

## ETHEPHON APPLICATION AT KIMRI STAGE ACCELERATES THE FRUIT MATURATION PERIOD AND IMPROVES PHYTONUTRIENTS STATUS (HILLAWI AND KHADRAWI (C.V.)) OF DATE PALM FRUIT

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Monsoon rains at the time of harvesting / ripening cause heavy losses to date fruit crop. The objective of current study was to investigate the potential of ethephon on ripening acceleration and fruit quality of date palm. Ethephon was applied through spray on fruit (2 ml, 4 ml and 6 ml/L) and by injection in fruit peduncle (1 ml, 2 ml and 3 ml/bunch) at final kimri stage of both cultivars during two seasons (2011 and 2012). Spray method performed well as foliar application of ethephon @ 4 ml/L reduced the fruit maturation period between kimri to rutab stage up to 14 days with uniform fruit ripening index of 61.21% and rutab fruit yield of 6.32 kg per bunch as compared to untreated fruit. Biochemical parameters such as ascorbic acid (1.33 and 1.45 mg/100 g), glucose (23.79 and 22.85%), fructose (21.97 and 21.32%), sucrose (6.06 and 5.55%), total phenolics, (155.55 and 161.90 mg GAE/100 g), total flavonoids (51.95 and 47.08 mg CEQ/100 g), total antioxidants (59.74 and 63.85%) were also higher in these fruits. Hillawi cultivar performed better by ethephon application as compared to Khadrawi. It is concluded that losses of date fruit can be minimized by the application of ethephon at final kimri stage by shortening the fruit transition period between final kimri and rutab stage with good biochemical composition.

**Keywords:** *Phoenix dactylifera* L., ethephon, ripening, phytonutrients

### INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, dioecious in nature and belongs to family Arecaceae (Barrow, 1998). Date palm has 14 recognized species; *dactylifera* is one of the most cultivated fruit crop in the world. *Dactylifera* is widely spread in the tropical and subtropical regions of Africa and southern Asia with varied geographical, soil and climatic conditions (El-Hadrami *et al.*, 2011). Date palm has a unique significance over all other fruits due to historical cultivation, multi-nutrition, consumption and use of every plant part (Barreveld, 1993). Date fruit is highly nutritious and an important source of sugars (glucose, fructose and sucrose), fibers, mineral elements, vitamins, minute amount of fats and proteins (Mohamed, 2000; Al-Farsi *et al.*, 2005; Baloch *et al.*, 2006). Muslims widely consume dates in the Holy month of 'Ramzan' at 'Iftar' time as it provides a quick source of energy. Therefore, dates are considered as an ideal food with abundant of potential health benefits (Barreveld, 1993; Al-Shahib *et al.*, 2003; Al-Farsi *et al.*, 2005; Elleuch *et al.*, 2008). However, in date fruit, a great variation exist regarding the biochemical composition which mainly depends upon the cultivars and different fruit maturation phases (Haider *et al.*, 2013).

Pakistan ranks fifth in the world dates production with an annual production of 735 thousand tons on an area of 90.1 thousand hectares, contributing 10% share in global production (FAOSTAT, 2014). Date palm is an important

fruit crop of Pakistan. Hillawi and Khadrawi are considered as very important date palm cultivars of Punjab, Pakistan due to their sweetness at late khalal and early rutab stage and these can be fully consumed at these stages. The ripening period of these cultivars starts from mid-July to early August which is a peak monsoon period, a real bottleneck for date crop. Every year monsoon clashes with the dates ripening period and a few minutes of rainfall can destroy up to 80% of the date fruit crop (ASF, 2010). Date palm growers started to harvest the fruit at early khalal stage and do not left the fruit on the tree up to rutab and tamer stage due to the threat of monsoon rains and sold them in the market at very low price due to uneven and poor fruit quality.

