Leakage Compositional Changes Accompanying Exposure Some Mango Cultivars to Low Temperature under *Vitro* Conditions

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Abstract. Under in *vitro* conditions (controlled temperature), the relationship between low temperature stress and the response of some different mango cultivars was monitored. Some biochemical events that occur following cold exposure of detached leaves of mango trees were detected to evaluate their ability to acquire cold injury during exposure to low temperature (5°C and 10°C degrees). The cultivars of Alphonso, Baladi, Bullock's Heart, Helmand, Hindi- Besennara, Mabrouka, Mestekawy, Nabeeh, Oweisi, Spates, Taimour and Zebda, which grown in A private orchard in Fayoum Governorate, Egypt were selected to verify this aim. This study was carried out during the period from November to March during years; 2012 and 2013. The following results were stated: the detected leakage compositional changes were significantly differed among the tested cultivars and sampling times. In this respect, electrolytes (%), Na⁺, K⁺, inorganic phosphate (Pi), Ca²⁺, total soluble sugars (TSS) and total free amino acids (TFAA) concentrations were detected in leachate of detached fresh leaves and showed significant differences in response to the cultivars and sampling times. However, in this study, Alphonso, Bullock's Heart, Helmand, Taimour and Zebda cultivars of mango had the best result in their cold tolerance under the conditions of this study, which is not exactly consistent with what the researchers found.

Introduction

Mango (*Mangifera indica* L.) is a popular, nutrient-rich tropical fruit, which is now one of the most important fruits crops in tropical and subtropical areas of the world (Mittra and Baldwin, 1997). Mango occupies third place in total world production of major tropical fruit crops after citrus and banana (Alonso and Blaikie, 2003). Environmental conditions outside the traditional areas for optimum growth of mango may impose stresses, which can result in physiological changes, reduced growth, or even permanent damage to the trees (Schaffer et al., 1994). Low temperature is acknowledged to be one of the most dominant environmental stresses that affect the growth, productivity and geographical distribution of crops and horticultural plants (Boyer, 1982). Exposure to low, nonfreezing temperatures induces genetic, morphological, metabolic and physiological changes in plants, which result in the development of cold hardiness and acquisition of freezing tolerance (Huner et al., 1993). Mango needs an optimum temperature range of 24-26.7°C and minimum threshold temperature is 10-12°C below this plant shows chilling injury (Allen and Ort, 2001) and show high susceptibility to low temperature (0-15°C). Young trees are damaged by low temperature variability among cultivars is apparent after a cold spell, but precise information on this subject is non-existent (Farooq and Azam, 2002). Electrolyte leakage is widely used to measure chilling damage as well as to quantify species resistance to cold and chilling injury (Mckay, 1992). Such conductivity measurements have also been used to estimate the positive permeability of membranes to electrolytes (Piotrowska and Kacperska, 1987). Sugars and some of the cold-induced proteins are suspected to have cryoprotective effects; they stabilize proteins and membranes during dehydration induced by low temperature. (Taiz and Zeiger, 1998). In cold tolerant tissues, the protective function of sugars has been ascribed to their ability to stabilize membranes and proteins during cold (freeze) induced dehydration (Minorsky, 2003). A Little is known about seasonal

changes in soluble carbohydrates in both of xylem and pith tissues, including whether the type and or concentration of specific sugars differ depending on how the tissues are adapted to cooling or freezing (Kasuga et al., 2007). It has been reported that under natural conditions, soluble sugars increase during the onset of winter when plants are subjected to low temperatures; conversely, soluble sugars decrease in spring when plants are deacclimating (Siminovich, 1981). Chilling of plants leads to an increase in inorganic P content and a drop in the proportion of organic P (Zia et al., 1994) which is a consequence of a breach of phosphorylation and enhanced decomposition of organic P compounds. Calcium is considered to be an important factor for the maintenance of cell membrane integrity and the regulation of ion transport. Ca^{2+} ions are essential for K^+ vs. Na^+ ion selectivity and membrane integrity (Hanson, 1984). Cold stress induces transient Ca^{2+} influx into the cytoplasm. Therefore, calcium permeable channels responsible for this Ca^{2+} influx are considered as sensors for low temperature (Monroy and Dindsa, 1995). They suggested that Na^+ ions acted by displacing Ca^{2+} ions from membranes, leading to increased membrane permeability and higher intracellular Na^+ ions concentrations. Cytoplasmic calcium levels increase rapidly in response to low temperature, largely due to an influx of calcium from extracellular stores (Polisensky and Braam, 1996). Solutes (Ca^{2+} , Na^+ , K^+ ions) leak from the leaves of chill sensitive, but not from those of chill resistant. Loss of solutes to water reflects damage to the plasma membrane and possibly also the tonoplast (Taiz and Zeiger, 1998). The effects of the high K^+ content of the cell in increasing frost tolerance have also been related to regulation of osmotic and water potential of the cell sap and reduction of electrolyte leakage caused by chilling temperature (Singer et al., 1996). In response to cold and other osmotic stresses, plants accumulate a range of compatible (Osmolytes) solutes including soluble sugars, amino acids (Ruelland and Zachowski, 2010). The metabolism of nitrogenous compounds is also responsive to low-temperature stress (Usadel et al. 2008), in particular that of certain amino acids and polyamine compounds (Davey et al. 2009). Factors implicated in cold acclimation include expression of cold-stress proteins (Hughes and Dunn, 1996), accumulation of sugars, particularly sucrose (Strand et al., 2003), other cryoprotectants, such as proline (Bravo et al., 2001). Changes in water soluble carbohydrates (Leborgne et al., 1995), or in free amino acids, especially proline (Xin and Browse 1998), are associated with cold acclimation and acquisition of cold tolerance. The positive correlation between the accumulation of endogenous proline and improved cold tolerance has been found mostly in low temperature-insensitive plants such as barley, grapevine and potato (Kaur et al., 2011).

