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INFLUENCE OF STORAGE TEMPERATURE ON FUNGAL PREVALENCE AND QUALITY OF CITRUS FRUIT (CV. BLOOD RED)

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Abstract

The influence of storage temperature on post storage fungal prevalence and quality of citrus fruit (sweet orange cv. Blood Red) was investigated at the Horticultural Laboratory of Khyber Pakhtunkhwa Agricultural University, Peshawar. The fruits were exposed to 5, 10 and 20 °C for 45 days and then kept at ambient temperature for 25 days. The fruits were evaluated for disease incidence and other quality attributes at 5 days incubation to the maximum of 25 days. Results indicated that disease incidence and prevalence of *Penecillium itallicum* and *Penecillium digitatum* were higher in fruits stored at 5 and 20°C and were lower at 10°C. Weight loss was higher in the fruits stored at 20°C followed by 5°C and was lower at 10°C Ascorbic acid was higher in fruits stored at 10°C followed by 5 and was lower at 20°C. Disease incidence and prevalence of *Penecillium itallicum* and *Penecillium digitatum* and weight loss increased while ascorbic acid decreased with increase in post storage incubation from 0 to 25 days. It is concluded that storage of citrus fruits at 10°C performed better in terms of fight against disease and fungal prevalence and perseverance of quality.

Introduction

Sweet orange is a high value fruit due to its taste and nutrition throughout the world. Among the sweet orange, cultivars Blood Red is generally preferred because of its red flesh caused by anthocyanins (Dougo *et al*., 2003) and possibly its therapeutic properties (Sajia, 1994). Citrus species is however susceptible to both pre and post-harvest pathogens (Pienar, 1969) that limit its export (Mahmood *et al*., 2008). Post harvest quality losses in citrus fruits have been commonly observed (Tariq *et al., 2001*a). Chemical (Smilanick *et al*., 2003; Palou *et al*., 2004). Biological (Kinay *et al*., 2001) and physical measures are usually adopted to remove spores from the surface of fruits and these are effective methods to decrease disease incidence (Abdel-El-Aziz & Mansour, 2006; Smilanick *et al*., 2003).

Post-harvest diseases destroy 10-30% of the total yield of crops but in perishable crops, especially in developing countries, they destroy more than 30% of the crop yield (Kader, 2002). The fungal pathogens, *Penicillium digitatum* and *Penicillium italicum*, are the predominant pathogens of citrus fruits (Snowdon, 1990, Brown & Eckert, 1988). Disease incidence in storage of sweet orange fruit increases with increasing storage duration (D' hallewin and Schirra, 2000) which could be as high as 50% (Abd-El-Aziz & Mansour, 2006).

Infection of oranges by *Penicillium digitatum* results in green mold, a predominant post-harvest disease of citrus (Brown & Eckert, 1988). Initial infection of the fruit occurs prior to or after harvest via wounds in the fruit peel (Snowdon, 1990). Another common fungal disease of oranges is the blue mold, caused by the fungus *Penicillium italicum* which can be decreased by storage at low but non-chilling temperatures. However, chilling for prolong duration may actually damage the tissue to the level where it may become highly sensitive to fungal infections (Ritenour *et al*., 2004). Both the fungi grow best at a temperature of 75° F (23.8°C) and their development is slowed by lower temperatures. While fungicides can be used to decrease diseases on citrus fruit (Agostini *et al*., 2006) but chemical residues limits their application. Thus, non-chemical measures of disease control are generally preferred in postharvest disease suppression (Al-Obeed & Harhash, 2006). Therefore, cooling citrus fruit at optimal temperature during storage may be effective to decrease blue mold. But exposure to suboptimal low chilling temperatures may cause chilling injury, which may favor the decay causing organisms (Kader, 2002, Arpaia & Kader, 2009).

Thus long term low temperature storage of citrus fruits is limited by their sensitivity to chilling (Arpaia & Kader, 2009) that may enhance susceptibility to decay causing organisms (Chalutz *et al*., 1985). The objective of this experiment was to investigate the influence of storage temperature on fungal and disease prevalence and citrus fruit quality during 25 days post-storage period.

