

## **EFFECT OF SPRAYING SOME MINERAL NUTRIENTS AND METHANOL ON BERRY SUNBURN AND BUNCH ROT IN REDGLOBE GRAPEVINES**

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### **ABSTRACT**

Field experiments were conducted during 2005 and 2006 on 7-year-old grapevines cv. Redglobe grown in a clay loamy soil at 1.5x3 meters, used flood irrigation trained to double cordon with a load of 60 buds per vine ( 20 fruiting spurs: 3 buds / spur) pruning was carried out at the third week of January of each season to examine the effect of foliar sprays of some micro nutrients and methanol on berry sunburn and bunch (berry) rot in seeded Redglobe. Leaf content of chlorophyll a, b and carotenoids increased after spraying Ca, Zn, Cu and methanol. Foliar application of methanol increased bud burst percentage, all treatments significantly improved vegetative growth of the vine. Maximum yield weight and biggest bunch components resulted from methanol application (25%) compared to the control. Untreated vines gave the highest percentage of infected berries whether sunburn or rots (16.24 % per vine). Spraying Ca, Zn, Cu and their combination or methanol reduced percentage of infected berries by about (84, 88, 90, 82.4 and 88% respectively). All treatments increased total soluble solids (TSS %) and TSS / acid ratio; while, total acidity decreased. Application of Ca had no effect on anthocyanin content of berry skin; however, the rest of treatments increased anthocyanin content. Total carbohydrates in canes and weight of prunings per vine were increased by all treatments. There was a strong significant positive correlation between yield weight: weight of prunings and percentage of infected berries ( $r^2 = 0.930$ ). Most of the positive effects resulted from spraying 20% methanol.

**Keywords:** table grape, fruit quality, yield, micronutrients, methanol, sunburn, bunch rot

### **INTRODUCTION**

Redglobe is a promising seeded table grape cultivar grown in Egypt. Redglobe produces the largest berries among the table grape cultivars currently grown in Egypt; consumers prefer large berries and the presence of seeds in the berries is not considered a problem.

The influence of minerals on the suppression of berry sunburn and bunch rot pathogens has received little attention, and the potential for utilizing mineral amendments for optimizing bio-control remains largely unexplored. Micro- and macro-element amendments have been used commercially on a limited scale to manage certain soil borne diseases of tomato and other vegetable crops (Engelhard, 1989). Disease reduction is most often attributed to improve nutrition that boosts host defenses or to direct inhibition of fungal growth and activity. Pathogen suppression may also result indirectly from amendment-mediated modification of chemical and physical properties, such as soil and rhizosphere pH (Simon, and Sivasithamparam (1989) or from modification of host root exudates to disfavor pathogenic activity. In a few cases, though, mineral amendments appear to reduce disease by indirectly

stimulating indigenous populations of microorganisms that are beneficial to plant growth and antagonistic to pathogens (Huber, 1989).

The production of high quality Redglobe grapes faced several challenges. Redglobe grapevines are of moderate vigor when planted on their own roots and over cropping can be a problem.. Clusters are highly susceptible to sunburn damage, and the moderate vigor likely contributed to this problem.

Most studies have focused only on NPK with few reports including the important plant nutrients. Micronutrients are essential for good plant growth and vigor. Ca, Zn and Cu are important components of various enzymes responsible for many metabolic reactions (Salisbury & Ross, 1992).

Many experiments showed that foliar application of methanol on plants increased growth vigor and yield ( Nonomura & Benson, 1992 b; Dèvlin et al. 1994 and Dwivedi et al. 2001).

Calcium is a divalent cation important for maintaining the strength of stem and stalk of plants. This mineral also regulates the absorption of nutrients across plasma cell membrane. Furthermore, calcium has a role in plant cell elongation and division, structure and permeability of cell membrane, nitrogen metabolism, and carbohydrate translocation (Plieth, 2005). Application of methanol directly affects metabolic pathways related to plant growth and development. However, the role of methanol as a plant growth regulator (Dwivedi et al. 2001) or as an agent to enhance vine vigor, fruit quality (Ramadan & Omran, 2005) and to advance bud burst and maturity needs more studies.

The objectives were to determine the influence of calcium, zinc, copper amendments and methanol on vegetative growth and fruit quality of Redglobe grapevines.

## **MATERIALS AND METHODS**

This study was carried out during two successive seasons 2005 and 2006 on 180 seeded Redglobe fruitfull grapevines planted on their own roots at the orchard of EL-Keissey, Minia Governorate. The vines were 7- year-old, grown in calay loamy soil at 1.5x 3 meters; trained to double cordon with a load of 60 buds per vine (20 fruiting spurs 3 buds spur pruning was carried out at the third week of January of each season. Crop load was adjusted to 20 clusters per vine immediately after fruit set. The treatments were repeated on the same vines for two seasons. The vines were divided into 6 groups, 30 / each. Group one was used as control; the 2nd was treated with 1.5g/ litre calcium ( CaCl<sub>2</sub>, 15% Ca), the 3 rd was treated with 1.5g/litre zinc ( ZnSO<sub>4</sub>, 20% Zn), the 4th are treated with 1.5g/litre copper (CuSO<sub>4</sub>, 3.5% Cu), the 5th was treated with a mixture of the three aforementioned compounds, and the sixth was treated with 20 % methanol. Vines were sprayed three times: at 2 weeks before bud burst, 2 weeks before fruit set and 2 weeks before veraison. Three liter of solution was sprayed on each vine. Cultural operations including Dormex spray were performed in accordance with the standard commercial practices already carried out for Redglobe table grapes.