The fruit ripening cycle of date palm (*Phoenix dactylifera* L.) passes through five developmental phases named as; Hababouk (baby fruit), Kimri (unripe fruit), Khalal (edible fruit), Rutab (soft fruit) and Tamer (dry fruit). Harvesting of date fruit depends upon the cultivar characteristics, climatic conditions and market demand (Glasner *et al.*, 1999). One of the ways to force early ripening is by use of plant growth regulators that alter the internal physiological processes of the plants. Various plant growth regulators can be used to advance the fruit harvest maturity (Rademacher *et al.*, 2004). Among these, ethephon directly stimulates the ethylene production and accelerates the physiological process of fruit maturation (Lieberman, 1979). Ethylene released from ethephon stimulates synthesis of endogenous ethylene (Hartmann, 1992; El-Kereamy *et al.*, 2003) that increases the

fruit sugar and colour by accelerating the fruit ripening process (Lopez *et al.*, 2000; Awad and de Jager, 2002). Pre-harvest application of ethephon accelerates the fruit ripening and noticeably increased the fruit quality parameters (Mougheith and Hassaballa, 1979). Globally, the use of ethephon on ripening acceleration and fruit quality has been studied but in Pakistan there is no single evidence regarding this aspect. Therefore, current study was carried out to evaluate the potential of ethephon on ripening acceleration and fruit quality of 'Hillawi' and 'Khadrawi' date palm cultivars.

## MATERIALS AND METHODS

The plants of two date palm (*Phoenix dactylifera* L.) cultivars 'Hillawi' and 'Khadrawi' having the age of about 20-22 years grown at Experimental Fruit Orchard Sq. No. 9 and Post Graduate Agricultural Research Station (Latitude 31°-26' N, Longitude 73°-06' E and Altitude 184.4 m), Institute of Horticultural Sciences, University of Agriculture Faisalabad, Punjab Province, Pakistan, were selected for this study work. All the selected plants were uniform in size, manually pollinated and uniform agronomic practices (fertilizers application and irrigation) were adopted throughout the period of investigation (2011-2012). A single date palm plant was selected as one replicate (2 bunches per replication). A liquid plant growth regulator ethephon [(Ethephon 480g/l SL (48% m/v)] was purchased from Shanghai Mingdou Agrochemical Co., Ltd. China. Various ethephon concentrations by two different methods foliar spray (2, 4 and 6 ml/L) and by injection (1, 2 and 3 ml/bunch) were applied at final kimri stage. For injection a small pit was made in the fruit peduncle with a sharp budding knife made up of stainless steel. The pit was made 20 cm away from the start of fruiting and ethephon solution was injected into the pit. After injection pit was covered with cellotape to avoid any contamination. A wooden step ladder was used for climbing up on the date palm plants during the experimentation.

**Fruit harvesting and analysis:** During the entire harvesting season, rutab or ripe fruit was periodically collected from the selected bunches and weighted. Fruit showing soft brownish tip was considered as ripe or rutab fruit (Slide 1 and 2). Fruit ripening/ maturity parameters (time to late khalal stage and time to rutab stage) were recorded from tagged bunches after the application of ethephon treatments and then average was calculated as total number of days. Rutab fruit yield per bunch was calculated from tagged bunches with a digital weighing balance during the entire harvesting season and then average was calculated. Uniform fruit ripening index was calculated by using the following formula as given below.

$$\text{Uniform fruit ripening index (\%)} = X/Y \times 100$$

Where, X = Number of ripe fruits

Y = Total number of fruits



Slide 1. Rutab fruit of Khadrawi date palm (soft/brownish tip)



Slide 2. Rutab fruit of Hillawi date palm (soft/brownish tip)

From each replication 100 rutab fruits were randomly selected for the biochemical (TSS, acidity, ascorbic acid, glucose, fructose, sucrose) and phytochemical (TPC, TFC, total tannins and total antioxidants) analysis. Total soluble solids concentration was measured by using digital refractometer (ATAGO, RX-5000, Japan) at room temperature (20°C) and readings were expressed as °Brix. Ascorbic acid contents were determined following the method described by Ruck (1961) by titrating the juice samples against 2, 6-dichlorophenolindophenol dye, to light pink color end point, persisted at least for 15 seconds. Total titratable acidity was determined by the method described by Hortwitz (1960) by titrating the juice samples against 0.1N NaOH, using 2-3 drops of phenolphthalein as an indicator till pink color end point was achieved. Total phenolic contents (TPC) were calculated by using Folin-Ciocalteu reagent method as reported by Ainsworth and Gillespie (2007). Total antioxidants activity was assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radicals as described by Amira *et al.* (2012). Total flavonoids contents were determined according to the method described by Kim *et al.* (2004). Total tannins contents were estimated according to the method of (AOAC, 1980) by titrating the extracted fruit samples with standard potassium permanganate solution by using indigo carmine as an indicator until colour changed to faint pink and values were expressed as percentage. For the estimation of sugars date fruits were extracted from the date flesh (5 g) in HPLC grade ethanol (80% v/v). The available extracts were then centrifuged at 13000 xg for 10 min and then supernatants