Thus, check some putative biochemical events that occur following cold exposure and Evaluation of some mango cultivars (grown under Fayoum Governorate conditions) according to resistance to these events were aimed.

Materials and Methods

In a private orchard, Fayoum, Egypt, located at $29^{\circ} 22' \text{N}$ and $30^{\circ} 47' \text{E}$. and in the laboratory of Agricultural Botany Dept., Faculty of Agric., Fayoum University. A trial was conducted during 2012 and 2013. The experiment assess to evaluate the cold tolerance of some mango cultivars grown in the orchard and check the leaf compositional changes that occur following cold exposure.

Climate of experiment site.

At the site of experiment, temperatures fluctuated during the duration of study and five years before the beginning of the study. In this respect, the mean monthly maximum temperature (T_{Max}) ranging from about 17.7°C in January 2008 to about 40.2°C in August 2010 and the fluctuation in mean monthly minimum temperature (T_{Min}) was ranging from about 5.7°C in January 2007/2008 to about 25.2°C in August 2012. An extreme minimum temperature of 0.6°C was recorded in January 2008; however, there were large differences between the selected years (Table 1)*.

Plant materials.

The plant materials comprised 12 mango cultivars which collected from their natural growing location in the period of November 2012 to march 2013. This is approximately the time at which

the cold hardiness may be occurring. Mango trees were about 25 years old, planted in a clay soil at 5x5 m apart. The cultivars were used in this study including most of the popular cultivars which grown in Fayoum Governorate. The tested cultivars were, Alphonso, Baladi, Bullock's Heart, Helmand, Hindi-Besennara, Mabrouka, Mestekawy, Nabeeh, Oweisi, Spates, Taimour, and Zebda.

(Table 1): The Monthly mean of maximum and minimum temperatures (open air-temperature) during the period from January 2006 to March 2013

Month Year	Temp.	2006	2007	2008	2009	2010	2011	2012	2013
January	Max.	19.4	19.4	17.7	20.7	21.9	20.7	20.4	23.0
	Min.	6.6	5.7	5.7	6.7	7.6	8.0	7.2	10.3
February	Max.	22.2	21.4	20.0	22.3	24.4	22.0	22.4	24.3
	Min.	8.4	7.8	6.5	6.4	8.2	9.4	8.2	10.4
March	Max.	26.3	25.0	28.6	23.2	27.5	25.6	25.4	29.0
	Min.	9.7	9.8	11.6	7.9	11.4	9.5	11.8	12.6
April	Max.	30.4	28.6	31.6	30.8	31.8	28.5	29.1	
	Min.	13.3	12.5	13.7	12.5	14.3	13.7	13.6	
May	Max.	33.4	35.2	35.4	32.8	34.1	32.8	34.1	
	Min.	16.9	17.7	18.2	16.7	16.7	17.4	18.3	
June	Max.	36.5	39.3	39.4	38.2	38.4	35.7	38.4	
	Min.	20.0	20.7	22.0	20.4	21.4	20.6	21.8	
July	Max.	37.4	38.9	37.7	38.5	36.3	38.7	39.8	
	Min.	21.3	21.8	22.1	22.7	22.4	21.5	23.6	
August	Max.	38.3	37.8	38.6	37.0	40.2	38.6	38.0	
	Min.	22.1	21.7	22.2	21.8	24.5	22.9	25.2	
September	Max.	34.8	34.3	35.9	35.2	36.2	36.1	35.2	
	Min.	20.3	20.5	20.0	20.7	21.9	22.1	23.4	
October	Max.	31.0	32.1	31.5	31.7	35.9	30.2	34.4	
	Min.	17.2	18.3	17.2	18.1	21.3	17.7	22.2	
November	Max.	23.6	26.7	26.6	25.0	31.3	26.5	27.9	
	Min.	11.1	12.8	13.1	11.7	16.5	12.6	16.4	
December	Max.	19.9	21.3	22.2	22.4	24.1	22.0	21.8	
	Min.	7.5	8.2	9.1	8.9	10.3	9.7	11.0	

* Data applied by Bureau of Meteorology, Agriculture Ademonstration, Fayoum Governorate, Egypt.

Thirty-six trees of similar phenotype in the field (size, vigor...etc.) and with management prehistory were selected for this experiment (no further tests were carried out confirm genetic uniformity). All selected trees were allocated at random with each replicate. The trees were derived from seedlings; named Baladi, while the others they had been grafted onto seedling rootstocks. Studying cold tolerance in the field is difficult. Fields sites often exhibit either complete survival or complete winter kill. Because of this variability, the laboratory procedures to measure cold tolerance have been developed by a number of investigators (**Ghassemi-Golezani et al., 2008**), **Rapacz (2002)** and **Rife and Zeinali (2003)**. The results have usually been in a good agreement with field observations of natural cold injury (**Pellett et al., 1981**).

At 30th of November, the mature fully expanded leaves of each cultivar were collected, cleaned, washed and towed dry then putted in a vinyl plastic bags and subjected to various low temperature treatments.

In this respect, the collected leaves from each cultivar were divided in two batches; each one was composed of 10 leaves in three replicates. These batches were stored in a refrigerator at 5°C and 10°C in darkness and ten leaves were taken from each batch (three replicates) per cultivar every 2 days up to 10 days. Samples from these leaves were selected for the study of leakage

compositional changes. All treatments were applied in a factorial randomized complete block design with three replicates.

Electrolytes leakage (%) estimation.

