

**IMPACT OF WATER WITHHOLDING BEFORE RIPENING,
ZINC AND GLUTATHIONE ON FRUIT SPLITTING AND
PRODUCTIVITY OF MANFALOUTY POMEGRANATE TREES.**

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ABSTRACT

Manfalouty pomegranate trees were subjected to one, two or three weeks water withholding before fruit ripening as well as foliar fertilized with zinc sulphate or glutathione each at 0.3 % once at two weeks after fruit setting.

Results showed that increasing duration of water withholding from one to three weeks before maturation caused a gradual increase in percentages of fruit splitting, fruit peel weight , total soluble solids and total sugars , while yield, pulp , peel thickness, total soluble tannins and total acidity were progressively reduced. Water withholding duration had no effects on fruit weight and dimensions. Spraying the trees once with zinc sulphate or glutathione each at 0.3 % was responsible for improving yield, fruit weight and dimensions, percentages of pulp , total soluble solids % and fruit peel weight and thickness, total acidity and total soluble tannins compared to the unsprayed. Application of glutathione was preferable than zinc sulphate in this connection.

Subjecting manfalouty pomegranate trees to one week water withholding before ripening. Accompanied with glutathione spraying once at two weeks after fruit setting at 0.3 % is suggested for improving yield and fruit.

INTRODUCTION

Manfalouty is considered one of the most important pomegranate cvs grown successfully under Sohage region. The problem of fruit splitting in this cv. substantially declined production, shelf-life of fruits and the consumption value. In addition, the incidence of cracked fruits effectively facilitated the different pest and fungus attack. The loss of yield as a result of fruit splitting reached more than 50%. Therefore, Manfalouty pomegranate orchards were gradually reduced and were replaced by another fruit crops. Many trials were initiated for searching about the reasons for this problems.

The deficiency of Zn is considered one of the main reasons for the appearance of misshape fruits in fruit crops. It is responsible for the biosynthesis of IAA and the activation of many enzymes that promote the biosynthesis of most organic foods. It regulates cell division and water relations within plant tissues. (Nijjar, 1985).

Also, the use of antioxidants is favourable for protecting the trees from free radicals that responsible for destroying plant tissues. Antioxidants are responsible for enhancing the cell division, the biosynthesis of organic foods and controlling the incidence of fungal attack (Prusky, 1988) . It is well known that during plant metabolism many free radicals or active oxygen species namely, singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radicals and ozone are produced. Production of these radicals lead to oxidation of lipids and the loss of cells permeability. They are also used instead of auxins and chemical compounds for producing organic fruits and lowering pollution occurred in our environment (Lee *et al.*, 1994). The beneficial effect of antioxidants on fruit crops was appeared through their catching or chelating effect on the unfavourable radicals (Raskin , 1992).

The term glutathione is typically used as collective term to refer to the tripeptide L- gama- glutamyl- L-cysteinylglycine in both reduced and dimeric forms. Glutathione is widely found in the forms of life and plays an essential role in the health of organisms. In plants, glutathione is the predominant non- protein thiol and functions as a redox buffer, keeping with its own SH groups those proteins in a reduced condition among other antioxidant activities. It is known

Fruit splitting and productivity of pomegranate trees

chemically as N-L-gamma- glutamyl- L cysteinyl glycine and is abbreviated as GSH. Its molecular formula is $C_{10}H_{17}N_3O_6S$. Glutathione is the principal intracellular non protein thiol and plays a major role in the maintenance of the intracellular redox state. It may be thought of as an intracellular redox buffer. Glutathione is a scavenger cabenger and an electron donor via the sulfhydryl group of its buniness reside cysteine. Its reducing ability maintains molecules such as ascorbate and proteins in their reduced state. It is also the cofactor for the major antioxidant enzymes Another antioxidant activity of glutathione is the maintenance of the antioxidant reducing agent ascorbate in its reduced state. Thereby, the idea of using glutathione as antioxidant and zinc sulphate for alleviating fruit splitting and improving productivity of pomegranate trees was arised.