were separated and analyzed by high performance liquid chromatography (HPLC). Results were expressed as percentage of fresh weight.

**Statistical Analysis:** Collected data were statistically analyzed using computer software MSTAT-C. Analysis of variance was used to test the significance of variance. While difference among treatment means were compared using LSD test ( $p=0.05$ ) (Steel *et al.*, 1997).

## RESULTS AND DISCUSSION

**Fruit maturity / ripening traits:** Statistically significant differences ( $p \leq 0.05$ ) were found regarding the effects of ethephon treatments and cultivars while interaction between them showed non-significant results for fruit maturity/ripening traits during the both years (Fig. 2-5). Fruit sprayed with ethephon (6 ml/L and 4 ml/L) accelerates the fruit maturity period from final kimri to rutab stage about two weeks as compared to untreated fruit. Hillawi fruit took shorter time to complete that period than Khadrawi. Higher rutab fruit yield (7.16, 6.62 and 6.54, 6.03 kg/bunch) was recorded in fruit sprayed with ethephon (6 ml/L and 4 ml/L) than untreated fruit during both years. Hillawi cultivar attained maximum rutab fruit yield (4.60 and 4.25 kg/bunch) than Khadrawi (Fig. 4). Maximum uniform ripening index

(62.49, 62.22 and 60.73, 60.20%) was achieved in fruit subjected to ethephon (6 ml/L and 4 ml/L) as compared to untreated fruit during both seasons. The Hillawi fruit attained higher uniform fruit ripening index (50.75 and 49.27%) as compared to Khadrawi (44.36 and 45.14%) (Fig. 5). Our results are correlated with Mougheith and Hassaballa (1979) who reported that pre-harvest ethephon application accelerates the fruit maturation period in 'Hayany' date palm. These findings are also correlated with Kamal (1995) who reported that foliar application of ethephon hasten the fruit ripening about one month in 'Zaghloul' and 'Samani' date palm. These findings are also correlated with Awad (2007) who reported that higher rutab fruit yield per bunch or evenly ripe fruits were obtained from ethephon treated fruits in 'Hilali' date palm as compared to untreated fruits. He also reported that no significant difference was found between the foliar and injection application methods. This effect of foliar ethephon application is also reported in other fruit crops such as in ber (*Ziziphus mauritiana* L) fruit ripening period accelerated about 2 weeks (Bal *et al.*, 1996), 13 to 22 days in persimmon (Kim *et al.*, 2004) and 8 days in fig fruit (Puech *et al.*, 1976). In the present study, method of ethephon application showed a great variation in their response as foliar spray performed better as compared to injection application. It is because;

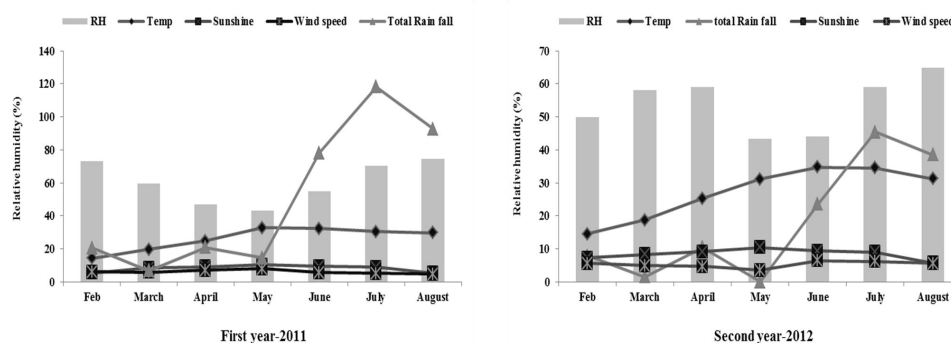


Figure 1. Meteorological data during the date palm season 2011 and 2012 at experimental site.

