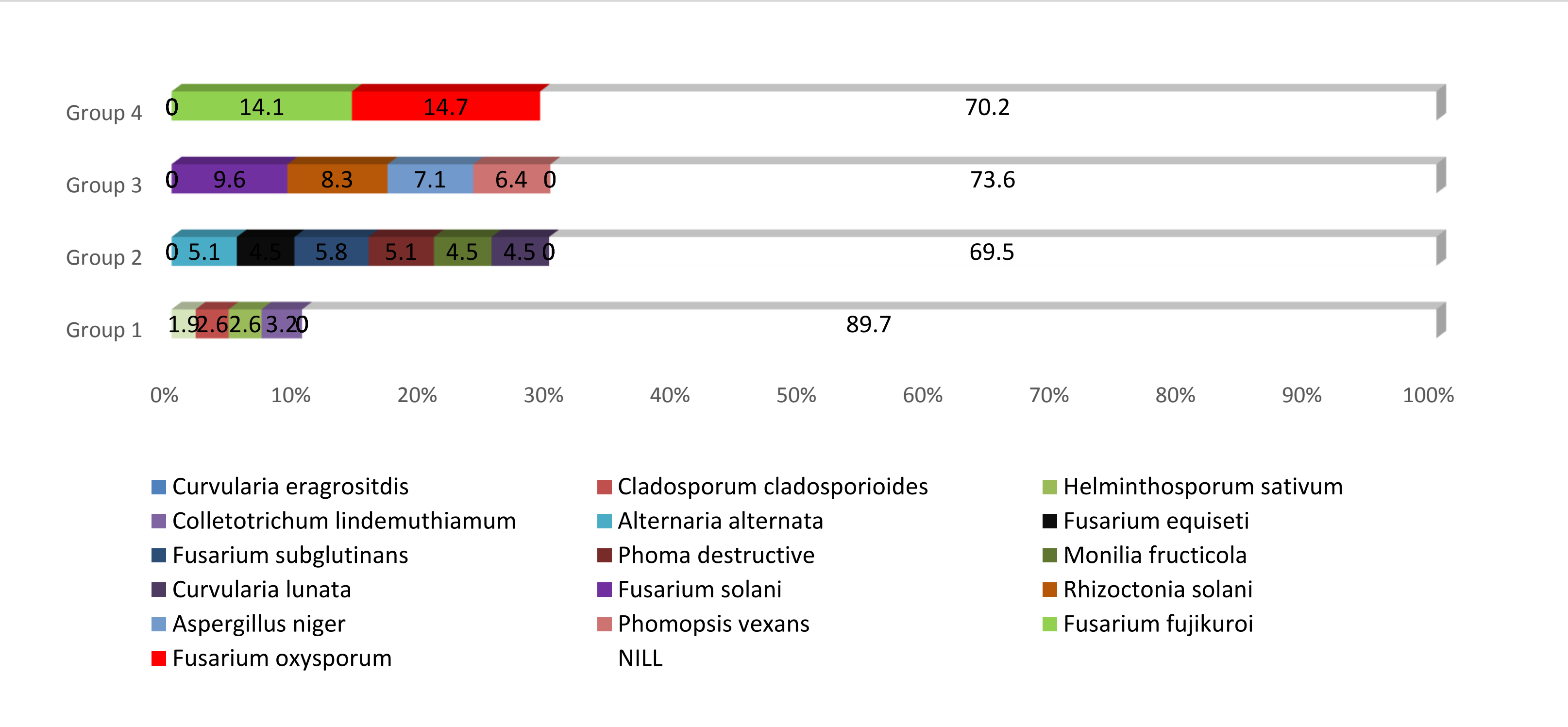

Figure 1. Percentage Occurrence of Fungal Isolates

Table 3: Frequency Occurrence (%) of Fungal Isolates from Spoilt Tomato Fruits Based on Tomato Cultivars

Fungal Isolates	UTC		RONITA		ROMA VF		TOTAL	
Fusarium solani	5	11.9	4	7.8	6	9.5	15	9.6
Fusarium fujikuroi	8	19.0	6	11.8	8	12.7	22	14.1
Fusarium equiseti	2	4.8	3	5.9	2	3.2	7	4.5
Fusarium subglutinans	2	4.8	3	5.9	4	6.3	9	5.8
Fusarium oxysporum	6	14.3	7	13.7	10	15.9	23	14.7
Curvularia eragrostidis	1	2.4	1	2.0	1	1.6	3	1.9
Phomopsis vexans	4	9.5	4	7.8	2	3.2	10	6.4
Phoma destructiva	2	4.8	2	3.9	4	6.3	8	5.1
Cladosporium cladosporioides	1	2.4	1	2.0	2	3.2	4	2.6
Alternaria alternata	1	2.4	4	7.8	3	4.8	8	5.1
Helminthosporium sativum	1	2.4	2	3.9	1	1.6	4	2.6
Colletotrichum lindemuthiamum	2	4.8	1	2.0	2	3.2	5	3.2
Monila fructicola	1	2.4	3	5.9	3	4.8	7	4.5
Curvularia lunata	2	4.8	2	3.9	3	4.8	7	4.5
Rhizoctonia solani	3	7.1	6	11.8	4	6.3	13	8.3
Aspergillus niger	1	2.4	2	3.9	8	12.7	11	7.1
Total	42	26.9	51	32.7	63	40.4	156	100.0

key: *N=number of occurrence of organism,%=percentage of occurrence of organisms*