The promoting effect of zinc on yield and quality of fruit trees were mentioned. (Yogaratham and Greenham 1982; Mohamed and Ahmed, 1991; Sandhu *et al.*, 1993 and 1994 ; Ahmed *et al.*, 1995 and 1997; Guo and Xu, 1998 and Gobara, 1998) and antioxidants (Ahmed *et al.*, 1998 ; Vorobev, 1999; Ahmed and Morsy, 2001 ; Abd El- Aziz, 2001 ; Ahmed *et al.*, 2003 , Mansour *et al.*, 2006 and Ahmed and Abdel aal , 2007)

Water stress during all stages of fruit crops development was responsible for reducing yield and improving fruit quality (Israelson and Hanson, 1962; Kramer, 1983 ; Li *et al.*, 1989; Johnson, *et al.*, 1992 Lawand and Patil, 1994; Hibbs *et al.*, 1994, Gupta *et al.*, 1999 ad El- Sisy, 2001)

The objective of this study was to examine the effect of water withholding before ripening, zinc sulphate and glutathione on fruit splitting , yield and fruit quality of Manfalouty pomegranate trees.

MATERIALS AND METHODS

This study was carried out during 2003 and 2004 seasons on 27 uniform in vigour 12 years old own on rooted Manfalouty pomegranate trees in a private orchard situated at Meat Dawood village, Gerga district, Sohag Governorate where the texture of the soil is sandy. The selected trees are planted at 3.5 x 3.5meters apart.

Surface irrigation system was followed. Soil analysis of the tested soil according to Wilde *et al.*, (1985) are shown in Table 1.

Table 1 : Analysis of the tested soil

Sand %	71.2
Silt %	20.0
Clay %	8.8
Texture	Sandy
pH	7.85
O.M. %	0.6
Total CaCO ₃ %	1.9
Total N % (Olsen)	0.03
Available P (Olson, ppm)	0.5
Available K (ammonium acetate, ppm)	40

This experiment involved two factors (A and B) . The first factor (A) consisted from the following three durations of water withholding before fruit ripening:

- a₁) 7 days water with holding before ripening
- a₂) 14 days water with holding before ripening
- a₃) 21 days water with holding before ripening

While, the second factor (B) comprised from the following three treatments from zinc and glutathione applications.

- b₁) spraying the trees with water (untreated trees)
- b₂) spraying the trees with zinc sulphate (13 % zn) at 0.3 %
- b₃) spraying the trees with glutathione at 0.3 %

Therefore, the present experiment involved nine treatments. Each treatment was replicated three times, one tree per each. Both zinc sulphate and glutathione was applied once at two weeks after fruit setting. Triton B as a wetting agent at 0.1 % was added to all solutions of zinc sulphate and glutathione. Spraying was done till run off. Randomized completely block design in split plot arrangement was adopted. The three treatments of duration of water withholding before fruit ripening occupied the main plots, and the three treatments of zinc and glutathione ranked the sub- plots.

Fruit splitting and productivity of pomegranate trees

All the selected trees (27 trees) were subjected to the same common horticultural practices that are usually applied in the orchard except the application of zinc sulphate and glutathione.

At harvesting time (1st week of Oct. in both seasons), the yield / tree expressed in weight (kg.) was recorded. Fruit splitting was estimated by counting the number of splitting fruits / tree and dividing this number by the total number of fruits / tree and multiplying the product by 100.

Ten fruits per tree was taken randomly for determining the following physical and chemical characters of the fruits.

- 1- Fruit weight (g.) and dimensions (length and width in cm.)
- 2- Percentages of fruit pulp and peels .
- 3- Fruit peel thickness(cm.)
- 4- Percentage of total soluble solids.
- 5- Percentage of total acidity (expressed as g citric acid / 100 ml juice (A.O.A.C., 1985).
- 6- Percentage of total sugars and total soluble tannins according to A.O.A.C. (1985)

All obtained data were tabulated and statistically analyzed according to Mead *et al.*, (1993).