The total leakage of inorganic ions was estimated (Lutts et al., 1996). Fresh leaf samples were cut into equal sized pieces (1g replicate^{-1}). Each sample was placed in boiling glass bottle (100 ml brown glass bottle) containing 50 ml deionized water. Bottles were then incubated at 25°C in an incubator for 24 hours, and then the electrical conductivity of the solution was recorded using a conductivity meter EC1 (Mod: HI99300). The bottles were then boiled at 100°C for 7 min, and re-incubated at 25°C in an incubator for 24 hours, and then the electrical conductivity of the solution was recorded EC₂. Electrolyte leakage (EL%) was calculated using the following formula:

$$\text{EL (\%)} = (\text{EC}_1 / \text{EC}_2) \times 100$$

Total soluble sugars determination (TSS).

Soluble sugars in leakage ($\text{mg } 100\text{g}^{-1}\text{ FW}$) were determined based on phenolsulfuric acid method by (Dubois et al., 1956). A glucose was used to standardize the procedure for quantifying sample values.

Total free amino acids determination (TFAA).

Total free amino acids in leakage ($\text{mg } 100\text{g}^{-1}\text{ FW}$) were determined calorimetrically according to (Jayarman, 1981). To determine the content of total free amino acids in each fresh leaf sample, a standard curve was done based on arginine.

Elements determination.

In leakage, Na^+ and K^+ concentration (PPM) was determined by using flamephotometer (Gallenkamp CO., England) (Page et al., 1982).

Pi concentraion ($\text{mg } 100^{-1}\text{g FW}$) was colorimetrically estimated by using chlorimetrically estimated by using chlorostonnous molybdophospheric blue colour method in sulfuric acid system as described by Jackson (1967).

Calcium determination.

A titration with ethylene diamine tetra acetate (EDTA) method is that of Diehl et al. (1950), was used for calcium determination in leakage ($\text{mg } 100^{-1}\text{g FW}$) as described by the U.S. salinity Laboratory (1954), U.S.D.A. Handbook 60.

Statistical analysis.

All data were subjected to analysis of variance (ANOVA) using the statistical software package of Genstat (version 11) (VSN International Ltd., Oxford, UK). Means comparison among the different treatments were performed using the least significant differences procedure (LSD) at the $P \leq 0.05$ level as illustrated by Snedecor and Cochran (1980).

Results

The results of one season were discussed because no significant differences were observed between results of the studied seasons.

Electrolytes leakage (%).

The present results in Table (2) and Fig. (1) exhibit that an increase in electrolytes leakage (%) from the leaves of the most cultivars under cold storage condition reached its maximum level by the cultivar of Mestekawy and the minimum level by the cultivar of Zebda at the temperature of 5°C . at storage of leaves at 10°C , electrolytes leakage (%) level showed a maximum value by the cultivar of Hindy-Besennara and the minimal was recorded by Ewais cultivar. In this respect, the electrolytes leakage (%) increased abruptly from 10°C to 5°C and reached a plateau at 5°C in the most of the tested cultivars. When different mango cultivars were exposed to low temperature either 5°C or 10°C , no clear trend in electrolytes leakage (%) was detected between the intervals of the cold storage for the leaves.

Leakage compositional changes.

Inorganic solutes.

Sodium (Na^+).

Data in Table (3) and Fig. (2) for the leakage of Na^+ from the stored leaves into imbibition medium as affected by cold storage at 5°C and 10°C indicate that the amount of leached Na^+ from the stored leaves at 5°C was a maximal value from leaves of Spates cultivar than those of the others. However, the lowest value was given by the leaves of Ewais and Zebda cultivars. At 10°C, the greatest amount of leaked Na^+ was exhibited by the cultivar of Taimour while, the lowest one was recorded by Ewais cultivar. Moreover, the level of Na^+ efflux was significantly differed among the studied cultivars. However, the trend of leaked Na^+ at 5°C showed a similar behavior the cultivars stored at 10°C along the ten days of storage. Since, Na^+ impedance of leaves tissue has been found to increase as the period of storage is proceeded in the most of tested cultivars. At the same time, the means of leaked Na^+ from the stored leaves at 10°C were the highest as comparing to those at 5°C.

Potassium (K^+).

Relative leakage of K^+ from the stored leaves for the tested cultivars under the conditions of cold storage (5°C and 10°C) is shown in Table (4) and Fig. (3). It is obvious that the amount of leached K^+ from the stored leaves at 5°C was a maximal level by the cultivar of spates and the minimal one was recorded by Mestekawy cultivar. Also, it is clear that the maximum leakage of K^+ was obtained by the cultivar of Balady and minimum value by Taimour one from the stored leaves at 10°C. However, the leaked K^+ level was significantly differed among the studied cultivars. In addition, it is noticed that the efflux of K^+ was increased as the period of exposure to low temperature was lengthened in the most of tested cultivars under the temperature of 5°C and 10°C. Comparatively, the means of K^+ efflux from the stored leaves under 10°C were the higher in all the studied cultivars as compared to those stored at 5°C.

Inorganic phosphorus (P_i).

Results presented in Table (5) and Fig. (4) show a relative P_i leakage from stored leaves of the different cultivars of mango. It is clear that the amount of leached P_i reached to its maximal level by Mestekawy cultivar but the minimal one was recorded by the cultivars of Helmand and Hindy-Besennara at the temperature of 5°C. While, at the temperature of 10°C the highest value of leaked P_i was exhibited by the cultivar of Mestekawy and the lowest value was obtained by Helmand cultivar. However, leaked P_i values were significantly differed among the tested cultivars during the all storage times. In addition, an increase in the most of leaked P_i had developed in response to the time of leaves storage at the degree of 5°C and 10°C as compared to the control (Zero time). Moreover, increase in the most of leaked P_i for the entire storage period from the leaves of the tested cultivars at 5°C and 10°C was recorded and the means of leaked P_i as affected by the storage of leaves at 10°C was a higher than those stored at C except Taimour cultivar leaves.