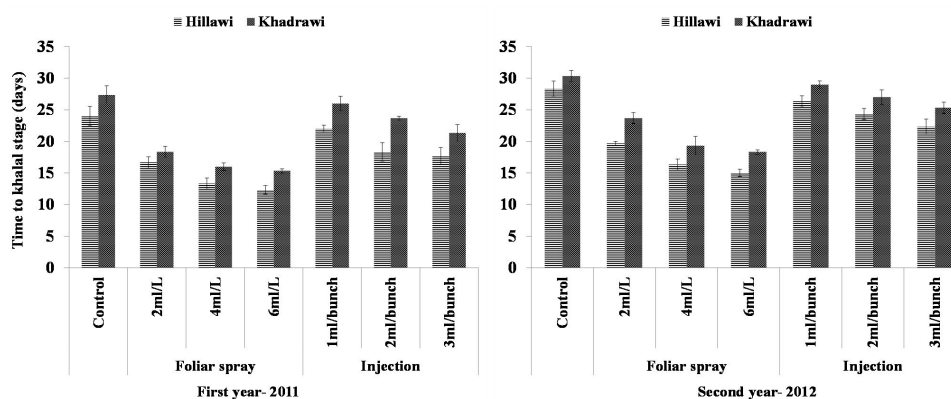


Figure 2. Effects of ethephon application (foliar and injection) on the ripening time from final kimri to late khalal stage of Hillawi and Khadrawi date palm cultivars  $\pm$  S.E.

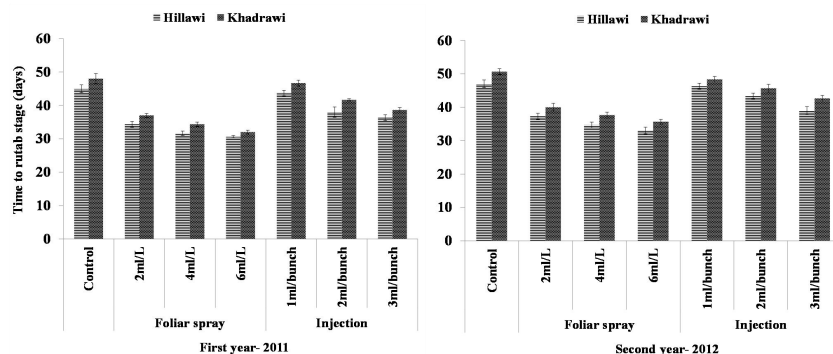


Figure 3. Effects of ethephon application (foliar and injection) on the ripening time from final kimri to rutab stage of Hillawi and Khadrawi date palm cultivars  $\pm$  S.E.

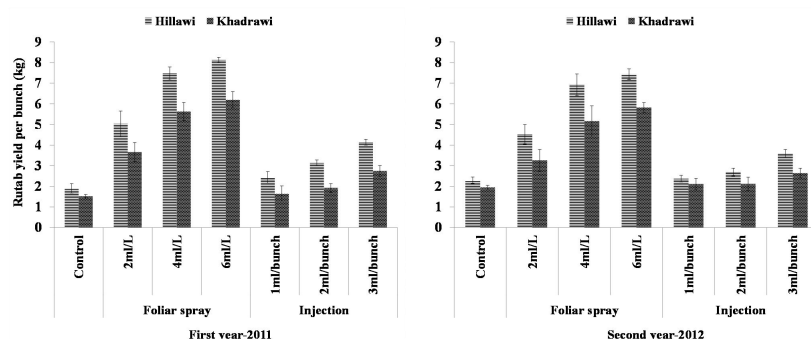


Figure 4. Effects of ethephon application (foliar and injection) on rutab fruit yield (kg) per bunch of Hillawi and Khadrawi date palm cultivars  $\pm$  S.E.