Materials and Methods

To study the influence of storage temperature on fungal and disease prevalence and quality of citrus fruits, an experiment was carried out at Khyber Pakhtunkhwa Agricultural University, Peshawar. The sweet orange fruits cv. Blood Red with about similar size and maturity and free from bruises and other mechanical injuries were procured from an orchard in Rustum, Mardan. The fruits were immediately transported to the laboratory and were carefully washed with distilled water and dried with a blower before allotting to designed storage conditions. The experiment was laid out in randomized complete block (RCB) design with three replications. The treatments consisted of three storage temperatures (5, 10 and 20°C) and post-storage incubation (0-25 days). There were 20 fruits per treatment. The fruits were stored at 5, 10 and 20°C for 45 days and then incubated at ambient temperature for 0-25 days. The fruits were evaluated for fungal prevalence and disease incidence and other quality attributes at 5 days interval to the maximum of 25 days. Data were recorded on the following parameters.

Disease incidence (%): Total disease incidence was recorded at each post-storage interval by visual

observation of diseases symptoms. It was presented as percentage of fruits with the symptoms of disease.

Fungal prevalence (%): The fungal prevalence was determined by identification of predominant pathogens. For this purpose fruits after 45 days exposure to different temperatures were incubated at ambient temperatures for 0-25 days. The diseased fruits were analyzed for specific pathogens. The pathogens (spores) were sampled from the diseased fruit, cultured and grown on PDA in Petri dishes at 25°C and identified in plant pathology laboratory of Khyber Pakhtunkhwa Agricultural University, Peshawar. The data on prevalence of pathogens were presented as percentage of total fruits.

Quality attributes

Weight loss: Weight loss was approximated as percent of original weight. For this purpose initial and final weight of the fruits was determined at time zero and after specified post-storage incubation at ambient temperature with the help of an electronic balance. The percentage of weight loss was calculated using the following formula:

$$\text{Percent weight loss} = \frac{\text{Weight of fresh fruit} - \text{Weight after interval}}{\text{Weight of fresh fruit}} \times 100$$

Percent ascorbic acid: For the determination of ascorbic acid, one ml of juice was diluted in 0.4% oxalic acid solution and volume was made up to 10 ml. This 10ml diluted sample was titrated against the standard dye solution until light pink color appeared, which persisted for 15 seconds. A blank titration was also carried out. Vitamin C was calculated at seven days interval according to following formula (Anon., 1990)

$$\text{Ascorbic acid} = \frac{F \times T \times 10}{D \times S} \times 100$$

$$F = \text{Factor for standardization} = \frac{\text{Where, (ml of ascorbic acid)}}{\text{Ml of dye}}$$

T = ml of dye used for sample – ml of dye used for blank
D = ml of sample taken for dilution
S = ml of dilute sample taken for titration
Percent ascorbic acid was determined as described by Anon., 1990.

Statistical analysis: The data were statistically analyzed using analysis of variance appropriate for RCB design and means were compared using LSD test at 5% level of probability when the F-values were significant (Steel & Torrie, 1984).

Results

Prevalence of *Penecillium digitatum*: The prevalence of *Penecillium italicaum* was significantly affected by ST and PSI (Table 1). The ST x PSI interaction was also significant (Fig. 1a). The prevalence of *Penecillium digitatum* was lower on fruits stored at 10°C as compared to 5 and 20°C. Amazingly, the prevalence was similar at 5 and 20°C. The ST x PSI interaction revealed that prevalence of *Penecillium digitatum* increased with increase in PSI duration at all storage temperatures but the increase was comparatively slower in fruits stored at 10°C. The prevalence reached to the maximum of 50% on fruits stored at 10°C as compared to 55 and 70% at 5 and 20°C, respectively.

Table 1. Effect of storage temperature and post storage incubation on *P. digitatum, P. italiaum*, disease incidence, weight loss and ascorbic acid of sweet orange fruit.

Storage temperature (ST)	*Penecillium digitatum* (%)	*Penecillium italicaum* (%)	Disease incidence (%)	Weight loss (%)	Ascorbic acid (%)
20°C	27.22 a	33.06 a	33.06 a	4.815 a	55.99 c
10°C	19.72 b	20.00 b	23.89 b	3.092 c	66.04 a
05°C	28.89 a	34.44 a	33.89 a	4.211 b	60.42 b
LSD	3.314	3.104	3.9743	0.5073	1.411
Post-storage incubation (PSI)					
0 day	5.56 e	8.33 e	8.33d e	1.039 f	71.15 a
5 days	9.45 e	11.67 e	13.33 e	1.580 e	69.68 a
10 days	16.11 d	19.44 d	20.56 d	2.590 d	65.75 b
15 days	25.00 c	28.33 c	30.55 c	4.650 c	60.59 c
20 days	37.22 b	45.00 b	43.89 b	6.343 b	51.98 d
25 days	58.33 a	62.22 a	65.01 a	8.014 a	45.73 e
LSD	3.314	4.390	5.6205	0.507	1.996
Interaction ST x PSI	* Fig 1a.	* Fig 1b.	* Fig 1c.	* Fig 2.	* Fig 2