Calcium (Ca^{2+}).

Regarding the changes of leaked Ca^{2+} from stored leaves for the different cultivars, data presented in Table (6) and Fig. (5) show that the levels of leaked Ca^{2+} from the stored leaves at 5°C reached its peaked by the cultivar of Ewais and the lowest one was recorded by the cultivar of Mestekawy. The results obtained here also show that the cultivar of Balady exhibited the greater value of leaked Ca^{2+} from the leaves and the lower was obtained by Taimour cultivar as the leaves stored at 10°C. In general, the level of leaked Ca^{2+} was significantly differed among the studied cultivars. Moreover, when different mango cultivars were exposed to low temperature either 5°C or 10°C, no clear trend was detected between the intervals of the cold storage for the leaves.

Organic solutes.

Total soluble sugars (TSS).

Data in Table (7) and Fig. (6) show the effect of exposed mango leaves to low temperature degrees on TSS in leachates of stored leaves. From the mentioned results in this Table, it could be stated that the effect of low temperature was mainly differed according to the cultivar. In this respect, the cultivar of Balady recorded the highest value of TSS in leachates of stored leaves and

the lowest one was given by the cultivar of Mabrouka when the leaves stored at 5°C. TSS reached its peak level by the cultivar of Zebda and the lowest one was recorded by Helmand cultivar at the temperature of 10°C. In general, the level of TSS in leachates of leaves was significantly differed among the studied cultivars. No a clear pattern in TSS changes along the periods of storage was found between the tested intervals.

Total free amino acids (TFAA).

As it can be seen from the data in Table (8) and Fig. (7) that, in the leachates of stored leaves, there is an increase in TFAA leakage to reach a maximal level by the cultivar of Mabrouka and a minimal level was recorded by the cultivar of Mestekawy at the temperature of 5°C. The amount of leached TFAA from stored leaves into the imbibing medium recorded the highest value by the cultivar of Bullock's Heart and the lowest was given by Hindy-Besennara at the temperature of 10°C. However, the level of leached TFAA was significantly differed among the tested cultivars. The changes in TFAA along the period of storage have not any trend between the times of storage for all the tested cultivars.

Discussion

Chilling temperatures (lower than 15°C) lead to numerous physiological disturbances in the cells of chilling-sensitive plant and result in chilling injury and death of tropical and subtropical (Lukatkin et al., 2012). Obviously, the problem of plant resistance to chilling temperature, which often occurs in spring and autumn in many countries, is important for practical plant breeding. A change in the Egyptian climate has already been observed during the last decades, and this is expected to continue throughout this century. This change in climate affects the behavior of fruit trees and their productivity. In 2008, Egypt was exposed to a wave of frost that caused severe damage to mango trees; Hence, there is a need to know which cultivars are resistant to winter cold (Ismail, 2014).

As a result of this sensitivity, low temperature produce a range of physiological and metabolic disorders that lead to serious losses. The various dysfunctions that arise under low temperature conditions result in various physical and metabolic changes that are easily scored and which can therefore be used to assess the degree of chilling injury. Thus, the objective of this trail is to study the some of physiological changes as indicators for determine index of some mango cultivars and to know which of them are resistant to chilling temperatures. In this respect, the following physiological indices were detected. Electrolytes leakage is widely used to measure chilling damage as well as to quantity species resistance to cold and chilling injury in conifer (Mckay, 1992) and in apple (Suwapanich and Haesungcharoen, 2006).

Low temperature alters the physical properties of cell membranes. Chilling of sensitive plants leads to multiple changes in their membranes, which enhance permeable properties of cell membrane (Lukatkin et al., 2012). These changes in the state of membranes may lead to secondary of irreversible reactions, depending on temperature, exposure duration and sensitivity of the species (or cultivars). After a prolonged chilling, these changes will cause loss of membrane integrity and compartmentation, the leakage of solutes, decrease of oxidative activity of mitochondria, increase of the activation energy of membrane-bond enzymes including H⁺-ATPase, reduce the rate of photosynthesis, cause disruption and imbalance of metabolism, the accumulation of toxic substances and the symptoms of chilling injury (Kasamo et al., 2000). In addition, A number of species of tropical have the lateral phase separation temperature some higher (15°C) than in plants from temperate zones (6-8°C) suggesting that plants reduce the freezing point of membranes with the distance from zone of tropical origin (Terzaghi et al., 1989). However, the increased leakage of electrolytes from the cultivar of Hindy-Besennara (Table 2) was interpreted as resulting from deteriorative changes in membranes and corresponds to the presence of released inorganic and organic ions. Similar results were obtained by Chinnusamy et al. (2007). Whereas ion uptake in plants receives considerable attention, the release of solutes from the plant into the environment is less investigated. Considerable amounts of solutes are lost after cell damage and cell death. In this

