

THE FATE OF DICHLORVOS (DDVP) IN FIELD-TREATED FOUR MATURITY STAGES OF DATES

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ABSTRACT

The fate of dichlorvos (DDVP) on dates after application in the field has been investigated. Two methods of application with two different concentrations were used, the traditional aerial application of 0.15% DDVP and a new method which is called the surgical method, with the new method 0.25% DDVP was directly applied into a hole in the trunk of the tree. Residues were determined using GC-ECD after extraction of the fruits. DDVP residues detected in Khalal fruits (mature full coloured stage) using aerial and surgical methods were 192.9 ± 1.9 and 352.0 ± 5.6 ng/g, respectively. For Rutab fruits (soft brown stage) DDVP residues were 24.05 ± 0.87 and 50.21 ± 3.6 ng/g, respectively. No residues were detected in the mature Tamer fruits (hard raisin-like stage).

INTRODUCTION

In the UAE, as well as in other Arabic and Islamic countries, dates are considered as one of the most important crops because of their religious and nutritional importance. The per capita daily consumption of dates in Abu Dhabi city (UAE) is between 10 to 200 g (El-Behissy, 1998) varies depending on the time of the year and consumer's age.

As with any agricultural crops, dates are a target for pest attack, so there is a need to treat them with pesticides. About 20% of the

total date palm trees in the Emirate of Abu Dhabi were infested with red palm weevils (Plant Protection Section, Agricultural Section, Abu Dhabi Municipality, 1995). The current practice in the UAE is to apply pesticides such as DDVP to control this insect species and other insect species on date palm trees as well as other trees (Plant Protection Section, Agricultural Section, Abu Dhabi Municipality, 1994). Bashir A. A. was investigating new method of application named the surgical method, which involved the direct application of DDVP into surgically opened date palm's tissues. This method proposed to control red palm weevils that destroyed large numbers of date palms. The harmful stage of the red palm weevils is the larvae, which have the capacity to chew out the entire tree if it is not detected in time and eradicated in the proper way. The symptoms of infestation are the exudation of a slimy gelatinous liquid, excretion of chewed fibres and on close observation a gnawing sound (Ministry of Agriculture and Fisheries, 1990).

Application of pesticides means that potentially high levels of pesticide residues may be consumed. There is a need to set regulations that control the use of pesticides in terms of types and doses in field applications. Dates are different from most fruits as there are three different edible stages: khalal (mature full coloured stage), rutab (soft brown stage) and tamer (hard-raisin-like stage) stages, each with different chemical composition. Khalal and rutab are mostly consumed fresh, while tamer may be consumed fresh or after storage or processing. Therefore, Good Agriculture Practice (GAP) for the application of pesticides on date palm trees is required to minimize the risk to human health.

Ciba Geigy company (1972) (FAO/WHO [I], 1993) carried out the only unpublished report to evaluate the residue levels of DDVP in date palms in Iraq. According to the Good Agricultural Practice (GAP) in the UAE where date trees are sprayed once aerially with DDVP at 0.95-2.0 kg per hectare, no measurable residue (<0.03 ppm) should be detected in mature fruits 138 days after the treatment.

The main objective of this study is to evaluate DDVP residues in fruits of varying maturity, from date palms treated using the current

practice for pesticide application and the new method proposed by Bashir A.A.

MATERIALS AND METHODS

Chemical used:

- Insecticide: An emulsifiable concentration 50% (w/v) of DDVP supplied by Denka international, Holland as Denkavepon 50[®], and analytical master standard, 99.8%, supplied by Ciba Giegy, ltd.
- Solvents: Acetone, Petroleum ether (40-60 °C) and Iso-octane that were of analytical grade. Solvents were used without further purification.
- Chemicals: Sodium chloride and sodium sulfate, which were of reagent grade. They were heated at 550°C for 12 hr.

Apparatus and instruments:

- Waring Blender (USA) with low and high speeds and stainless steel jar
- XA analytical balance (Fisher Scientific,)
- Whatman filter paper no. 4 (15 cm, fast, retains coarse and gelatinous precipitates), no. 1 (9 cm, medium fast, retains medium crystalline) and no. 5 (7 cm, slow, retains fine crystalline).
- Rotvapor RE 121 Rotary evaporator (Büchi, Switzerland) equipped with water bath and General Electric vacuum pump. The water bath was set at 35°C, while the vacuum pump was set at 300 mmHg.
- GC-ECD, Varian 3600, equipped with Electron Capture detector was used in the following conditions.