RESULTS AND DISCUSSION

Yield :

Data in Table 2 clearly show that varying durations of water withholding before fruit ripening significantly affected the yield. Increasing days of water withholding from 7 to 21 days before ripening caused a gradual decline in yield. Significant differences in yield was observed among the three durations . The maximum and minimum yields were recorded on the trees water withholding with 7 and 21 days before ripening, respectively. These results were true in both seasons.

The beneficial of water on cell division, bud transformation, photosynthesis and the translocation of nutrients from roots to top parts of the trees explain the present results (Israelson and Hanson, 1962).

Table 2: Effect of duration of water withholding before fruit ripening on some physical characters of fruits, zinc sulphate and Glutathione on yield (kg.), fruit splitting % of Manfalouty pomegranate trees during 2003 and 2004 seasons.

Spraying zinc sulphate and Glutathione (B)	Yield (tree / kg)								Fruit splitting %							
	2003				2004				2003				2004			
	Duration of water withholding before fruit ripening (A)															
	7	14	21	Mean (B)	7	14	21	Mean (B)	7	14	21	Mean (B)	7	14	21	Mean (B)
Sp raying water	74.0	72.0	70.0	72.0	31.0	29.0	27.0	29.0	26.0	28.0	30.0	28.0	22.0	24.0	26.5	24.2
Sp raying Zn sulphate at 0.3%	77.1	75.1	73.0	75.1	36.0	34.0	31.5	33.8	22.0	24.0	27.0	24.3	19.0	21.1	23.5	21.2
Sp raying Glutathione at 0.3%	81.1	79.0	77.0	79.0	39.0	36.0	34.0	36.3	21.5	23.0	25.0	23.2	16.0	18.0	20.1	18.0
Mean (A)	77.4	75.4	73.3		35.3	33.0	30.8		23.2	25.0	27.3		19.0	21.1	23.4	
L.S.D. at 5 %	A	B	AB		A	B	AB		A	B	AB		A	B	AB	
	1.3	1.8	3.1		1.8	1.9	3.3		1.7	0.8	1.4		1.4	1.1	1.9	
	Fruit weight (g.)								Fruit length (cm.)							
Sp raying water	225	233	222	223.3	250	247	245	247.3	7.1	7.0	6.9	7.0	7.5	7.4	7.3	7.4
Sp raying Zn sulphate at 0.3%	300	299	297	298.7	315	311	310	312.0	8.0	7.9	7.8	7.9	8.5	8.4	8.3	8.4
Sp raying Glutathione at 0.3%	350	348	346	348.0	380	377	375	377.3	8.9	8.8	8.7	8.8	9.2	9.2	9.1	9.2
Mean (A)	291.7	290.0	288.3		315	311.7	310		8.0	7.9	7.8		8.4	8.3	8.2	
L.S.D. at 5 %	A	B	AB		A	B	AB		A	B	AB		A	B	AB	
	NS	15.0	26.0		NS	14.0	24.2		NS	0.6	1.0		NS	0.6	1.0	

Fruit splitting and productivity of pomegranate trees

The reduction on the yield in response to water stress was also reported by Hibbs *et al.*, (1994), Gupta *et al.*, (1999) and El- Sisy (2001).

It is clear from the data in Table 2 that foliar application of zinc sulphate or glutathione each at 0.3 % was significantly very effective in enhancing the yield compared to the check treatment. Spraying glutathione was preferable than zinc sulphate in improving the yield. These results were true in both seasons.

The interaction between water stress before ripening and the application of zinc sulphate and glutathione had significant effect on the yield. The maximum yield (81.1 and 39.0 kg / tree) was recorded on the trees one week water withholding before ripening and treated once with glutathione at 0.3 % in both seasons.

The trees three weeks water withholding before maturation and did not spray with any material gave the minimum yield (70 and 27 kg / tree in both seasons). These results were true in both seasons.