The total frequency and percentage occurrence of fungal isolates in tomato cultivars UTC, Ronita, and Roma VF are 42 (26.9%), 51 (32.7%), and 63(40.4%). The tomato cultivar with the highest frequency of occurrence of fungal isolates was Roma VF, with 63 (40.4%), while the lowest was UTC with 42(26.9%). The ANOVA showed that there are statistically significant differences in the mean number of occurrences among the fungal isolates ($p = 0.0000 < 0.05$) and the tomato varieties ($p=0.0260 < 0.05$) (Table 3).

Table 4: Factors that predispose tomato to fungal-induced spoilage

Factors	Number of respondents (%)	R	p-value	Inference
Tomato varieties				
No response	22 (11.2)	0.200	0.006	S
UTC	77 (39.3)			
Roma VF	32 (16.3)			
Ronita	10 (5.1)			
Griffaton	22 (11.2)			
all varieties	33 (16.8)			
Grade				
No response	9 (4.6)	0.305	0.008	S
Ripe	185 (94.4)			
unripe	2 (1.0)			
Sorting				
Yes	130(66.3)	0.147	0.040	S
No	66 (33.7)			
Storage condition				
No response	2 (1.0)	0.242	0.001	S
Shade and covering with leaves	76 (38.8)			
Storage under shade	101 (51.5)			
Sorting before storage	7 (3.6)			
Concrete floor	10 (5.1)			
Packaging materials				
Basket covered with leaves/paper	145(74.0)	0.233	0.001	S
plastics crates	40 (20.4)			
Sacks	11 (5.6)			
Means of transportation				
Pick-up van	32 (16.3)	0.204	0.004	S
Long vehicle	52 (26.5)			
Car	75 (38.3)			
Motorbike	29 (14.8)			
Others	8 (4.1)			
Storing before selling				
Yes	74 (37.8)	0.230	0.001	S
No	122 (62.2)			
Nature of damage				
No response	3 (1.5)	0.356	0.000	S
Physical	98 (50.0)			
Fungi	53 (27.0)			
Insect	11 (5.6)			
Over ripe	31 (15.8)			

Dependent variable: % fungal spoilage, Independent variables: factors R: Regression

DISCUSSION

Sixteen different species were isolated from the two hundred and twenty-five (225) tomato samples from the five locations at five different times. These include *Fusarium solani, Fusarium fujikuroi, Fusarium equiseti, Fusarium subglutinans, Fusarium oxysporum, Curvularia eragrostidis, Phomopsis vexans, Phoma destuctiva, Cladosporium cladosporioides, Alternaria alternata, Helminthosporium sativum, Colletotichum lindemuthiamum, Monilia fructicola, Curvularia lunata, Rhizoctonia solani,* and *Aspergillus niger*. They were the main fungal species found to be responsible for the post-harvest deterioration of tomato fruits at different frequencies from the five locations. These species were previously reported for causing damage to tomato fruits from different markets in Nigeria (Sinno *et al.*, 2020).