Column: Chrompack WCOT Fused Silica, CP-Sil 5 CB stationary phase (100% methyl silicone); length, 10 m; i.d., 0.32 mm; o.d., 0.45 mm; film thickness, 1.20 µm; column oven; programmed, 100 °C (1 min.), to 120 °C by 2 °C/min. (1 min.), then to 250 °C by 5 °C/min. (10 min.). Detector: Electron capture detector (ECD, Ni⁶³), setted at 320 °C. Injector: 230 °C, splitless mode (1 min.). Gases: carrier gas; helium; 2.2 ml/min., makeup gas; nitrogen, at a flow of 20-22 ml/min. Data handling: built-in data handling processor, chart speed; 0.3, Offset; 20%.

The application of dichlorvos:

Eighteen mixed varieties of date palms (*Phoenix dactylifera*) were treated with dichlorvos in open field. Palm trees were about 5-6

years old. Trunk height was 1.5 to 2.0 metres. The treatments were as follow: six trees were treated with water as control (Group 1), six trees were treated with DDVP using the aerial spraying with the maximum concentration (0.15%) allowed for agricultural use in Abu Dhabi (Group 2) and six trees were treated using the surgical method which involves the direct application into a surgically made hole in the tree trunk, with 0.25% DDVP (Group 3). The 0.25% DDVP was reported to be 100% effective against red palm weevils. After application, the hole made in each tree in group 3 was covered by white cement to avoid microbial action. The applications were done using an application pump (9 H.P. machine) connected to a reservoir where DDVP solutions were prepared. Date palms were applied three times in the season. The first application was done before flowering on Dec 12, 1995, the second was on March 17, 1996 during flowering and artificial pollination and the third was on May 28, 1996 at the end of hababouk stage (tricarpeialate stage) and the beginning of jimri (immature green) stage.

Samples were collected from each group in four different stages of maturation, jimri, khalal, rutab and tamer. The first collection was done on May 21, 1996 at jimri stage. The second collection was done on June 28, 1996 during khalal stage, the third collection on July 23, 1996 at the rutab stage and the fourth collection on August 9, 1996 at the tamer stage. From the six trees in each group, a total sample of about 10 kg were collected by random selection of about 1-2 kg date fruits from each tree. After mixing the 10 kg sample, 2 kg were taken as the representative sample for the group and packed in tightly closed plastic bags and stored at -18 °C. Six sub-samples from each sample were analyzed within one week.

Analysis of dichlorvos residues:

Preparation of date samples: Seeds and caps were removed manually and dates were chopped using a knife. Chopped dates were mixed using a mortar to produce a homogenized paste.

Measurement of moisture content: Six sub-samples of about 2-3 g each was spread out in dried and weighed metallic dish. Samples were then dried in an oven maintained at 100 °C under vacuum for 12 hr (practically was enough time to get constant weight). Hot dried dishes were removed from the oven and placed into a desiccator,

cooled and weighed. Moisture content was calculated as a percentage of the wet sample.

Extraction of dichlorvos residues: The residues were extracted using the methods of Miller (1992) and Nakamura et al. (1994) with modifications. 20 g (± 1 g) of dates were blended with 100 ml acetone and 20 ml water for 2 minutes. The mixture was filtered through a fast filter paper (Whatman no. 4) then the retained residues were blended again with another 50 ml acetone and 20 ml water. Then the combined extract was transferred to a separating funnel. About 5-6 g of sodium chloride were added, and extracted by 100 ml petroleum ether. The aqueous layer was transferred to another separating funnel and extracted with another 50 ml of petroleum ether. The organic fraction was then filtered through 20 g of anhydrous sodium sulfate on medium fast filter paper (Whatman no. 1) and evaporated at 35 °C using a rotary evaporator to about 5 ml. About 2 to 3 ml of iso-octane were then added, and the evaporation of petroleum ether completed. The extract was filtered into a measuring cylinder fitted with a glass stopper using slow filter paper (Whatman no.5) then the solution was completed to a total volume of 20 ml using iso-octane. Aliquots of 0.5-1 μ l were injected onto the GC-ECD injector. Recovery experiment: 20 g of dates prepared as stated above were blended with 100 ml acetone and 20 ml water for 2 min. Then a known amount of standard DDVP solution was added to the mixture in the blender, and re-blended for further 2 min. DDVP was extracted and measured using the procedures as described above.