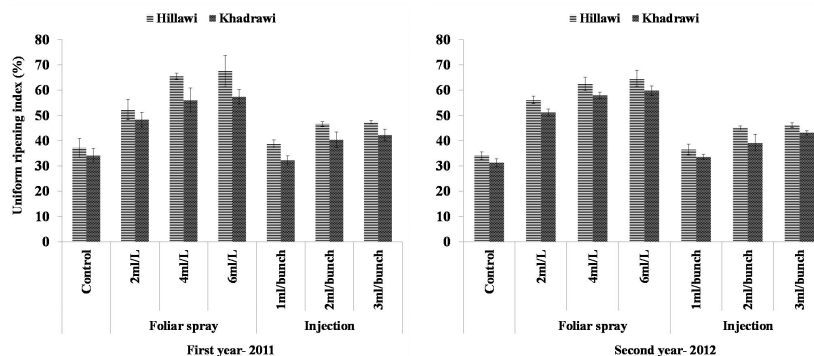
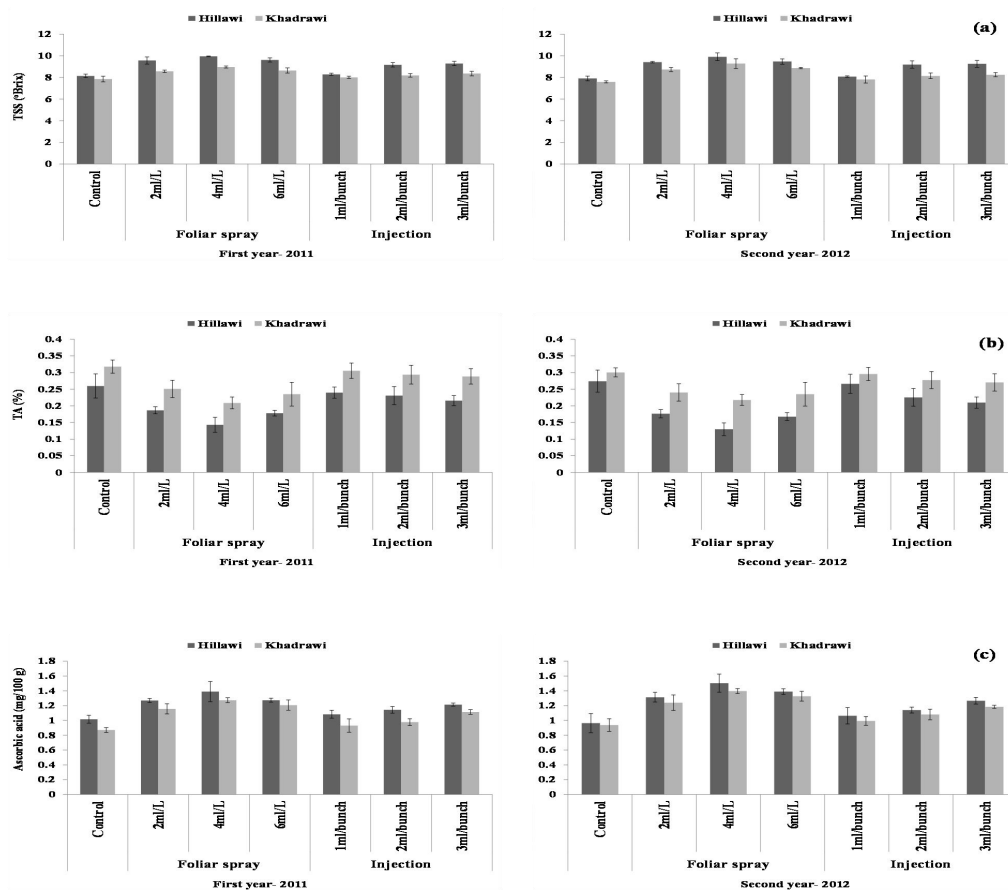


Figure 5. Effects of ethephon application (foliar and injection) on uniform fruit ripening index (%) per bunch of Hillawi and Khadrawi date palm cultivars  $\pm$  S.E.

foliar spray might have a direct contact with fruit cuticle layer that fasten up the respiration process and fruit reached at maturity within shorter time. Whereas, ethephon application by injection could not showed quick and fast response due to its slow translocation from the peduncle to individual fruit strands. It is also possible that the whole amount of ethephon concentrations used could not reach in proper way to the desired place.

