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Full Length Research Paper

Effects of stages of maturity on the susceptibility of tomato fruits to postharvest fungal pathogens

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The tomato is popularly grown in Kenya as a fruit vegetable, and hence provides employment and income to rural communities. Besides, it is a source of minerals and vitamins and therefore improves the quality of stew and salads. However, adequate amounts of tomatoes of good quality are rarely available due to production constraints, notably lack of varieties that are resistant to pre- and postharvest rots, not prone to spoilage during postharvest handling, and do not have a "short shelf life". Postharvest tomato rots are principally caused by fungi and bacteria. Other factors that determine the magnitude of postharvest losses include: the fruit maturity stage at harvest, the fruit cultivar and the postharvest pathogen identity. Hence, a project was designed to find out the effect of colour maturity stage at harvest on the susceptibility of tomato fruits cultivar Cal J to the postharvest fungal pathogens (*Fusarium oxysporum f. sp. lycoperisici, Fusarium solani, Alternaria alternata and Geotrichum candidum).* Tomato fruits, cultivar Cal J, were harvested at the green, yellow and red colour maturity stages and inoculated with the 4 fungi (*Fusarium oxysporum f. sp. lycoperisici, Fusarium solani, Alternaria alternata and Geotrichum candidum)* that were isolated from diseased fruits sampled from tomatoes sold in a Nairobi market. The pathogenicity of the fungi was assessed by comparing the lesion development and amount of damage caused on inoculated tomato fruits. The susceptibility of the tomato fruits to the fungi at different stages of maturity was determined by comparing lesion development and fruit damage on the mature green, yellow and red cultivar Cal J tomato fruits. The fungus *G. candidum* caused significantly (P≤0.05) highest damage with respect to lesion diameters and fruit damage (97.2%) compared to the other fungi: *F. solani* (82.4%), *F. oxysporum f. sp. lycopersici* (62.6%) and *A. alternata* (52.2%) on mature red fruits. The damage caused by *A. alternata* was significantly (P≤0.05) smallest. This is probably because *G. candidum* produces enzymes that effectively degrade the tomato tissues while those produced *A. alternata* are not as effective. Similarly, *G. candidum* caused significantly (P≤0.05) highest (97.2%) damage on the mature red fruits while the damage (20.5%) caused on the green fruits was significantly (P≤0.05) lowest. The trend prevailed for the other fungi. This could be due to higher concentration of biochemical defence mechanisms in green tomato fruits, which reduce as the tomato fruits ripen. Storage trails indicated that the "shelf-life" of the mature green Cal J tomato fruits was up to 3 times longer than that of the mature red ones, confirming that Cal J tomato fruits harvested at the green maturity stage have a significantly (P≤0.05) longer "shelf-life" than those harvested at the other maturity stages. The results, from the study, makes it necessary to advice farmers to: harvest tomatoes at the green maturity stage, avoid postharvest handling process that inflict damage; plant tomato varieties that withstand mechanical damage, and are resistant to postharvest infection; and prevent contact between tomato fruits with sources of fungal inoculums.

Keywords: Maturity stage, tomato fruits, cultivar cal j, postharvest fungi, "shelf-life", pathogenicity, susceptibility.

INTRODUCTION

The tomato, which is scientifically called *Solanum lycopersicum* L. (Bello *et al*., 2016) is a major fruit vegetable grown in many parts of the world. In Kenya it is mostly grown by small-scale rural farmers (MAFK, 2014; Mungai, 2000;

Seif, 1995), and therefore provides employment to the people hired to work in the farms, and a source of income to the tomato farmers. Tomato fruits contain calories, vitamins and minerals (MAFK. 2014; Rice *et al*., 1993; Minja, 1993; Atherton and Rudich, 1986). Therefore it improves the nutrient quality of the stews and salads (when used as one of the ingredients), that are part of the wide variety of dishes (foods) prepared in hotels and homes of different Kenyan communities. However, due to production constraints (Barki-Golan, 2001; Sommer *et al*., 2002; Bello *et al*., 2016) there is seasonal fluctuations in the quantity and quality of the tomatoes available to the tomato handlers: the farmers; traders; retailers; and consumers in hotels, especially the tourist class ones, in the major towns in the country that include Nairobi, Mombasa, Nakuru, Kisumu and Eldoret. The fluctuations inevitably affect the demand and supply of the commodity, leading to unpredictable earnings, and revenue collection.