The Detection limit of the GC-ECD: The detection limit of the GC system was estimated by observing the detector response for decreasing amounts of standard solutions of DDVP in iso-octane. It is considered as the nanograms that give response after injecting 2 μ l of the standard solution into the GC-ECD. The 2 μ l are the maximum volume that could be injected in the detection instrument.

RESULTS AND DISCUSSION:

Analytical Methodology:

The DDVP was estimated by GLC using a Chrompack WCOT Fused Silica, CP-Sil 5 CB column and ECD detector (Figure 1). A linear calibration curve was obtained by plotting the concentrations of standard DDVP verses the detector response. The correlation

coefficients was 0.9947-1.00 for concentrations up to 2267 ng/ml. Data used in establishing the calibration curve were the overall means of two series of concentrations, 8.85-283.2 ng/ml and 566.8-2267 ng/ml, (Table 1 and Figure 2).

Table 1: Concentrations of analytical standard DDVP that were used in establishing the standard curve and their responses.

Conc. (ng/ml)	n	mean area (x10 ⁶)	SD
8.851	6	0.38	0.18
56.70	10	2.98	0.42
113.4	13	6.25	0.62
226.7	16	12.4	0.46
283.2	11	14.5	1.7
566.8	16	31.2	2.8
1134	14	59.3	15
2267	20	121	8.4

n: number of replicates.

The recovery of DDVP was estimated by spiking the blended mixture with standard DDVP solution. The recovery of residues was $81.2 \pm 2.9\%$ (mean \pm SD). The recovery range 70-120 % is accepted by the Codex Alimentarius (1993). Also, between 87-97 % of DDVP were recovered from six different types of fortified vegetables and fruits that detected by GC-FPD as reported by Nakamura et al. (1993). They also reported the detection limit of DDVP by GC-FPD was 0.002 ppm (2 ng/g). Nakamura et al. (1994) also reported that between 83.6-113.0 % of DDVP were recovered from various fruits and vegetables and detected by GC-NPD. The detection limit of the GC system was 2.2 ng/ml (4.4 pg DDVP).

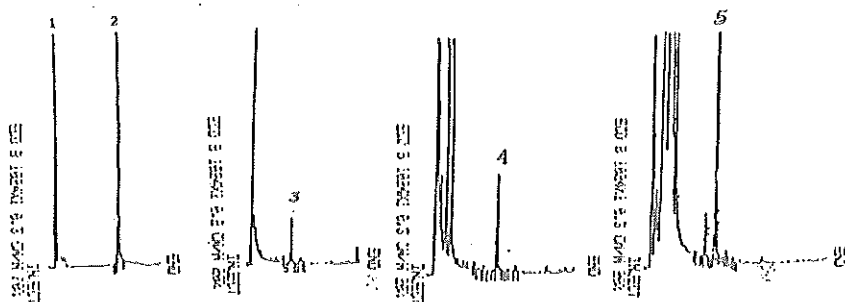


Figure 1:GC-ECD chromatograms of DDVP in standard solution, khalal, rutab and tamer extracts. The retention time of DDVP peaks was between 6.072 to 6.199 minutes. Peak 1 was the iso-octane, peak 2 was standard working solutions of 2267 ng/ml, while peaks 3 to 5 were detected DDVP residues in Khalal extract, rutab extract and tamer extract, respectively.

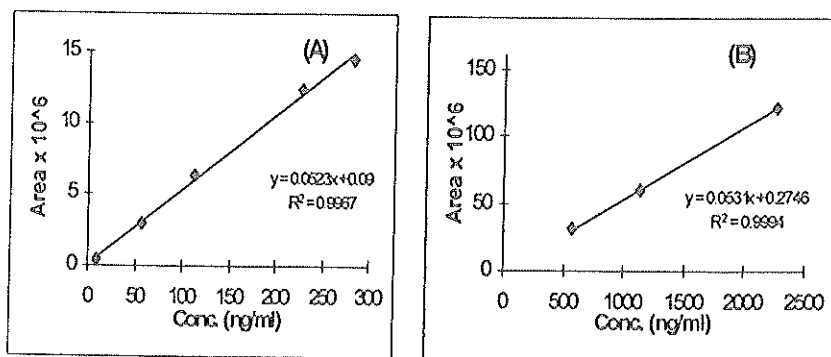


Figure 2:DDVP standard curve, (A) first series, 8.85-283.2 ng/ml and (B) second series, 566.8-2267 ng/ml.