**Biochemical composition of date fruit:** Pre-harvest ethephon application (foliar and injection) significantly ( $p \leq 0.05$ ) affected the fruit biochemical composition in both

tested date palm cultivars during two study seasons (Fig. 6 & 7). The fruit sprayed with ethephon (4 ml/L) showed higher amounts of total soluble solids (9.45 and 9.59 °Brix), ascorbic acid (1.33 and 1.45 mg/100 g) and lower acidity level (0.175 and 0.173%) as compared to untreated fruit during both years. Higher level of glucose (23.79 and 22.85%), fructose (21.97 and 21.32%) and sucrose (6.06 and 5.55%) was noted in fruit subjected to ethephon (4 ml/L) as compared to control fruit during both study seasons. The Hillawi cultivar attained maximum level of sugars than Khadrawi. The increase in TSS in ethephon-treated fruit

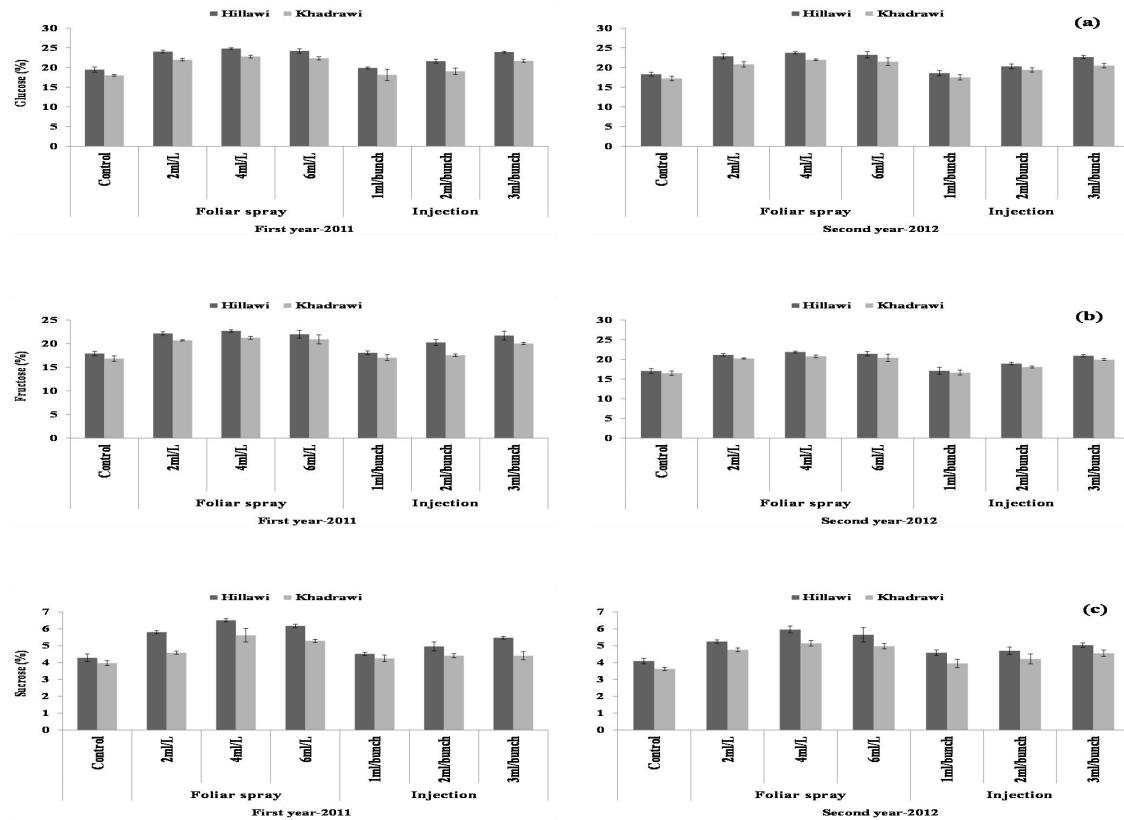


**Figure 6. Effects of ethephon application (foliar and injection) on TSS (°Brix) (a), total titratable acidity (%) (b) and ascorbic acid contents (mg/100 g) (c) of Hillawi and Khadrawi date palm cultivars ± S.E.**