Also, Sajad and Jamaluddin (2017) reported that the major post-harvest losses of fruits and vegetables are caused by species of fungi such as *Alternaria, Botrytis, Monilia, Penicillium, Fusarium, Geotrichum, Helminthosporium, Curvularia* which were similar to findings in this study. Chohan *et al.* (2016) reported that tomatoes are vulnerable to attack by different fungal pathogens, which are soil-borne, airborne, and seed-borne and inflict heavy losses in producing tomato fruits. The isolated and identified fungal species from tomato fruit rots are mainly caused by several fungi species (Sajad and Jamaluddin 2017). The study of Kvas *et al.* (2009) reappraised the numerous constituents of this group, including several economically significant plant pathogens and some that may produce secondary metabolite mycotoxins that can be harmful to human and animal health. Members of this group, such as *Fusarium solani, Fusarium fujikuroi, Fusarium subglutinans,* and other isolates in this study, are of worldwide distribution. Some are commonly saprobic on plant debris and in soil. Some strains are plant pathogens causing seedling blights, root/bulb rots, and vascular wilts. A number of strains have been isolated from human clinical specimens, and some members of this group of fungi isolated in this study are assigned to hazard group 2 by the ACDP (UK). The sixteen fungal species obtained in this study revealed that the market locations studied have diverse fungal species responsible for the post-harvest deterioration of tomato fruits. The frequency and percentage occurrence of fungal isolates from Dakachi, Damangaji, Sabo Gari, Basawa, and Samaru; 16(10.3%), 28(17.9%), 26(23.1%), 34(21.8%) and 42(26.9%) respectively. The market with the highest frequency and percentage occurrence is Samaru 42(26.9%), while the market with the lowest frequency is Dakachi at 16(10.3%).Variation in frequency of occurrence may have stemmed from improved methods of tomato handling and also due to a healthy market environment. Improper handling practices during post-harvest operations and at retail locations can cause mechanical injuries to tomatoes. Even minor injuries can create entry points for fungi to invade the fruits. Inadequate handling equipment and surface sanitation can lead to cross-contamination between healthy and spoilt tomatoes (Yuan *et al.*, 2019). The fungal isolate with the highest percentage frequency of occurrence is *Fusarium oxysporum* 23(14.7%), while the fungal isolate with the lowest frequency of occurrence is *Curvularia eragrostidis* with 3(1.9%). *Fusarium species* are well-known plant pathogens causing diseases in various crops, including tomatoes (Thomma *et al.*, 2011). The presence of *Fusarium spp* in tomato crops can devastate agriculture by yielding losses (Leslie *et al.*, 2006). These species in this group are widespread and frequently isolated from soil and many different plant species worldwide. Some strains occur as saprobes on plant debris, others cause disease of a wide range of plants, including seedling blight, root/bulb rots, and vascular wilts. Members of this group also occur as contaminants from industrial sources, e.g., in machine cooling fluids, and have been reported as opportunistic pathogens of human eyes and skin (Proctor *et al.*, 2018). The frequency and percentage occurrence of fungal isolates in tomato cultivars UTC, Ronita, and Roma VF were 42 (26.9%), 51 (32.7%), and 63(40.4%), respectively. The tomato cultivar with the highest % frequency of occurrence of fungal isolates is Roma VF 63(40.4%), while the lowest was observed in UTC 42(26.9%). Yousuf *et al.* (2021) reported that different tomato cultivars are susceptible to fungal infections. Some cultivars may possess genetic traits that make them more susceptible to fungal pathogens, whereas others may display resistance or tolerance. The choice of cultivar can significantly influence the susceptibility of tomato fruits to fungal-induced spoilage. The occurrence varied significantly between fungal isolates but not between the tomato varieties. *Fusarium fujikuroi* had the highest occurrence, while the organism with the least occurrence in relation to tomato cultivars was *Curvularia eragrostidis*. Mc colloch *et al.* (2002) reported that fungi cause different types of deterioration in post-harvest fruits, which occurs largely as blight and fruit rot of market vegetables. *Drosophila melanogaster* can carry spores and mycelia fragments on its body, from decaying tomatoes to cracks and wounds in healthy ones. Tomato varieties had a significant effect on fungal-induced spoilage. In this study, most respondents sold UTC, followed by Roma and other varieties. Their choice of tomato cultivars could be due to their unique characteristics. However, all varieties had varied percentages of fungal occurrence. Apart from unique characteristics, other factors such as grading, sorting, packaging materials, and means of transportation predisposed tomatoes to fungal-induced spoilage. In this study, 94.4% of respondents purchased ripe tomatoes, which are more susceptible to infection by fungal species. 33.7% did not sort their tomatoes, 74.0% used raffia baskets for packaging tomatoes, while a higher percentage of respondents used cars as a means of transportation of tomato fruits, which may not provide all the protection needed for the fruits. These findings align with the report of Ritenour *et al.* (2017) that inappropriate harvesting practices may cause damage such as wounds, breakage, or squeezing of the cellular and tissue structure. This generally increases the susceptibility of to mato fruits to the invasion and growth of fungi and speeds fungal decay rate. Lima *et al.* (2020) also stated that if tomatoes are not efficiently separated, fungal spores can spread to healthy fruits, accelerating the spoilage process.

CONCLUSION

Fungal isolates of eleven genera have been isolated, which include *Fusarium spp, Phomopsis spp, Phoma spp, Cladosporium spp, Alternaria spp, Helminthosporium spp, Colletotrichum spp, Monilia spp, Curvularia spp, Rhizoctonia spp,* and *Aspergillus spp* have been isolated. The frequency and percentage occurrence of fungal isolates from location ranged from 16(10.3%) – 42 (26.9%). The frequency and percentage occurrence of fungal isolates in UTC, Ronita, and Roma VF ranged from 42(26.9%) to 63 (40.4%), respectively. The frequency and percentage occurrence of fungal isolates in UTC, Ronita, and Roma VF ranged from 42(26.9%) to 63(40.4%), respectively. Tomato varieties, sorting, storage condition, pa ckaging material, means of transportation, and nature of damage significantly affected tomato fungal-induced spoilage.

RECOMMENDATIONS

Maintaining good sanitation practices in the field and during post-harvest handling of tomato fruits is crucial in minimizing the introduction and spread of fungi. Removal and proper disposal of infected plant debris and regular disinfection of equipment can help reduce the risk of fungal-induced spoilage. Inadequate handling equipment and surface sanitation can lead to cross-contamination between healthy and infected tomatoes

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