The variation in the quantity and quality of the available tomato fruits results from negative production factors, which include pre-harvest diseases, notably early blight, mould rot (Yazici, *et al*., 2013; Mohammed *et al*., 2015; Olaniyi *et al*., 2010), tomato wilt (Mwangi, *et al*. 2011), and infection by pathogens after harvest leading to fruit rots (Liu *et al*., 2013; Bello et al., 2016). Postharvest fungal and bacterial infections cause fruit rot and spoilage, thus, negatively affecting the value of the harvested fruits (Snowdon, 2010; Shi, *et al*., 2013). The spoilt, rotting tomatoes are generally discarded by the commodity handlers, between harvesting and consumption. The discarded spoilt tomatoes are regarded as postharvest loss, and are a reflection of the economic loss to tomato handlers and revenue authority.

Postharvest fungi, not only, cause the tomato fruit spoilage, but, some produce mycotoxins (toxic fungal metabolites) (Pose *et al*., 2010; Van de Perre, *et al.,* 2014), that cause health problems on ingestion or dermal contact (Kocić-Tanackov *et al*., 2010). For example aflatoxins produced by *Aspergillus flavus* cause liver cancer and fumonisins by *Fusarium moniliforme* cause esophageal cancer (Pereica, 1999, Van de Perre, *et al*., 2014; López *et al*., 2016).

The quantity of tomato fruits losses after harvest, is however, determined by the tomato variety and the fruit colour maturity stage at harvest (Wang *et al*., 2010; Xu *et al*., 2010; Lolas, *et al*., 1998; Subedi *et al*., 1998), and magnitude and nature of losses is influenced by postharvest handling procedures. The easily damaged varieties are prone to injury during postharvest handling (Zhu and Zhang, 2016). The level of injury determines the amount of postharvest losses because the injuries are avenues through which infection occurs (Barkai-Golan, 2001; Pitt and Hocking, 2009; Ilham., *et al*., 2003; Kazempour, 2000;), resulting to the rotting of the fruits. Besides, losses are even greater for tomatoes that are harvested when the fruits are over mature (red stage of maturity) because such fruits are easily damaged.(Wang *et al*., 2010; Xu *et al*., 2010). The degree of spoilage on the tomatoes harvested at the different fruit colour (green, yellow, red) maturity stages is similarly affected by the postharvest handling procedures (Droby *et al*., 2010; Pia *et al*., 2010; Burdon, 1997), particularly sorting, packaging, transport containers and means of transport (Snowdon, 2010; Burdon, 1997; Ryall, 1979). All these factors cumulatively determine the extent of fruit spoilage by the time they reach the consumer (Abd-Allah *et al*., 2013; Zao *et al*., 2010; Janisiewicz, 1998; Lurie, *et al*., 1997; Dasgupta and Mandal, 1989; Onesirosan and Fatunla, 1979). The discarded heaps of rotting tomatoes found around the open air markets demonstrates the magnitude of loss incurred by tomato farmers (Dijksterhus *et al*., 2013, Tijjani *et al*., 2014; Mohammed *et al*., 2004; Kader, 2002). In addition, rotting tomatoes smell, thus polluting the air; make the environment dirty; and therefore attract flies that visit smelly, rotting substrates (Birmingham *et al*., 2015; Walsh *et al*., 2011; Sommer *et al*., 2002; Bickley, 1956). The flies may pick microorganisms some of which may be disease agents, as they move about on the rotting tomato fruits (Mitchell, 2004; Wells and Buttefield, 1999; Onesirosan and Fatunala, 1976). These flies may find their into the dining rooms of the communities residing in the neighbourhood, thus endangering their health.