respect, the basic assumption is that the greater the injury of the living tissue, the greater the efflux of ions from stressed cells (Palta et al., 1977). A maximal level of Na^+ leakage was observed by the cultivar of Hindy-Besennara (Table 3). The differences in the rate of Na^+ efflux among these leaves of different cultivars reflect actual differences in membrane permeability and have protective effect on membrane damage. Thus, may be attributed to the cultivars had lower Na^+ content in the tissues resulted in lesser damage to the cells. These results are in accordance with those of Taiz and Zeiger (1998) who found that Na^+ ions leak from the leaves of chill sensitive more than those of chill resistant. Loss of Na^+ to water reflects damage to the plasma membrane and possibly also the tonoplast. The leakage of K^+ from the leaves into the imbibing medium was differed between the tested cultivars under the condition of field. The highest rate of K^+ efflux was observed at the leaves of Helmand (Table 3) this, reflects the impairment of membrane under environmental stress. It is also clear from data in Table (4) that, the amount of leaked K^+ was similar to that of leaked Na^+ at field conditions. Thus, may be attributed to the cultivars had lower K^+ content in the tissues resulted in lesser damage to the cells. Similar results had found by (Taiz and Zeiger, 1998). Relative Pi leakage as a function of the conditions of field recorded the highest values of leached Pi from leaves of Bullock's heart (Table 5). This increased leakage with these cultivars suggests a marked damage to the membrane which limits leakage from tissues. Also, the origin of Pi could be from decomposition of different phosphate-containing metabolites and structural components by various phosphatases and subsequent leaching of Pi under cold stress. These results are in a good agreement with those reported by Taiz and Zeiger (1998). Leaf electrolytes values of Ca^{2+} in Table (6) indicate that Nabeeh cultivar are more resistant to leakage of Ca^{2+} than the others. Increased resistance to cold stress in these cultivars may occur as a result of the role of Ca^{2+} in conforming cold resistance (Percival et al., 1999). Ca^{2+} is involved in the control and maintenance of physiological plant responses to chilling injury such as the maintenance of membrane integrity and transport function (Palta, 1996). Additionally, cold stress induces transient Ca^{2+} permeable channels for this Ca^{2+} influx are considered as sensors for low temperature (Monroy and Dindsa, 1995). The activation of Ca^{2+} channels by cold is thought to the result of physical alterations in cellular structure. Previous studies had the same trends (Catala et al., 2003). Differential rates of total soluble sugars (TSS) leakage had developed in response to the conditions of field (Table 7). Maximum leakage of TSS was obtained by from the leaves of Ewais. While, the lowest values were recorded by the cultivar of Nabeeh. This trend reflects the membrane dysfunction with chilling sensitive cultivars to the conditions of study more than the tolerant ones. It has been reported that under natural conditions, TSS increase during the onset of winter when plants are subjected to low temperatures; conversely, decrease in spring when plants are deacclimating (Sauter et al., 1996). These results are similar to those obtained by Lennartsson and Ögren (2004). Relative leakage of total free amino acids (TFAA) from the collected leaves from the field explained that the loss of TFAA in the leakage of the cultivar of Zebda more than others (Table 8) may be associated with loss of membrane integrity. This result is in good agreement with that obtained by Ghosh et al. (1981).

(Table 2): Electrolytes leakage (%) in leachate of mango leaves after exposure to low temperature degrees (5°C and 10°C) storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	44.00	69.15	92.73	83.82	56.38	68.09	74.03	59.42	80.00	73.77	50.46	52.71	63.27
Balady	49.00	68.45	90.86	81.17	56.76	67.13	72.88	60.30	81.06	68.09	74.26	67.08	70.16
Bullock's Heart	82.00	83.52	75.91	73.45	66.62	61.69	72.24	74.82	75.63	51.65	47.59	48.57	59.65
Helmand	46.00	66.32	49.99	66.06	44.96	48.86	55.24	53.63	65.99	51.78	44.37	45.24	52.20
Hindy Besennara	47.00	73.09	90.60	81.34	49.54	64.00	71.71	78.29	75.54	80.30	61.80	76.81	74.55
Mabrouka	57.00	75.78	61.78	77.27	38.65	47.96	60.29	89.98	70.51	51.33	67.64	78.56	71.60
Mestekawy	63.00	77.87	92.15	84.38	73.46	70.75	79.72	59.26	59.66	56.47	59.94	52.85	57.64
Nabeeh	33.00	56.43	37.38	67.06	55.86	55.97	54.54	51.94	44.88	46.05	50.68	43.92	47.50
Ewais	67.00	84.77	83.21	79.84	69.88	53.91	74.32	58.00	64.87	53.79	37.51	37.59	50.35
Spates	74.00	79.65	83.32	81.68	54.74	43.43	68.57	73.91	65.09	58.33	50.14	61.02	61.70
Taimour	47.00	68.45	68.67	58.64	56.50	52.32	60.92	51.52	75.17	65.07	55.94	69.14	63.37
Zebda	50.00	54.41	50.52	63.44	51.83	49.81	54.00	72.54	68.01	55.15	61.95	57.31	62.99
Mean	55.00	71.49	73.09	74.85	56.26	56.99		65.3	68.87	59.32	55.19	57.57	
LSD (5%)	Cultivars (A)								4.28				
	Temprature (C°) (B)								1.75				
	Storage period (day) (C)								2.76				
	(AxB)								6.05				
	(BxC)								9.57				
	(AxC)								3.91				
	(AxBxC)								13.54				