might be due to the rise of sugar contents which depends upon the conversion of starch on hydrolysis or it might be due to the enhanced ripening initiated by the ethylene. These results are confirmed with the findings of (Khalifa *et al.*, 1975; Rouhani and Bassiri, 1977) who reported that ethephon application efficiently improved the total soluble solid contents in date fruits than untreated fruits. According to Sandhu *et al.*, (1989) application of ethephon in ber (*Ziziphus mauritiana* L.) fruit increased the amount of total soluble solids as compared to fruit where no ethephon was applied. Pre-harvest spray of ethephon increased the TSS contents in table grapes (Nikolaou *et al.*, 2003) and 'Cripp's Pink' apple (Whale *et al.*, 2008) at the time of commercial fruit harvest. The reduction in fruit acidity might be due to the effect of ethylene on the activity of enzymes that promotes the fruit ripening process and lower down the acidity level (Sisler, 1984; McGlasson, 1985; Yang, 1985). Our findings are also in line with Hashinaga and Ito (1985) who reported that ethephon application significantly reduced the acidity level in 'Meiwa Kumquat' fruit. The increased in ascorbic acid contents in ethephon treated fruit might be due

to quick ripening process and fruit have less time to loss the ascorbic acid contents. These findings are supported by various co-workers who reported that ethephon treated fruit have higher ascorbic acid in cape gooseberry (Yadava *et al.*, 2008), mango (Mann, 1985) and 'Allahabad Safeda' guava cultivar (Brar *et al.*, 2012). In the present study ethephon treatments at final kimri stage significantly increased the accumulation of sugar contents in treated fruit as compared to untreated one. These results are in line with (Bal *et al.*, 1996; Gala *et al.*, 2001; Suresh and Zora, 2003; Kulkarni *et al.*, 2004) they all reported that ethephon treatments significantly improved the sugar contents in different fruits as compared to the fruits where no ethephon was applied.

**Fruit phytonutrient composition:** Total phenolics, total flavonoids, total tannins and total antioxidants showed significant differences ( $p \leq 0.05$ ) regarding the effects of ethephon treatments and cultivars while interaction between them was found non-significant during the both years (Fig. 8). Fruit sprayed with ethephon (4 ml/L) showed higher TPC (155.55 and 161.90 mg GAE/100 g), TFC (51.95 and 47.08 mg CEQ/100 g), total antioxidants (59.74 and 63.85%) and



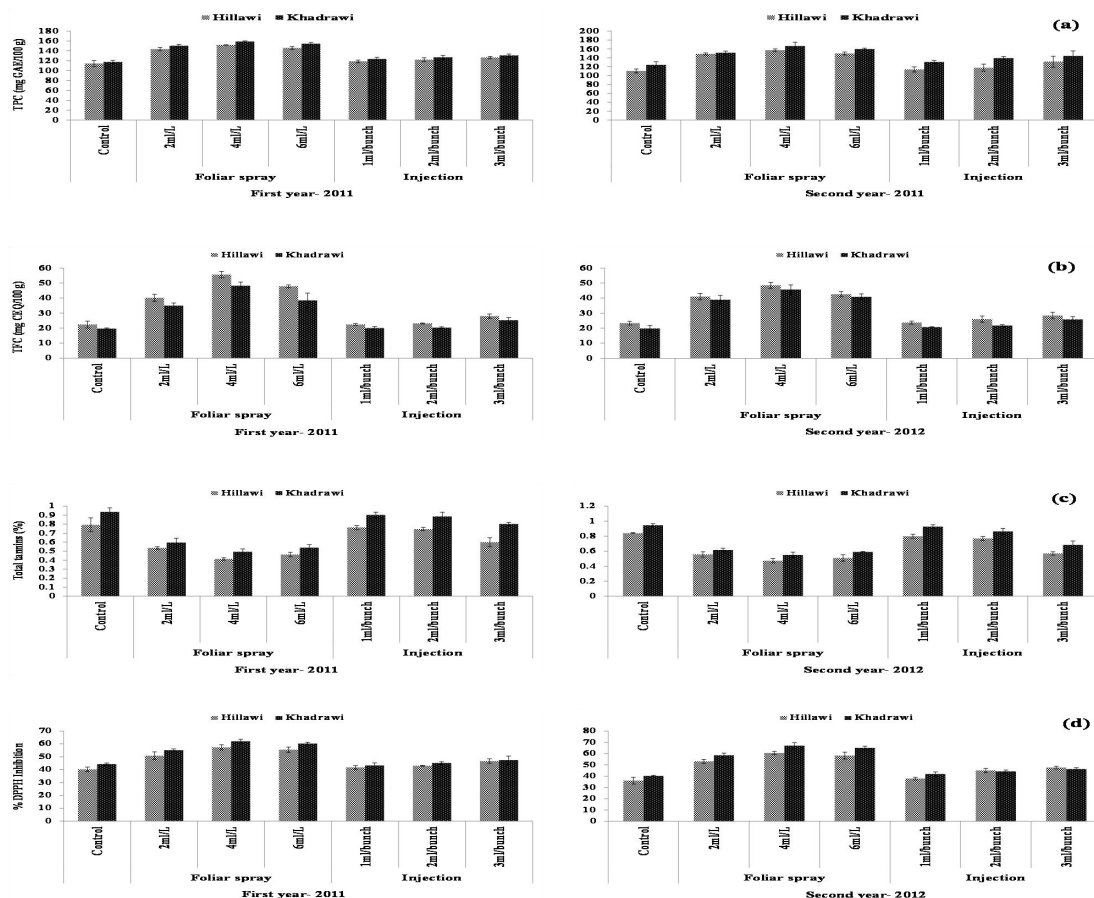
**Figure 7. Effects of ethephon application (foliar and injection) on glucose (%) (a), fructose (%) (b) and sucrose contents (%) (c) of Hillawi and Khadrawi date palm cultivars  $\pm$  S.E.**