Due to the magnitude of tomato postharvest loss, it necessary to come up with a postharvest tomato loss control strategy that targets one or more of the tomato postharvest loss predisposing factors. Consequently, a study was undertaken to find out the susceptibility of fruits of tomato cultivar Cal J, harvested at different maturity colour (Green, Yellow, and Red) stages (Wang *et al*., 2010; Xu *et al*., 2010) to four postharvest tomato rot fungi (isolated from diseased tomato fruits). The study aimed at determining the maturity colour stage at which harvested Cal J tomato fruits are least susceptible to mechanical damage during postharvest handling processes. Consequently, it is expected that the amount of tomato postharvest losses due to infection, particularly by fungi is controlled.

The information will be disseminated to tomato farmers and other handlers through The Ministry of Agriculture Field Officers regarding: the appropriate maturity colour stage at which tomatoes are harvested in order to minimize losses after harvest; the need to grow varieties that withstand mechanical damage, resist infection by fungi, and have a “long shelf life”; and the necessity to avoid contaminating harvested tomato fruits with fungi and any debri that bears fungal inoculums. Besides, the findings will be a reference for use by tomato stakeholders.

MATERIALS AND METHODS

Growth and management of the tomatoes

Soil was dug from a non-cultivated portion near the Department of Plant Sciences garden at Kenyatta

Table 1. Duration taken by the tomato fruits in the field (unharvested) to attain the green, yellow and red colour maturity stages after flowering.

Colour Maturity stage	No. of days taken by replicate fruits to attain the colour maturity stage					Mean*no. of days
Mature green	42	43	43	41	43	42.0 ± 0.422 c
	44	42	42	40	40	
Mature yellow	47	48	49	50	46	48.7 ±0.473 b
	49	48	50	51	49	
Mature red	53	54	55	55	55	54.0 ± 0.258 a
	53	53	54	54	54	

Means of 10 replicates.
Mean ± SE (Standard Error) in the same column followed by a different letter differ significantly (p≤ 0.05) following Tukey's test.

University, sterilized (by autoclaving twice at 24-hour intervals) and apportioned into wooden seedling boxes (40 cm by 30 cm by 15 cm). Certified seeds of tomato cultivar Cal J, bought from the Kenya Seed Company outlet at Kijabe Street, Nairobi City, Kenya, were sown in triplicate seedling boxes, and three times at 7 days interval in furrows (2 cm deep and 10 cm apart) prepared on the soil in the seedling boxes. At the end of planting, there were three sets of seedling boxes: just planted, seven days old, and fourteen days old. The growing seedlings were thinned leaving 10 plants per furrow 14 days after planting.

Four weeks old seedlings were transplanted into 10 cm deep holes, 45 cm by 60 cm prepared at a previously fallow plot at the Department of Plant Sciences garden that had been cleared of vegetation and dug twice. Weeding was done during the third and the fifth week after planting. The tomatoes were staked in the sixth week. Diseases were controlled by spraying with the organic sulphur fungicide (Dithiocarbonate) Mancozeb that is sold as Dithane M-45, which is a broad spectrum fungicide for control of foliage and fruit diseases of vegetables that include tomatoes and potatoes.

A minimum of ten plants bearing yellow open flowers, from each set of tomatoes that were transplanted first, second and last at 7 days interval were tagged with labels showing dates. The period taken by the fruits of the tomatoes that were transplanted first, second and last to attain the red, yellow and green maturity colour stages respectively were recorded (Table 1)

Tomato fruits storage trials.

Clean/unblemished mature red, yellow and green fruits harvested from the tomatoes grown as already described (Section 2.1) were stored in open carton boxes kept in a lockable shelf in the laboratory at ambient temperature. The tomatoes were observed regularly to determine the period it took each fruits to be spoilt. A tomato fruit was considered spoilt when it lost firmness or started to rot.