Levels of DDVP during the maturation of field treated date fruits:

Results of DDVP field application by aerial and surgical methods are summarized in Table 2. DDVP residues were estimated in fruits at the edible stages as well as in the inedible jimri stage (the stage before the last application of pesticide).

No analysis was done before jimri stage because there were no edible date fruits available for analysis. The main aim for following the levels of DDVP was to know if the levels are within the acceptable limits or not in edible stages which is an important factor

for the fitness of fruits for consumption. People mostly consume khalal, rutab and tamer fruits.

Table 2: DDVP levels in dates, treated using aerial and surgical methods, at four stages of maturity.

Stage	Control	Aerial spraying, conc. \pm SD (ng/g)		Surgical method, conc. \pm SD (ng/g)	
		Before last application	After last application	Before last application	After last application
Jimri	nd	182.5 \pm 5.6	-	84.85 \pm 0.78	-
Khalal	nd	-	192.9 \pm 1.9	-	352.0 \pm 5.6
Rutab	nd	-	24.05 \pm 0.87	-	50.21 \pm 3.6
Tamer	nd	-	nd	-	nd

Results are on wet base., nd: non detected, less than 2.2 ng/g.

As shown in table (2), DDVP residues ranged between 24 to 352 ng/kg. The residues varied according to the tested stage and method of DDVP application.

After one month of last application, 0.0141% of the initial residues remained in khalal fruits. This calculation based on the assumption that all the applied DDVP was absorbed by trees from group (3) and reached the fruits. This very low remaining level demonstrates the rapid breakdown of DDVP in the field.

Generally there are many factors that influence the levels of DDVP, as any other pesticide, in the fruits such as the physical and chemical properties of the pesticide (Abou-El-Soud et al., 1995, Westlake et al., 1970), the method of application (Westlake et al., 1970), the applied doses (Ciba-Geigy Ltd, 1971a and 1971b), the number of doses (Cabras et al., 1985), the nature of the crop (Vidal et al., 1998), and the weather in the field (Vidal et al., 1998).

The chemical and physical properties of DDVP are important factors affect the fate of DDVP after application. Highly reactive DDVP can be degraded by enzymatic and non-enzymatic systems (FAO/WHO [I], 1993, Fest and Schmidt, 1982). Also, it might be lost by weather because of its high volatility. Although the applied

doses were relatively very high, the rate of degradation was very fast that left low residues in the edible stages.

In Switzerland in 1971 (from FAO/WHO [1], 1993), DDVP was applied aurally once on apple trees at a concentration of 0.05 % (50 g active ingredient per 100 litre). Residues were 0.45, 0.14 and 0.1 mg/kg at days 0-1, 3-4 and 5-7 after treatment. Assuming that the tree absorbed all the DDVP, at day 7, 0.01 % of the initial applied residues remained. In our study DDVP persisted longer and this difference is probably because of the variations in tested crops, in the application methods (aurally in the case of apples and direct application into the internal tissues in the case of palms), the applied doses, and in the weather.

DDVP residues measured in the date fruits were greater in the case of surgical method than those of aerial application. This differences may be related to the differences in the applied concentrations which were 0.25% and 0.15 % in case of surgical and aerial methods, respectively. The higher the applied dose, the higher were the deposits of residues in each stage of maturity. In samples of mature fruits (khalal, rutab, and tamer), group 3 fruits applied with the 0.25% DDVP had higher residue levels than group 2 trees applied with 0.15% DDVP.