(Table 3): Concentration of Na⁺ (ppm) in leachate of mango leaves after exposure to low temperature degrees (5°C and 10°C) storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	3.00	4.25	2.34	4.21	5.61	6.23	4.53	3.88	3.74	4.21	8.41	5.76	5.20
Balady	3.00	2.99	3.74	3.74	4.44	9.58	4.90	3.29	5.61	4.28	10.05	8.18	6.28
Bullock's Heart	4.00	4.21	3.50	3.82	3.82	8.41	4.75	3.97	4.44	4.44	7.94	8.18	5.79
Helmand	4.00	3.35	4.91	4.44	5.84	8.18	5.34	3.97	5.61	4.44	8.57	8.57	6.23
Hindy Besennara	5.00	3.50	5.14	4.67	4.67	8.10	5.22	4.21	2.57	4.28	5.61	8.88	5.11
Mabrouka	4.00	3.46	3.74	4.05	4.05	8.41	4.74	3.74	5.61	5.14	7.94	7.94	6.07
Mestekawy	4.00	3.36	1.17	4.21	4.67	7.94	4.27	3.50	3.27	5.61	7.01	8.57	5.59
Nabeeh	4.00	3.50	4.91	4.21	5.61	8.88	5.42	2.80	3.74	5.14	9.11	8.18	5.79
Ewais	3.00	2.80	3.27	4.21	4.67	6.78	4.35	3.27	4.21	4.91	4.28	7.48	4.83
Spates	3.00	3.64	5.61	4.67	5.61	8.88	5.68	2.48	4.44	4.67	8.88	9.50	5.99
Taimour	4.00	3.79	4.21	4.21	5.37	8.41	5.20	3.41	4.21	5.38	6.70	8.88	5.72
Zebda	3.00	3.74	3.74	4.21	3.04	7.01	4.35	3.74	4.21	4.67	5.14	6.54	4.86
Mean	4.00	3.55	3.86	4.22	4.78	8.07		4.77	4.30	4.76	7.47	8.05	
LSD (5%)	Cultivars (A)								1.15				
	Temprature (C°) (B)								0.47				
	Storage period (day) (C)								0.74				
	(AxB)								1.62				
	(BxC)								2.57				
	(AxC)								1.05				
	(AxBxC)								3.63				

(Table 4): Concentration of K⁺ (ppm) in leachate of mango leaves after exposure to low temperature degrees (5°C and 10°C) storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	4.68	8.13	4.47	8.04	10.72	11.91	8.65	7.42	7.15	8.04	16.08	11.02	9.94
Balady	4.32	5.72	7.15	7.15	8.49	18.32	9.36	6.28	10.72	8.19	19.21	15.63	12.01
Bullock's Heart	5.95	8.04	6.70	7.30	7.30	16.08	9.08	7.59	8.49	8.49	15.19	15.63	11.08
Helmand	6.29	6.40	9.38	8.49	11.17	15.63	10.21	7.59	10.72	8.49	16.38	16.38	11.91
Hindy Besennara	6.72	6.70	7.15	8.93	8.93	15.49	9.98	8.04	4.91	8.19	10.72	16.97	9.77
Mabrouka	6.40	6.61	2.23	7.74	7.74	16.08	9.07	7.15	10.72	9.83	15.19	15.19	11.61
Mestekawy	5.47	6.43	2.23	8.04	8.93	15.19	8.17	6.70	6.25	10.72	13.40	16.38	10.69
Nabeeh	6.25	6.70	9.38	8.04	10.72	16.97	10.36	5.36	7.15	9.83	17.42	15.63	11.08
Ewais	3.90	5.36	6.25	8.04	8.93	12.95	8.31	6.25	8.04	9.38	8.19	14.29	9.23
Spates	4.11	6.97	10.72	8.93	10.72	16.97	10.86	4.74	8.49	8.93	16.97	18.17	11.46
Taimour	5.70	7.24	8.04	8.04	10.27	16.08	9.93	3.52	8.04	10.27	12.81	16.97	10.32
Zebda	4.92	7.15	7.15	8.04	5.81	13.40	8.31	7.15	8.04	8.93	9.83	12.51	9.29
Mean	5.38	6.79	7.37	8.07	9.15	15.42		9.12	8.23	9.11	14.28	15.40	
LSD (5%)	Cultivars (A)							1.19					
	Temprature (C°) (B)							0.69					
	Storage period (day) (C)							0.82					
	(AxB)							1.92					
	(BxC)							3.01					
	(AxC)							2.02					
	(AxBxC)							3.83					

(Table 5): Inorganic phosphorus concentration (mg 100g⁻¹ FW) in leachate of mango leaves after exposure to low temperature degrees (5°C and 10°C) storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	4.52	4.88	5.24	5.71	6.31	6.90	5.81	5.35	5.83	6.43	7.14	7.50	6.45
Balady	5.47	6.66	6.90	7.50	7.73	7.50	7.26	6.54	7.38	8.09	7.73	9.04	7.76
Bullock's Heart	6.54	6.78	7.26	7.62	8.21	9.28	7.83	6.90	8.33	8.57	8.69	8.81	8.26
Helmand	4.64	4.88	5.00	5.24	5.47	5.71	5.26	4.88	5.24	5.71	6.43	6.66	5.78
Hindy Besennara	6.43	4.88	5.12	5.35	5.00	5.95	5.26	6.07	6.43	7.14	7.62	7.97	7.04
Mabrouka	5.35	5.35	5.59	5.95	6.90	7.38	6.24	5.71	7.02	7.14	7.73	9.28	7.38
Mestekawy	8.21	8.57	11.90	12.02	12.49	12.85	11.57	10.59	10.23	10.83	12.26	14.04	11.59
Nabeeh	7.38	7.62	7.85	7.73	8.09	8.57	7.97	7.50	8.33	9.04	9.16	9.28	8.66
Ewais	7.50	6.90	7.02	8.69	7.73	8.09	7.69	7.14	7.50	7.97	8.45	7.85	7.78
Spates	6.31	6.78	6.66	7.14	7.26	7.73	7.12	6.43	7.38	8.81	9.04	9.28	8.19
Taimour	5.71	6.07	6.54	7.38	9.16	9.64	7.76	6.43	6.78	7.85	8.21	9.28	7.71
Zebda	6.90	6.78	8.09	8.69	7.97	7.62	7.83	7.02	8.09	7.14	9.52	10.00	8.35
Mean	6.25	6.35	6.93	7.42	7.70	8.10		6.71	7.38	7.89	8.50	9.08	
LSD (5%)	Cultivars (A)							0.58					
	Temprature (C°) (B)							0.24					
	Storage period (day) (C)							0.37					
	(AxB)							0.82					
	(BxC)							1.29					
	(AxC)							0.53					
	(AxBxC)							1.83					