Isolation of postharvest tomato fungi

Fifty tomato fruits showing disease symptoms were collected from the Retail Market in Nairobi (10 tomatoes from each of 5 stalls) and carried in paper bags to the laboratory. The infected fruits were surface sterilized using cotton wool saturated with 70% alcohol. Tissues (approximately 4 mm square) were cut from the margins of lesions from the fruits using flame sterilized forceps and aseptically transferred to the centre of Petri dishes of (90 mm size), one tissue per Petri dish containing approximately 15 ml Potato Dextrose Agar (PDA) Oxoid medium. The medium was prepared by dissolving 39 g of the PDA powder in 1 litre of sterile distilled water before autoclaving at 121 ^{o}C for 15 minutes.

The inoculated Petri dishes were incubated at 25 ^{o}C for 10 days. The growing fungi were sub cultured into Petri dishes containing fresh PDA medium to obtain pure cultures. The cultures were identified by examining (under the microscope) specimens of sporulating portions of the cultures mounted on a microscope slide.

Fungal pathogenicity and damage (spoilage) assessment tests on the tomato fruits

Mature red, yellow and green fruits were harvested from the tomatoes grown as already described (Section 2.1) and carried to the laboratory in paper bags, and the fruits washed in running tap water for five minutes and sterilized using cotton wool saturated with 70% alcohol.

A cut, 2 mm deep was made with a cooled, flame sterilized cork borer (6 mm in diameter) on the side of each of the red, yellow and green tomato fruits and the

Table 2. Storage duration (in good condition) of the tomato fruits that were harvested at the red, yellow and green colour maturity stages before they rot/get spoilt.

Maturity stage	No. of days for replicate fruits to rot/get spoilt	Mean*no. of days
Mature green	76 86 82 80 78 80 76 81 79 82	80.0 ± 0.955 a
Mature yellow	38 36 37 40 39 41 38 37 39 40	38.5 ± 0.748 b
Mature red	23 17 18 22 20 21 18 19 24 22	20.4 ± 0.500 c

Mean of 10 replicate fruits.
Mean ± SE in the same column followed by different a letter differ significantly ($p \leq 0.05$) following Tukey's test.

loose tissue removed. The wounds were inoculated with 6 mm discs of mycelium, cut with a cork borer from the periphery of 10 days old cultures of the 4 postharvest tomato rot fungi (*Fusarium oxysporum f. sp. lycopersici, Fusarium solani, Alternaria alternata* and *Geotrichum candidum*) isolated from infected tomato fruits as already described (Section 2.3). Controls comprised fruits that were similarly wounded, but treated with sterile agar discs. Five replicate fruits were prepared for each treatment.

The treated fruits were put in transparent closed polythene bags (Onesirosan and Fatunla, 1976) and placed on a clean laboratory bench. The diameters of lesions on the treated fruits were measured on day 3, 6, 9 and 12 after inoculation using calipers, which was borrowed from The Physics Laboratory at Kenyatta University. On the last (Day 12), however, each fruit was diametrically cut open and the % rotted (damaged) area determined by expressing the estimated rotted area as % of the healthy fruits.

RESULTS

Field observations showed that the unharvested tomato fruits took significantly ($p \leq 0.05$) shortest period (42 days) to attain the mature green colour stage and significantly ($p \leq 0.05$) longest period (54 days) to attain the mature red colour stage (Table 1). The storage trials also indicated that the "shelf-life" (storage duration before the fruits rot/get spoilt) of the harvested tomatoes was significantly ($p \leq 0.05$) longest (80 days) for the mature green fruits and significantly ($p \leq 0.05$) shortest (24 days) for the mature red fruits (Table 2).

The pathogenicity and fruit damage assessment tests revealed that lesion development and amount of damage on inoculated tomato fruits depended on the fungi and the fruit colour (green, yellow and red) maturity stage (Table 3 and 4). The largest lesion diameters and percentage fruit damage were observed on the inoculated mature red tomato fruits and the least on the mature green tomato fruits (Table 3 and 4). With respect to lesion size on tomato fruits inoculated with fungi, *G. candidum* and *F. o. lycopersici* caused the significantly ($p \leq 0.05$) largest (87.6 mm) and second largest (74.6 mm) lesions on the red tomato fruits respectively, while *A. alternata* caused significantly ($p \leq 0.05$) the smallest (42.2 mm) lesions 12 days after inoculation (Table 3). Similarly, the fungus *G. canadidum* caused significantly ($p \leq 0.05$) highest percentage damage (97.2 %) on the red tomato fruits while *A. alternata* caused significantly ($p \leq 0.05$) the least percentage damage (52.2 %) (Table 4)