Also the two methods of application used in the study differ mainly in the target of application, which in sequence affected the levels and site of retained residues. In aerial spraying only a fraction of the applied residues will be retained on the surface of the palm leaves and fruits (residues could not retained by the trunk because of the presence of leaf bases and fibrous appendages on the trunk). Thus, the initial amount of residue on the palm was not known. Of the retained residues a portion was probably lost through weathering and a portion absorbed into the fruits and metabolized. With the trees treated by the surgical method involving a direct application into a hole in the trunk, the residue was initially at one location and not distributed over the whole of the tree as in aerial spraying. Most of the applied dose can be expected to be retained by the tree, and the initial amount of residues applied can be determined, but these residues were presumably spread throughout the body of the tree through the internal vessels. The rate of migration of the residues in

the tree and the rate at which it was metabolized then determined the final level in the fruits.

The calculated ratio between residue levels after last application in group 2 to group 3 was approximately 1:2. This ratio was expected because the calculated ratio between applied doses was 1:1.67, which is roughly 1:2. In contrast, in immature fruits (jimri stage) the residue levels in group 2 fruits before last application, was higher than that detected for group 3 fruits, the opposite might be expected as the applied dose in group 3 was more than group 2. The weather before last application was colder than after last application (see Table 3, Department of Civil Aviation, 1996). This meant that water transportation (ascent of sap) of residues in the case of group 3 from the trunk to the upper part, where fruits presented, was comparatively lower before last application than after last application where the weather was hotter.

In general, the weather had a significant effect for both group 2 and group 3 fruits specially after last application. The rates of degradation appeared to be much faster after the last application, compared to the earlier application. This reflects the low ambient temperature during the period between the first and last applications of the DDVP when the tree's physiological activity and water transportation was lower compared to the period after the final application when the temperatures were higher (Table 3, Department of Civil Aviation, 1996).

Table 3: Temperatures and relative humidity % during months.

Month, year	Temperature (°C)				Relative Humidity (%)			
	Dry bulb maximum		Dry bulb minimum		Maximum		Minimum	
	range	mean	range	mean	Range	mean	range	mean
Dec., 1995	19.6-30.7	24.9	14.4-21.7	17.9	66-99	88	31-86	56
Jan., 1996	20.5-29.0	23.8	11.5-18.2	15.6	59-96	87	27-82	49
Feb., 1996	20.9-32.4	26.0	12.0-19.0	16.0	73-99	88	12-73	43
Mar., 1996	23.0-35.5	29.0	13.6-23.6	18.6	65-100	87	22-76	41
Apr., 1996	27.7-41.4	34.1	14.0-24.2	19.4	58-99	83	8-48	26
May, 1996	35.6-45.4	40.8	18.8-26.6	23.1	37-98	75	7-30	16
June, 1996	37.8-46.0	41.9	24.5-30.0	27.5	50-98	77	6-43	24
July, 1996	40.9-47.1	45.0	25.6-32.3	29.2	30-98	69	8-39	18
Aug., 1996	39.7-47.1	43.9	25.4-31.4	29.1	42-98	74	11-30	19

Values were measured in Abu Dhabi Bateen Airport in Abu Dhabi, UAE. Shown months were the period between first application (26/12/95) and tamer stage (9/8/96).

Table 4: Moisture content of raw jimri, khalal, rutab and tamer fruits*

Commodity	n	Moisture content (%) (\pm SD)
Raw jimri	6	83.5 \pm 1.6
Raw medium mature khalal	6	57.4 \pm 1.3
Raw rutab	6	36.6 \pm 0.8
Raw tamer	6	22.7 \pm 1.0

*Moisture content of raw fruits for all the four stages were measured in mixed varieties date samples.

Another important factor was the nature of the crop. In dates there are five different maturity stages of which three are edible. The main compositional difference between the five stages was the moisture content (Table 4). Calculating the results on a dry weight basis showed different results (Table 5). In group 2, the comparison between DDVP residue levels measured in jimri and khalal fruits on a wet weight basis and on the dry weight basis, showed that on a wet weight basis the residues were approximately equal, while after calculating them on dry base, residues in jimri stage were double those in khalal stage. Other maturity stages in the two groups showed similar results. This difference suggested that the moisture content should be considered in setting tolerances for DDVP residue levels in different maturity stages of dates. It was also clear from the results that the rate of degradation of DDVP in jimri and khalal stages of higher moisture content was faster than that in rutab and tamer stages of lower moisture content. It was discussed previously (Switzerland, 1971, from FAO/WHO [I], 1993) that the rate of the decomposition of DDVP in apples was faster than in khalal. This difference may be related to the higher moisture content in apples than khalal fruits (about 84 and 57 %, respectively). This factor in addition to other factors, were discussed previously, could accelerate the decomposition of chemically active DDVP in apple trees in shorter time compared to khalal fruits.