Means in the same column followed by different letters are significantly different at p≤0.05
* = Significant at 5% level of probability

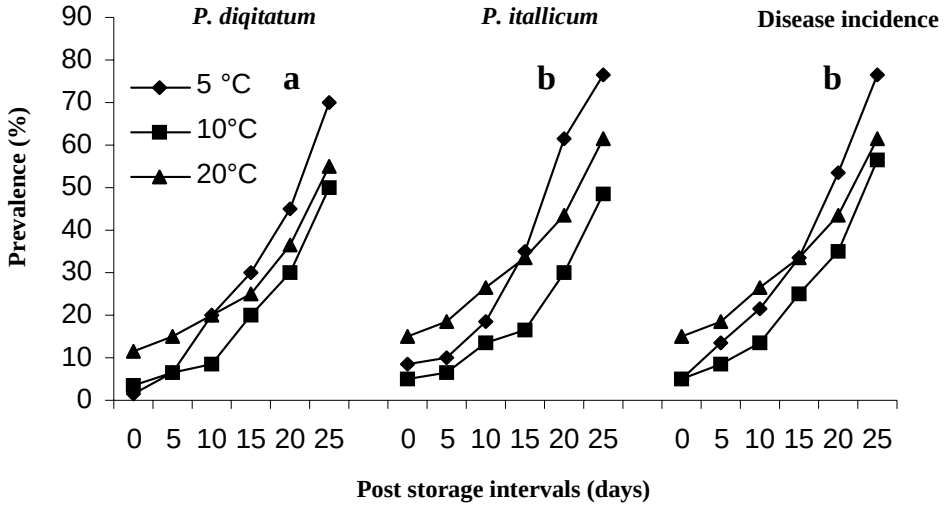

Fig. 1(a, b, c). Interactive effects of storage temperature and post storage incubation on *P. digitatum, P. itallicum* and disease incidence of citrus fruit.

Prevalence of *Penecillium italicaum*: The prevalence of *Penecillium italicaum* differed significantly with ST and PSI (Table 1). The ST x PSI interaction was also significant (Fig. 1b). The prevalence of *Penecillium italicaum* was lower on fruits stored at 10°C as compared to 5°C and 20°C. The ST x PSI interaction revealed that prevalence of *Penecillium italicaum* enhanced with increase in PSI at all storage temperature. However, the increase was slower in fruits stored at 10°C so that it was 48.33% as against 61.67 and 76. 37%, recorded for at 5 and 20°C, respectively.

Disease incidence (%): Disease incidence varied significantly with ST and PSI (Table 1). The ST x PSI interaction was also significant (Fig. 1c). The disease incidence was lower for fruits stored at 10°C as compared to fruits stored either at 5 or 20°C, which were statistically at par with each other. The disease incidence consistently enhanced with increase in PSI from 0 to 25 days. The ST x PSI interaction indicated that the disease incidence of fruits increased with increase in PSI at all storage temperatures but the increase was comparatively slower at 10°C.

Weight loss (%): The weight loss in citrus fruits was significantly affected by ST and PSI (Table 1). The ST x PSI interaction was also significant (Fig. 3). The higher weight loss was recorded when fruits were stored at 20°C (4.805%), which was significantly higher than storage at 10 °C (3.092%) or 5°C (4.2119%). Weight loss increased with increase in post-storage incubation at ambient temperature. The weight loss significantly increased from 1.039% to 8.014% with each 5 days interval from 0 to 25 days post storage incubation. The interaction between the two factors indicated that weight loss constantly increased with increase in PSI however the increase was much lower at 10°C as compared to 5°C and 20°C, respectively. The weight loss reached to a maximum value of 6.49% which was lower than the weight loss occurred at 5°C (9.54%) and 10°C (8.01%), respectively.