(Table 6): Concentration of Ca^{2+} (mg g⁻¹ FW) in leachate of mango leaves after exposure to low temperature degrees (5°C and 10°C) storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	3.33	1.67	2.67	5.00	5.33	4.67	3.87	2.67	2.33	3.00	4.33	4.67	3.40
Balady	8.00	6.00	3.67	2.33	7.33	1.67	4.20	2.67	7.00	5.67	3.00	4.00	4.47
Bullock's Heart	4.00	2.33	1.67	3.33	4.00	3.00	2.87	5.67	1.67	2.00	4.33	3.67	3.47
Helmand	5.00	5.00	2.67	2.33	6.00	4.33	4.07	2.67	2.00	2.67	4.33	2.00	2.73
Hindy Besennara	5.00	4.67	1.33	2.00	3.67	3.33	3.00	2.67	1.33	7.00	3.67	3.33	3.60
Mabrouka	3.33	2.67	5.67	2.00	3.67	2.67	3.33	1.33	1.33	6.00	1.67	4.67	3.00
Mestekawy	1.67	1.67	3.33	2.00	3.67	3.00	2.73	1.67	1.00	2.33	5.00	3.67	2.73
Nabeeh	2.33	5.00	2.00	1.67	3.33	2.67	2.93	2.67	3.00	3.00	2.33	2.33	2.67
Ewais	6.67	6.33	2.00	3.33	6.33	4.67	4.53	6.67	1.33	3.33	5.67	5.00	4.40
Spates	7.67	4.00	1.67	1.33	3.67	3.67	2.87	6.33	3.67	3.00	3.00	3.67	3.93
Taimour	2.67	5.33	5.00	1.67	5.00	1.33	3.67	1.33	2.00	2.67	1.67	3.33	2.20
Zebda	5.67	2.67	4.67	1.67	6.33	4.33	3.93	7.00	2.67	2.33	3.00	3.67	3.73
Mean	4.61	3.94	3.03	2.39	4.86	3.28		3.61	2.44	3.58	3.50	3.67	
LSD (5%)	Cultivars (A)								0.95				
	Temprature (C°) (B)								0.39				
	Storage period (day) (C)								0.61				
	(AxB)								1.35				
	(BxC)								2.13				
	(AxC)								0.87				
	(AxBxC)								3.0				

(Table 7): Total soluble sugars concentration (mg 100 g⁻¹ FW) in leachate of mango leaves after exposure to low temperature degrees (5°C and 10°C) storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	16.64	18.37	23.57	21.84	30.16	25.65	23.92	18.03	21.15	18.72	25.31	32.24	23.09
Balady	18.72	19.41	19.76	19.76	42.64	45.07	29.33	19.41	25.65	16.64	20.80	44.03	25.31
Bullock's Heart	16.99	18.72	21.84	24.27	20.80	16.64	20.45	21.15	24.27	22.53	35.71	34.32	27.59
Helmand	14.91	19.07	21.49	18.72	19.41	27.04	21.15	15.95	18.37	21.49	14.91	14.91	17.13
Hindy Besennara	13.52	17.68	18.72	22.19	19.41	42.64	24.13	19.41	25.31	32.93	27.39	28.77	26.76
Mabrouka	14.56	14.56	15.25	17.33	20.45	15.60	16.64	14.21	18.37	19.07	17.33	17.33	17.26
Mestekawy	19.41	23.23	23.23	21.84	25.65	26.69	24.13	15.60	17.33	40.21	16.29	24.61	22.81
Nabeeh	15.25	18.37	19.07	23.23	15.60	15.60	18.37	17.68	30.16	15.60	20.45	21.84	21.15
Ewais	18.37	20.45	20.45	25.31	28.08	39.17	26.69	18.03	29.47	16.29	25.65	30.51	23.99
Spates	20.45	19.41	20.45	19.76	26.35	18.72	20.94	24.61	27.04	25.31	20.80	24.96	24.54
Taimour	11.09	18.03	16.99	19.07	20.45	21.15	19.14	18.72	21.49	16.99	17.33	14.91	17.89
Zebda	16.64	13.87	15.95	15.60	36.40	37.79	23.92	29.47	29.81	34.32	42.99	38.48	35.01
Mean	16.38	18.43	19.73	20.74	25.45	27.65		19.36	24.04	23.34	23.75	27.24	
LSD (5%)	Cultivars (A)								4.19				
	Temprature (C°) (B)								1.71				
	Storage period (day) (C)								2.71				
	(AxB)								5.93				
	(BxC)								9.38				
	(AxC)								3.83				
	(AxBxC)								13.26				