DISCUSSION

The significant ($p \leq 0.05$) lesion development and fruit damage on the inoculated fruits (Tables 3 and 4) confirms that the fungi (*F. o. lycopercisi, F. solani, G. candidum* and *A. alternata*) are important postharvest spoilage organisms of tomato fruits. These observations agree with that of Wani, 2011, in whose document on an overview of the fungal rot of tomato, cites the fungi that are a subject of this study as tomato postharvet rot agents. The amount of spoilage (lesion spread and fruit damage) in the inoculated tomato fruits (Tables 3) varied probably due to the differences in the pathogenicity of the fungi and defense mechanisms of the plant. In this regard, *G. candidum* was the most pathogenic fungus, while *A. alternata* was the least pathogenic. This implies that, the mechanisms of pathogenesis and colonization of the tomato fruits tissues by the fungi vary in nature and amounts.

For example it has been established that colonization of plant tissues by fungi is facilitated by enzymes. Evidence is found in *Alternaria alternata*, that produces endo-1,4-β-glucanase that aids in colonization of infected plant tissues (Dijksterhus *et al*., 2013). Besides, pectic

Table 3. Lesion development on mature red, yellow and green tomato fruits inoculated with 4 fungi: *F. solani*,(*F.s), F.o. lycoperisici(F.o.l.)*, *G. candidum(G.c.)* and *A. alternata(A.a.)*.

Maturity Stage	Postharvest fungi	Mean* lesion diameter on tomato fruits in mm on day			
		3	6	9	12
Green	F.s	10.3±0.464 a	14.0±0.359 a	16.1±0.332 a	18.1 ± 0.400 a
	F.o.l	10.3±0.464 a	14.8±0.374 a	15.4±0.430 ab	17.7 ± 0.300 a
	G.c	6.8 ±0.200 b	12.4±0.374 b	14.0 ±0.316 b	15.0 ± 0.245 b
	A.a	9.4 ±0.292 a	12.3±0.374 b	14.5 ±0.354 b	14.6 ± 0.447 b
	Control	6.1 ±0.100 d	6.3 ±0.200 c	6.3 ± 0.200 c	6.3 ± 0.200 c
Yellow	F.s	10.3±0.464 b	15.6±0.458 b	22.4 ± 0.812 a	41.6 ± 0.510 a
	F.ol.	8.5±0.354 c	15.5±0.474 b	18.5 ± 0.500 b	26.3 ± 0.539 b
	G.c.	12.2±0.255 a	21.3±0.539 a	21.4 ± 0.510 a	41.9 ± 0.678 a
	A.a	10.0±0.652 b	12.8±0.406 c	15.2 ± 0.583 c	16.5 ± 0.447 c
	Control	6.1±0.100 d	6.4 ±0.245 d	6.4 ± 0.245 d	6.4 ± 0.245 d
Red	F.s	12.5±0.592 b	26.0±0.707 b	38.1 ± 0.640 c	51.3 ± 1.390 c
	F.o.l	11.8±0.800 b	25.0±0.707 b	51.7 ± 1.140 b	74.6 ± 1.330 b
	G.c	20.4±1.030 a	41.0±1.000 a	68.5 ± 1.200 a	87.5 ± 0.612 a
	A.a	12.4±0.583 b	17.8±1.060 c	24 3 ± 1.040 d	42.2 ± 1.160 d
	Control	6.1 ±0.100 c	6.4±0.245 d	6.3 ± 0.200 e	6.4 ± 0.245 e

Mean of replicate fruits.
Means ± SE in the same column followed by the same letter are not significantly different ($p \leq 0.05$) following Tukey's test.

enzymes produced by fungi also play a major role in macerating and killing of plant tissues, therefore, causing them to rot. This is confirmed by the fact that *Fusarium oxysporum* and *Colletotrichum lindermuthianum* produce pectic enzymes that aid in the colonization of plant tissues during infection (Collmer and Keen, 1986).