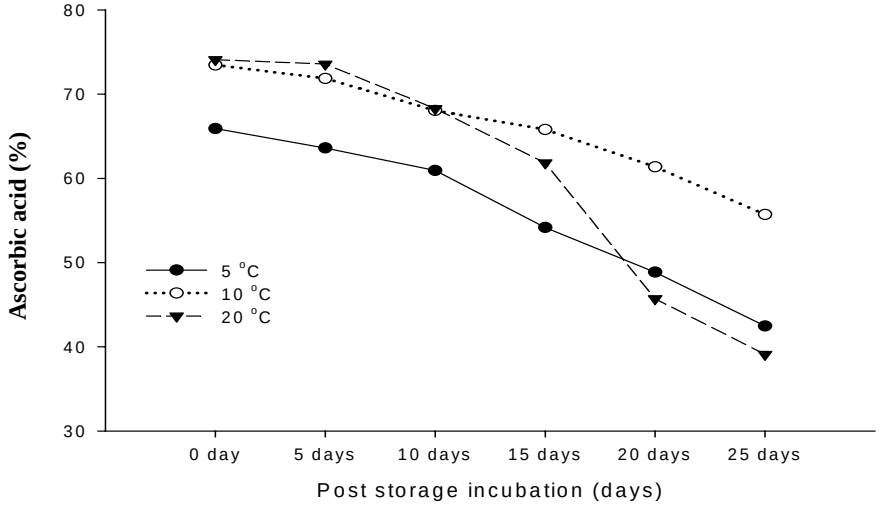

Fig. 2. Interactive effects of storage temperature and post storage incubation on ascorbic acid of citrus fruit.

Ascorbic acid content (%): The Ascorbic acid content of the sweet orange fruit was significantly affected by ST and PSI (Table 1). The ST x PSI interaction or ascorbic acid content was also significant (Fig. 2). Maximum ascorbic acid content of the fruit (66.038%) was recorded in fruits stored at 10°C followed by 5°C (60.41%). Minimum ascorbic acid content of 55.987% was recorded in fruits stored at 20°C. The ascorbic acid content consistently diminished with increase in PSI. It was the highest (71.149%) on day 0 and reached to a minimum value of 45.734% on 25th day of PSI. The interaction value showed that ascorbic acid content decreased with increase in PSI but the rate and amount of decrease was lower in fruits stored at 10^{o}C as compared to other temperatures.

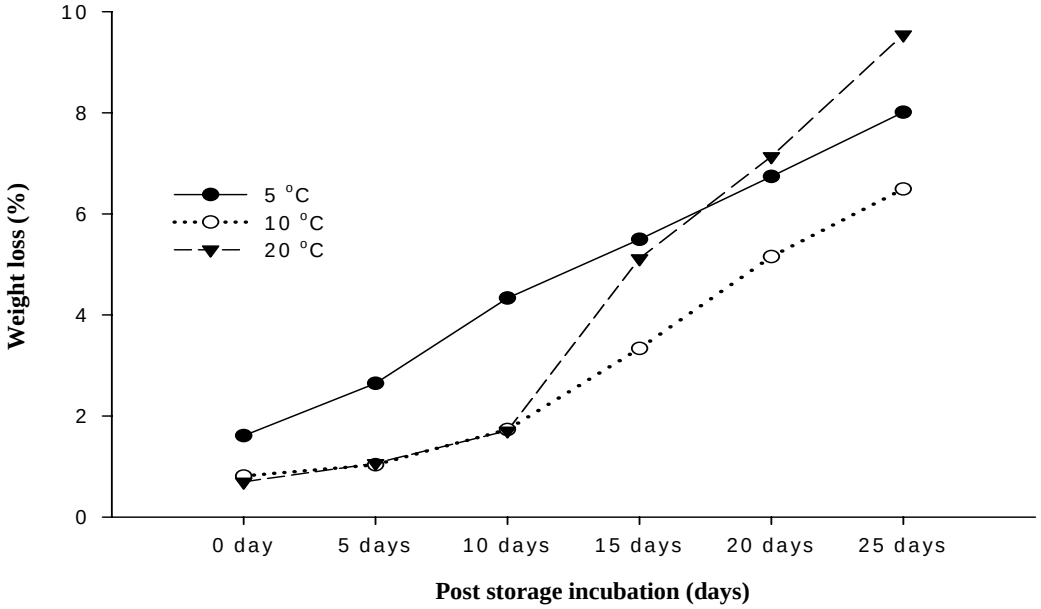

Fig. 3. Interactive effects of storage temperature and post storage incubation on weight loss of citrus fruit.