The positive action of Zn and Glutathione on stimulating growth and nutritional status of the tree could results in improving the yield.

The results of Ahmed *et al.*, (2003); Mansour *et al.*, (2006) who worked on antioxidants as well as Ahmed *et al.*, (1995) and (1997) , Guo and Xu (1998) and Gobara (1998) who worked on zinc supported the present results.

Fruit splitting %:

It is clear from the data in Table 2 that fruit splitting was adversely affected by water withholding before ripening. It increased significantly by water withholding with one to three weeks before ripening. There was a gradual increment on fruit splitting with increasing duration of water withholding from one to three weeks. The maximum values were recorded on the trees three weeks water withholding before ripening. One week water withholding before ripening effectively minimized such percentage. Similar results were obtained in both seasons.

The effect of water on building cell wall and the biosynthesis of cellulose and lignins could explain the present results (Kramer, 1983). The results of Li *et al.*, (1989) confirmed the present results.

It is evident from the obtained data that spraying zinc sulphate or glutathione each at 0.3 % significantly was accompanied with reducing fruit splitting rather than the check treatment. Application of glutathione at 0.3 % was preferable than using zinc sulphate in reducing fruit splitting. Significant differences were observed on fruit splitting among three studies treatments. The lowest values were detected on the trees sprayed once with glutathione at 0.3 % . Untreating the trees gave the maximum values. These results were true in 2003 and 2004 seasons.

Fruit splitting was significantly affected by the studied interactions. The minimum percentage of fruit splitting (21.5 and 16.0 % in both seasons) were recorded on the trees one week water withholding before ripening and sprayed with glutathione at 0.3 % . One week water withholding without the application of zinc sulphate and glutathione gave the maximum values (3.0 and 26.5 % in 2003 and 2004 seasons). Similar trend was noticed in both seasons.

The essential role of Zn and glutathione on building cell wall of fruits and adjusting water relations with plant tissues could explain the present results. The important role of Zn and glutathione on enhancing cell division could result in encouraging the growth of peels and pulp similarly. These results are in agreement with those obtained by Gupta *et al.*, (1999).

Physical fruit quality:

Data in Tables 2 and 3 clearly show that varying duration of water withholding before ripening had no effect on weight and dimensions of fruit. Percentage of pulp as well as weight and thickness of peels were significantly affected by varying durations of water withholding before ripening. Percentage of pulp and peel thickness were gradually reduced, while fruit peels was increased with increasing durations of water withholding before ripening from one to three weeks. These results could be attributed to water stress conditions which resulted in drying pulp and produced thinner fruit peel. These results are in agreement with those obtained by Li *et al.*, (1989), Lawand and Pratt (1994) and El- Sisy (2001).

Table 3: Effect of duration of water withholding before fruit ripening on some physical characters and chemical characters of the fruits of Manfalouty pomegranate trees during 2003 and 2004 seasons.