Table 5: DDVP levels (dry weight basis) of treated dates using aerial application and surgical method at four stages of maturity

Stage	Control	Aerial spraying, conc. ±SD (ng/g)		Surgical method, conc. ±SD (ng/g)	
		before last application	After last application	before last application	After last application
Jimri	nd	1080±33	-	514.3 ±4.7	-
Khalal	nd	-	452.8 ±4.4	-	826.3 ±13
Rutab	nd	-	37.9 ±1.6	-	79.2 ±5.7
Tamer	nd	-	nd	-	nd

nd: non detected, less than 2.2 ng/g.

CONCLUSIONS

It was difficult to have a fair comparison between the two methods of DDVP application (i.e. aerial spraying and surgical method) as each method had its specific factors, specially the applied doses and site of application, that affected the rate of DDVP retention by the plant.

Highly reactive and volatile DDVP residues decreased during the growth period, specially after the last application which was associated with a continuous increase in temperature. The hotter the weather, the greater was the maturation rate of the fruits as well as the degradation rate of DDVP. Temperature and moisture content positively affected the rate of DDVP degradation.

The MRL of 3 mg/kg dates that recommended by the FAO/WHO [1] (1993) was required to be amended according to differences in daily rates of consumption. Two important factors that affect the consumption of dates were the stage of maturity of the date, and the age of the consumer (Tables 6 and 7) (El-Behissy, 1998).

If a 70 kg body weight person consumes 1 g dates containing the MRL of 3 mg DDVP/kg that recommended by the FAO/WHO [1] (1993). Then estimated daily intake of DDVP by this person will be $[(3\text{mg/kg} \times 0.001\text{kg dates}) / 70\text{kg b.w.}] = 0.000043$ (or 4.3×10^{-5}) mg DDVP/kg b.w.

The average daily consumption of dates was in the range of 10 to 200g. For ages <18 of about 50 kg body weight, the daily intake of DDVP from dates containing the MRL concentration could be in the range of 0.0006-0.012 mg DDVP/kg b.w. It could be less in the range of 0.0004-0.0086 mg DDVP/kg b.w. for ages >18 with average b.w. of 70 kg. It was clear from these estimated ranges of daily intake of DDVP, that the consumption of more than 60 g dates by ages <18 and more than 70 g dates by ages >18 was greater than the ADI of 0.004 mg/kg b.w. (Tomlin, 1994).

Table 6: The consumption of dates by males and females of different two age ranges during the year and in Ramadan*.

Group	Gender	Age (years)		During the year			Ramadan			
				n	Number of dates		weight of dates (g) ^f	n	Number of dates	
					Mean	Range			Mean	Mean
1	male	12	6-18	14	6	1-10	60	16	4	1-10
2	female	13	4-17	20	6	1-20	60	17	4	1-15
3	male	40	24-59	30	9	1-20	90	31	8	3-20
4	female	33	21-49	20	7	2-20	70	26	6	2-10

* Considering that the weight of date fruit is 10 g, as the weight of date fruit is in the range 5-15 g (Al-Oqaidi,1987).

Table 7: The consumption of different maturity stages of dates among males and females of different two age ranges.

Group	Gender	Age (years)	Khalal		Rutab		Tamer	
			n	Number of dates	n	Number of dates	n	Number of dates
1	Male	mean	12	6	5	6	13	6
		range	6-18	2-10	1-15	1-10	1-10	1-10
2	Female	mean	13	8	5	5	14	5
		range	4-17	3-12	1-12	1-20	1-20	1-20
3	Male	mean	40	6	29	8	31	8
		range	24-59	1-20	1-20	1-20	1-20	2-20
4	Female	mean	33	5	26	8	30	7
		range	21-49	2-15	2-20	2-20	1-20	1-20

Applying detected residue levels shown in Table 2 and considering the daily consumption of different stages of maturity by different ages (Table 7), then the daily intakes were in the range $0-7.7 \times 10^{-4}$ mg DDVP/kg b.w., within the ADI. So the two methods of DDVP field application were acceptable from the viewpoint of the ADI specially if we consider that the fresh dates, including khalal and rutab, were consumed over a short period of about two months.

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