Discussion

The lower disease incidence for fruits stored at 10°C clearly indicates its effectiveness in decreasing disease incidence during post-storage period in sweet orange fruits. The higher storage temperature of 20°C may have allowed the growth of various pathogens. The higher disease incidence during post-storage incubation in fruits stored at 5°C may be due to chilling injury which may have increased disease susceptibility (Arpaia & Kader, 2009). The fruits developed disease symptoms even during storage with high disease incidence at 20°C (15%) as compared to 5% at 10 or 5°C at day zero of post storage period. This may be due to direct effect of temperature on growth of pathogens (Bulger *et al.,* 1987). Later on, however, fruits stored at 5°C for 45 days and subsequently incubated at ambient temperatures for 25 days showed the highest disease incidence, while it remained steady in fruits exposed to 10°C. It may be due to the fact that pathogens may not be able to develop while the fruits were stored at 5°C but it resulted in increased disease susceptibility after the fruits were shifted to warmer temperature (Porat, *et al*., 2000a; Smilanick, 2003). The low temperature (10°C) did not induce any disease susceptibility and hence the pathogens continued to show least disease incidence even when shifted to ambient temperature after storage (Porat *et al*., 2000 b; Arpaia & Kader, 2009).

Penecillium italicaum and *Penecillium digitatum* are the most common and abundant pathogens on sweet orange fruits (Plaza *et al*., 2003; Snowdon, 1990). These pathogens may infect the fruit either before harvest or during packing and storage (Snowdon, 1990). Since *Penecillium digitatum* is a wound requiring pathogen for the initiation of infection process (Snowdon, 1990) thus, it seems reasonable to assume that the incidence of *Penecillium digitatum* was enhanced by stored at 5°C for 45 days due to chilling injury. Similar results were reported by Lindhout *et al.,* (2004) who found that chilling-injured navel oranges showed rind breakdown, injury to the integrity of oil glands that may ultimately result in enhancing susceptibility to decay. Likewise, the incidence of *Penecillium italicaum* was higher in fruits stored at 5°C at the start of post-storage incubation which could be attributed to its ability to digest the plant cell wall enzymatically (McCollum, 2004).

Another interesting observation regarding both the pathogens is their presence immediately even after storage at 5°C. Though present in small intensity; it indicates their ability to survive at 5°C for 45 days. Furthermore, the incidence of *Penecillium itallicum* at 5°C was 33.3% higher than *Penecillium digitatum* during the first five days of post-storage incubation which indicates that *Penecillium itallicum* incidence was enhanced due to low temperature. Almost all the fruits showed mixed symptoms of both the pathogens during the

later parts of post-storage incubation. A small percentage of fruits also exhibited the symptoms of stem end rot (2%) caused by *Alternaria citri*. There were certain other infections as well which contributed 5% to the total diseased fruits (data not shown).

Weight loss was generally higher at high temperature of 20°C. The rate of weight loss per day for fruits stored at 20°C was 3.196% during post storage period which increased to 3.501and 3.708% per day for fruits stored at 10 and 5°C. Increased weight loss with increasing storage is common in fruits (Al-Obeed & Horhash, 2006) but the increased rate of weight loss in fruits exposed to low temperature may be due to their higher moisture content due low temperature storage and hence having the potential to lose more water but also due to chilling related damage to the rind of such fruits (Lindhout *et al*., 2004).

When the rate of decreases in ascorbic acid content was analyzed as a function of storage temperatures, the influence of storage temperatures became more evident. The fruits stored at 20°C lost ascorbic acid at rate of 0.9376/day while the fruits stored at 10 and 5°C lost ascorbic acid at the rate of 0.7096 and 1.4025 per day, respectively. This very clearly reveals disproportionate and excessive loss of ascorbic acid in sweet orange fruits stored at suboptimal temperatures (5°C). Ascorbic acid content is an important constituent of different fruits (Hussain *et al*., 2010). In citrus and other fruits, ascorbic acid is rapidly lost during storage (Rajwana *et al*., 2010). The retention of ascorbic acid is of major importance in post-harvest handling of citrus fruits. The results indicate that maximum retention can be achieved with storage of fruits at 10°C rather than storage at lower temperature of 5°C or high temperature of 20°C. Kaul & Saini (2000) reported 23.35% decline in ascorbic acid content of lemon juice with 6 months storage at 12-40°C, thus citrus fruits either held at high temperature (20°C) or injured by chilling (5°C) lose ascorbic acid at a faster rate than fruits held at optimum low temperature (10°C).

Conclusion

It is concluded from the results that citrus fruits stored at modest low temperature of 10°C performed better than fruit stored either at 5 or 20°C in terms of disease incidence and fungal prevalence and retention of quality.

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