(Table 8): Total free amino acids concentration (mg 100 g⁻¹ FW) in leachate of mango leaves after exposure to low temperature degrees (5°C and 10°C) storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	13.33	28.09	29.52	49.04	34.76	27.62	33.81	26.66	34.28	23.81	15.24	25.24	25.04
Balady	19.05	37.14	17.14	40.95	26.19	15.71	27.43	46.66	36.66	34.28	19.52	23.81	32.19
Bullock's Heart	12.86	37.61	27.14	36.19	35.23	20.00	31.23	51.90	50.95	24.76	16.66	22.38	33.33
Helmand	14.76	30.00	21.90	28.09	24.76	20.95	25.14	30.47	34.28	22.38	32.85	39.04	31.81
Hindy Besennara	20.00	20.47	25.71	30.00	27.62	23.19	25.40	21.43	20.47	18.09	20.95	22.38	20.66
Mabrouka	15.71	34.28	32.85	49.04	31.90	28.57	35.33	46.66	29.04	22.85	17.14	25.24	28.19
Mestekawy	17.62	25.71	20.00	20.00	18.57	18.09	20.47	31.42	30.95	21.43	21.90	21.43	25.43
Nabeeh	16.19	25.24	26.66	26.19	17.14	26.66	24.38	23.33	15.24	20.95	28.09	26.66	22.85
Ewais	13.81	16.66	20.95	24.28	26.66	19.52	21.62	21.43	30.95	18.09	19.52	20.00	22.00
Spates	14.28	28.09	32.85	28.57	33.33	26.19	29.81	23.33	25.24	25.24	21.90	19.05	22.95
Taimour	13.81	35.71	42.38	33.33	21.90	29.04	32.47	43.80	27.14	19.05	14.28	18.57	24.57
Zebda	13.81	35.71	42.38	33.33	21.90	29.04	24.66	24.28	18.09	15.71	19.05	32.85	22.00
Mean	10.95	18.57	18.09	39.04	26.66	20.95		32.62	29.44	22.22	20.59	24.72	
LSD (5%)	Cultivars (A)							3.36					
	Temprature (C°) (B)							1.37					
	Storage period (day) (C)							2.17					
	(AxB)							4.76					
	(BxC)							7.52					
	(AxC)							3.07					
	(AxBxC)							10.64

(Fig. 1): Electrolytes leakage (EL, %) in leachate of mango leaves after exposure to low temperature degrees (5 and 10°C) at various storage periods (2 to 10 days).

Cultivars: 1. Alphonso; 2. Balady; 3. Bullock's Heart; 4. Helmand; 5. Hindy Besennara; 6. Mabrouka; 7. Mestekawy; 8. Nabeeh; 9. Ewais; 10. Spates; 11. Taimour; 12. Zebda. Storage period (day): A= 2days; B=4days; C=6days; D=8days; E=10days; F=mean of all periods

(Fig. 2): Concentration of Na^+ (ppm) in leachate of mango leaves after exposure to low temperature degrees (5 and 10°C) at various storage periods (2 to 10 days).

Cultivars: 1. Alphonso; 2. Balady; 3. Bullock's Heart; 4. Helmand; 5. Hindy Besennara; 6. Mabrouka; 7. Mestekawy; 8. Nabeeh; 9. Ewais; 10. Spates; 11. Taimour; 12. Zebda. Storage period (day): A= 2days; B=4days; C=6days; D=8days; E=10days; F=mean of all periods

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(Fig. 3): Concentration of K^+ (ppm) in leachate of mango leaves after exposure to low temperature degrees (5 and 10°C) at various storage periods (2 to 10 days).

Cultivars: 1. Alphonso; 2. Balady; 3. Bullock's Heart; 4. Helmand; 5. Hindy Besennara; 6. Mabrouka; 7. Mestekawy; 8. Nabeeh; 9. Ewais; 10. Spates; 11. Taimour; 12. Zebda. Storage period (day): A= 2days; B=4days; C=6days; D=8days; E=10days; F=mean of all periods

(Fig. 4): Inorganic phosphorus concentration (P_i , $\text{mg } 100\text{g}^{-1}$ FW) in leachate of mango leaves after exposure to low temperature degrees (5 and 10°C) at various storage periods (2 to 10 days).

Cultivars: 1. Alphonso; 2. Balady; 3. Bullock's Heart; 4. Helmand; 5. Hindy Besennara; 6. Mabrouka; 7. Mestekawy; 8. Nabeeh; 9. Ewais; 10. Spates; 11. Taimour; 12. Zebda. Storage period (day): A= 2days; B=4days; C=6days; D=8days; E=10days; F=mean of all periods

(Fig. 5): Concentration of Ca^{2+} (mg g^{-1} FW) in leachate of mango leaves after exposure to low temperature degrees (5 and 10°C) at various storage periods (2 to 10 days).

Cultivars: 1. Alphonso; 2. Balady; 3. Bullock's Heart; 4. Helmand; 5. Hindy Besennara; 6. Mabrouka; 7. Mestekawy; 8. Nabeeh; 9. Ewais; 10. Spates; 11. Taimour; 12. Zebda. Storage period (day): A= 2days; B=4days; C=6days; D=8days; E=10days; F=mean of all periods

(Fig. 6): Total soluble sugars concentration (TSS, mg 100 g⁻¹ FW) in leachate of mango leaves after exposure to low temperature degrees (5 and 10°C) at various storage periods (2 to 10 days). Cultivars: 1. Alphonso; 2. Balady; 3. Bullock's Heart; 4. Helmand; 5. Hindy Besennara; 6. Mabrouka; 7. Mestekawy; 8. Nabeeh; 9. Ewais; 10. Spates; 11. Taimour; 12. Zebda. Storage period (day): A= 2days; B=4days; C=6days; D=8days; E=10days; F=mean of all periods

(Fig. 7): Total free amino acids concentration (TFAA, mg 100g⁻¹ FW) in leachate of mango leaves after exposure to low temperature degrees (5 and 10°C) at various storage periods (2 to 10 days). Cultivars: 1. Alphonso; 2. Balady; 3. Bullock's Heart; 4. Helmand; 5. Hindy Besennara; 6. Mabrouka; 7. Mestekawy; 8. Nabeeh; 9. Ewais; 10. Spates; 11. Taimour; 12. Zebda. Storage period (day): A= 2days; B=4days; C=6days; D=8days; E=10days; F=mean of all periods

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