lower total tannins (0.45 and 0.51%) as compared to untreated fruit during both study years. The fruit of Hillawi attained higher TFC, lower tannins while Khadrawi showed maximum TPC and total antioxidants during both seasons. Ethephon application at final kimri stage had a significant influence on the phytonutritional composition in both date palm cultivars. It is because the application of ethephon generates the ethylene that plays an important role for the synthesis of phenolic compounds under different stress conditions (Lin *et al.*, 2009). These findings correlates with Whale *et al.* (2008) who reported that pre-harvest ethephon application significantly increased the level of phenolics and flavonoids due to the increased concentration of cyaniding 3-galactoside in the exposed fruit skin at commercial harvest as compared to untreated fruit. According to El-Kereamy *et al.* (2000) ethephon treatments enhanced the gene expression of some enzymes that advanced the synthesis of phenolic compounds. The increase in antioxidant activity of both date palm cultivars treated with ethephon demonstrated an increase in their nutritional value. Ethephon treatments also build up the level of total flavonoids contents. These results are also supported by different workers (Nikolaou *et al.*, 2003; Lombard *et al.*, 2004; El-Kereamy *et al.*, 2000) they all reported that pre-harvest ethephon application enhanced

the level of total polyphenols, flavonoids and proanthocyanidins in table grapes. In our study total tannin contents were also decreased in fruit subjected to ethephon treatments as compared to untreated fruit. These results are associated with the findings of (Kato, 1990; Itamura *et al.*, 1991; Taira *et al.*, 1991; Park *et al.*, 1998; Tamura *et al.*, 1999) they reported that higher concentration of ethephon greatly accelerated the coagulation process of tannins in persimmon fruit due to the disappearance of astringency and fruit become sweeter in taste.

**Conclusion:** In conclusion, pre-harvest spray of ethephon (4 ml/L) at final kimri stage is effective to accelerate the fruit maturation period, maximum uniform ripening; higher rutab fruit yield and good fruit quality composition. Therefore, this could be an effective cultural practice for Hillawi and Khadrawi date palm cultivars to combat the seasonal fluctuations in the form of monsoon rains at the time of fruit harvesting/ripening.

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**Figure 8.** Effects of ethephon application (foliar and injection) on TPC (mg GAE/100 g) (a), TFC (mg CEQ/100 g) (b), total tannins (%) (c) and total antioxidants (%DPPH inhibition) (d) of Hillawi and Khadrawi date palm cultivars  $\pm$  S.E.

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