Spraying zinc sulphate and Glutathione (B)	Fruit width (cm.)								Pulp %							
	2003				2004				2003				2004			
	Duration of water withholding before fruit ripening (A)															
	7	14	21	Mean (B)	7	14	21	Mean (B)	7	14	21	Mean (B)	7	14	21	Mean (B)
Spraying water	5.5	5.5	5.4	5.5	6.0	5.9	5.8	5.9	55.0	53	51	53.0	57	55	54	55.3
Spraying Zn sulphate at 0.3%	6.3	6.2	6.1	6.2	7.1	7.0	7.0	7.0	61.0	60	57	59.3	62	61	60	61.0
Spraying Glutathione at 0.3%	7.5	7.4	7.4	7.4	8.2	8.1	8.0	8.1	66.0	64	63	64.3	67	65	63	65.0
Mean (A)	6.4	6.4	6.3		7.1	7.0	6.9		60.7	59.0	57.0		62.0	60.3	59.0	
L.S.D. at 5 %	A	B	AB		A	B	AB		A	B	AB		A	B	AB	
	NS	0.5	0.9		NS	.6	1.0		1.0	1.1	1.9		1.0	1.2	2.1	
	Fruit peels %								Fruit peel thickness (cm.)							
Spraying water	45	47	49	47.0	43	45	46	44.7	4.7	4.5	4.0	4.4	4.9	4.5	4.2	4.5
Spraying Zn sulphate at 0.3%	39	40	43	40.7	38	39	40	39.0	4.5	4.0	3.8	4.1	4.4	4.0	3.8	4.1
Spraying Glutathione at 0.3%	34	36	37	35.7	33	35	37	35.0	3.6	3.3	3.0	3.3	4.0	3.7	3.8	3.7
Mean (A)	39.3	41.0	43.0		38.0	39.7	41.0		4.3	3.9	3.6		4.4	4.1	3.8	
L.S.D. at 5 %	A	B	AB		A	B	AB		A	B	AB		A	B	AB	
	1.1	1.6	2.8		1.0	1.6	2.8		0.5	0.3	0.5		0.3	0.4	0.7	

It is obvious from the obtained data that spraying zinc sulphate or glutathione each at 0.3 % was significantly accompanied with improving fruit weight and dimensions as well as pulp and at the same time was responsible for reducing fruit peel weight and thickness compared to the check treatment. Application of glutathione was preferable than zinc sulphate in improving physical quality of the fruits. The lowest fruit weight and dimensions as well as pulp and the highest fruit peel weight and thickness were recorded on untreated trees. These results were true in both seasons.

The studied interaction had obvious effect on physical characters of the fruits. The maximum fruit weight and dimensions as well as the highest pulp and the minimum fruit peel weight and thickness were recorded on the trees one week water withholding before ripening and sprayed with glutathione at 0.3 % . Leaving the trees without irrigation before ripening with one week and neglecting the applications of these materials gave unfavourable effects. Similar trend was observed in both seasons. These results are in concordance with those obtained by Ahmed *et al.*, (1998) and (2003) and Mansour *et al.*, (2006) on antioxidants and Ahmed *et al.*, (1995) and (1997) on zinc.

Chemical fruit quality:

Data in Table 4 clearly show that increasing durations of water withholding from 7 to 21 days before ripening caused a gradual promotion on chemical quality of the fruits in terms of increasing total soluble solids and total sugars and decreasing both total acidity and total soluble tannins. Leaving the trees without irrigation for three weeks before ripening effectively enhanced chemical quality of the fruits in both seasons. The effect of water stress on concentrating of total soluble solids namely sugars, and pigments could explain the present results .The results of El- Sisy (2001) confirmed the present findings.

It is clear from the obtained data that foliar application of zinc sulphate or glutathione each at 0.3 % resulted in great stimulation on chemical quality of fruits. Spraying glutathione was favourable than zinc sulphate in this respect. The best results with regard to chemical

Table 4: Effect of duration of water withholding before fruit ripening on some physical characters and chemical characters of the fruits of Manfalouty pomegranate trees during 2003 and 2004 seasons.