The observed larger lesions and higher % damage on the red fruits compared to the green fruits (Table 3 and 4) indicate that the fruits became more susceptible to spoilage by the postharvest fungi as they ripen (change from mature green to mature red colour) probably because of the biochemical changes that occur during the ripening process (Bargel and Neinhuis, 2005; Gray *et al*., 1993).

During ripening of tomato fruit there are changes in oxidative and antioxidative parameters. In the fruit, the processes associated with hydrogen peroxidase content, lipid peroxidation and protein oxidation increase at the start of ripening (Jimenez, *et al*., 2002). The change in fruit colour of tomato is accompanied by degenerative reaction (Grieson and Kader, 1986). The changes are senescence related and results in deterioration of the cell membrane and death (Bouzayen*, et al*., 2010). These changes are controlled by fruit ripening related genes in which gene encoded enzymes participate. Ripening in tomato fruits is accompanied by increased ethylene production (Bouzayen, *et al*., 2010). The end result of all the changes include: the softening of the fruit texture. Such fruits are prone to mechanical damage and hence have a "short shelf life". Hence, mature red tomato fruits are prone to injury during harvesting, sorting, packaging, transport and storage. Such fruits are prone infection and consequently postharvest rots.

Therefore, the knowledge of the biochemicals produced during the tomato fruit ripening process and the mechanisms involved, can form a basis for designing an intervention strategy that slows down the fruit ripening process. For example, according to Bouzayen, *et al*. (2010), enzymes, genes, and growth hormones are involved in the metabolic pathway that lead to changes during ripening such as ethylene production by tomato fruits which bring about change in colour of the tomato from green to red, as well as the softening of the fruit texture. Slow down of the ripening process, can be achieved through strategies that interfere with the critical step(s) in the biochemical process that results to production of chemicals or compounds that are responsible for tomato fruit ripening. For instance, ripening of fruits may be slowed or controlled by intervening with the critical step in the metabolic pathway for the production of ethylene. This has the benefit of increasing the postharvest ''shelf life'' of the fruits, and consequently a reduction in the postharvest loses. This is confirmed by the fact that the tomatoes harvested at the

Table 4. Percentage (%) fruit damage of mature green, yellow and red tomato fruit inoculated with the fungi: *F. solani*, *F.o lycopersici, G. candidum* and *A. alternata* after 12 days incubation.

Fungi	Mean* % fruit damage/rot at different colour maturity stages		
	Green	Yellow	Red
F. solani	40.6 ± 1.44a	69.6 ± 0.921a	82.4 ± 0.927b
F. o. *lycopersici*	19.4 ± 1.29b	22.2 ± 1.07d	62.6 ± 0.927c
G. candidum	20.5 ± 0.866b	60.8 ± 1.39b	97.2 ± 0.374a
A. alternate	12.6 ± 0.927c	34.2 ± 1.07c	52.2 ± 1.09d

Mean of 5 replicate fruits.
Mean ± SE in the same column followed by a different letter differ significantly (p≤0.05) following Tukey's test.

green stage had a "shelf life" of up to 3 times (Table 2) that of the tomato fruits harvested at the red colour stage. This constitutes a further strategy for reducing postharvest losses because green fruits are more tolerant to the fruit spoilage fungi than the red fruits and have a longer "shelf-life" (Table 2, 3 and 4).

The proposed strategies, which emanate from this study that include: minimizing postharvest losses of tomato by harvesting at the right maturity stage, in addition to planting varieties that are tolerant to postharvest fungi, as well as avoiding the wounding of tomato fruits and minimizing contact with tomato rot fungi is supported by other researchers (Guillen, *et al.,* 2007; Lolas *et al*., 1998; Subedi; *et al*., 1998). Therefore, it is necessary to assess the postharvest qualities of the different tomato varieties grown by farmers with the view of identifying those that have long "shelf-life" and are tolerant to postharvest spoilage organisms. Further, farmers can be advised appropriately on the right tomato varieties that they can grow, and the relevant postharvest management practices that they can apply.

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