Spraying zinc sulphate and Glutathione (B)	T.S.S.								Total acidity %							
	2003				2004				2003				2004			
	Duration of water withholding before fruit ripening (A)															
	7	14	21	Mean (B)	7	14	21	Mean (B)	7	14	21	Mean (B)	7	14	21	Mean (B)
Spraying water	14.7	15.2	16.3	15.4	14.0	15.0	16	15.0	1.95	1.80	1.61	1.79	1.97	1.84	1.66	1.82
Spraying Zn sulphate at 0.3%	15.8	16.5	17.0	16.4	15.5	16.0	17	16.2	1.51	1.41	1.29	1.40	1.55	1.45	1.30	1.43
Spraying Glutathione at 0.3%	18.0	19.0	19.2	18.7	17.5	18.0	18.0	18.0	1.30	1.11	1.05	1.15	1.33	1.12	1.08	1.18
Mean (A)	16.2	16.9	17.5		15.7	16.3	17.2		1.59	1.44	1.32		1.62	1.47	1.35	
L.S.D. at 5 %	A	B	AB		A	B	AB		A	B	AB		A	B	AB	
	0.5	0.5	0.9		0.5	0.6	1.0		0.11	0.13	0.22		0.11	0.14	0.23	
	Total sugars %								Total soluble solids %							
Spraying water	13.2	13.9	14.5	13.9	12.0	12.5	12.8	12.4	2.8	2.5	2.0	2.4	3.0	2.4	2.2	2.5
Spraying Zn sulphate at 0.3%	14.4	15.0	15.6	15.0	14.0	15.0	15.5	14.8	2.0	1.6	1.2	1.6	1.8	1.5	1.3	1.5
Spraying Glutathione at 0.3%	16.2	16.9	17.1	16.7	16.0	17.0	17.3	16.8	1.2	1.0	0.9	1.0	1.5	1.2	1.1	1.3
Mean (A)	14.6	15.3	15.7		14.0	14.8	15.2		2.0	1.7	1.4		2.1	1.7	1.5	
L.S.D. at 5 %	A	B	AB		A	B	AB		A	B	AB		A	B	AB	
	0.3	0.5	0.9		0.5	0.5	0.9		0.3	0.3	0.5		0.2	0.2	0.3	

quality of the fruits were obtained on the trees three weeks water withholding before ripening as well as application of glutathione once at 0.3 %. Unfavourable effects were recorded on untreated trees and one week water withholding before ripening in both seasons. The advancing effect of Zn and glutathione on maturity explain the present results. These results are in concordance with those obtained by Mansour *et al.*, (2006) and Ahmed and Abde aal (2007) who worked on antioxidants and Gobara (1998) who worked on zinc.

As a conclusion, one week water withholding before ripening and application of Glutathione once two weeks after fruit setting at 0.3 % is suggested to be very beneficial for controlling fruit splitting as well as improving yield and fruit quality of Manfalouty pomegranate trees growing under sandy soil.

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تأثير منع الري عن الاشجار قبل النضج والزنك والجلوتاثيون على تشقق الثمار وإنتاجية أشجار الرمان المنفلوطى

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تم منع الري عن اشجار الرمان المنفلوطى قبل النضج باسبوع ، اسبوعين، ثلاثة اسابيع مع الرش الورقى لكبريتات الزنك او الجلوتاثيون بتركيز ٠,٣ % لكل منهما مرة واحدة بعد عقد الثمار باسبوعين.

اشارت نتائج الدراسة الى ان زيادة فترة التصويم من اسبوعين الى ثلاثة اسابيع قبل النضج يودى الى زيادة تدريجية فى النسبة المئوية لتساقط الثمار والنسبة المئوية لقشرة الثمار والنسبة المئوية للمواد الصلبة الذائبة الكلية والنسبة المئوية للسكريات الكلية اما كمية المحصول للشجرة والنسبة المئوية لللب وسمك القشرة والنسبة المئوية للتأين الذائب والحموضة الكلية فقد كانت تقل تدريجيا ولم يكن لفترات منع الري ايه تأثيرات على وزن وابعاد الثمرة كما أدى رش الاشجار مرة واحدة بكبريتات الزنك و الجلوتاثيون بتركيز ٠,٣ % والنسبة المئوية لتساقط الثمار والنسبة المئوية للقشرة وسمك القشرة والنسبة المئوية لكلامن الحموضة الكلية والتأين السذاب وذلك بالمقارنة بعدم الرش وكان استخدام الجلوتاثيون افضل من استخدام كبريتات الزنك فى هذا الصدد.

يقترح لرفع الانتاجية تصويم اشجار الرمان لمدة اسبوع قبل النضج مصحوبا برش الجلوتاثيون مرة واحدة بعد العقد باسبوعين بتركيز ٠,٣ %.