### **Photosynthetic pigments:**

Ten mature leaves per replicate were collected from the middle part of the shoot for the determination of chlorophyll a, b, and total carotenoids ( $\text{mg.g}^{-1}$  fresh weight) according to Wellburn (1994).

#### **Leaf analysis:**

petioles of leaves opposite to the clusters were collected at veraison for chemical analysis according to N (Pregl,1945),P (Jackson,1958), K and Ca (Brown and Lilleland,1946), Zn and Cu (Piper,1950).

#### **Vegetative growth:**

The number of young and fully expanded leaves per shoot was counted and leaf area was measured for 30 leaves per vine positioned opposite to the basal clusters. ultimate shoot length, number and diameter, density (shoot / m canopy) were estimated.

#### **Bud burst percentage:**

The number of bursted out buds was recorded for each treatment at the season followed that season of application and was calculated according to the following equation: Bud burst % = number of bursted out buds / total number of buds left per vine) x100

#### **Yield and bunch quality:**

Yield per vine (kg) was recorded at the time of harvest. A sample of 30 bunches per treatment was picked up and the following parameters were determined: (1) average bunch weight, berry weight and size , number of berries per bunch (2) fruit composition: 100 berries were taken from each replicate and crushed to determine total soluble solids (TSS%)by using a hand refractometer, total acidity (as g tartaric acid per 100 ml juice) according to the A.O.A.C (1975), and the ratio of TSS/acid was thus calculated. In addition ,the content of total anthocyanins in berry skin was determined according to Rabino *et al.*(1977).Total carbohydrates in the canes were determined according to Dubois *et al.* (1956).

**Statistical analysis:** Data were statistically analyzed using SPSS (SPSS Inc.). Analysis of variance was carried out using a general one –way model, and student Newman-Keuls(S-N-K) was used for comparison between particular means. Simple correlations or linear regressions were carried out between different parameters (Snedecor &Cochran, 1972).

## **RESULTS AND DISCUSSION**

### **Photosynthetic pigments:**

Fig.1 revealed that spraying Ca, Zn, Cu and methanol significantly increased leaf content of chlorophyll a (chl. a), chlorophyll b (chl b) and total carotenoids in both of the study seasons. It is evident that foliar application of ca, Zn, Cu or methanol induced significant increments of photosynthetic pigments .Considerable increases were obtained by all treatments especially copper or methanol. It is obvious that there is a strong significant positive correlation between nitrogen, phosphorous and potassium levels in the leaves at veraison stage and total carotenoids ( $r^2 = 0.926, 0.908$ and  $0.756$ ).Furthermore. There is a relationship between spraying copper and pigment photosynthetic content ( $r^2= 0.923, 0.629$  and  $0.742$ ).

The results showed that the application of Ca, Zn, Cu or methanol is effective in increasing photosynthetic pigments of Redglobe leaves. this may be due to element functions. Zinc plays a vital role in many important enzyme systems, exerts an effect on carbohydrate metabolism through its effects on photosynthesis and sugar transformation, a constituent of other enzymes involved in photosynthesis and formation of chlorophyll. Cu, is needed as a cofactor of plastocyanin to participate in electron transfer reactions of photosynthesis in the form of plastocyanin (Yruela *et al.* 2000), has a role in photosynthesis and chlorophyll formation (Raven *et al.* 1999). Calcium serves as a signaling messenger for ABA-induced stomatal closure. The significant effect of methanol application on Chl .a, b and total carotenoids of Redglobe grapevines may be due to the increase in number of leaves and leaf area, resulted from abundant CO<sub>2</sub> supply and the increased number of stomata per mm<sup>2</sup> at the abaxial leaf surface after spraying 10-50% of methanol (Ramadan and Omran, 2005).

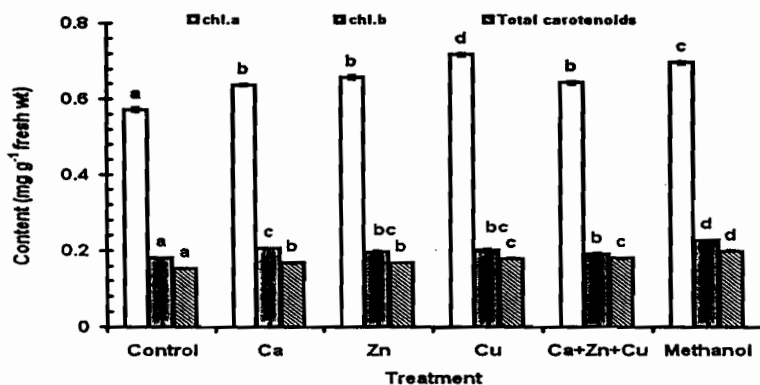


Figure 1: Content of chlorophyll a,b and total carotenoids in leaves of Redglobe grapevines. Values are means±SE,n= 3,means of each component with different letters are significantly different at p<0.05 according to the S-N-K test.

#### Mineral composition.:

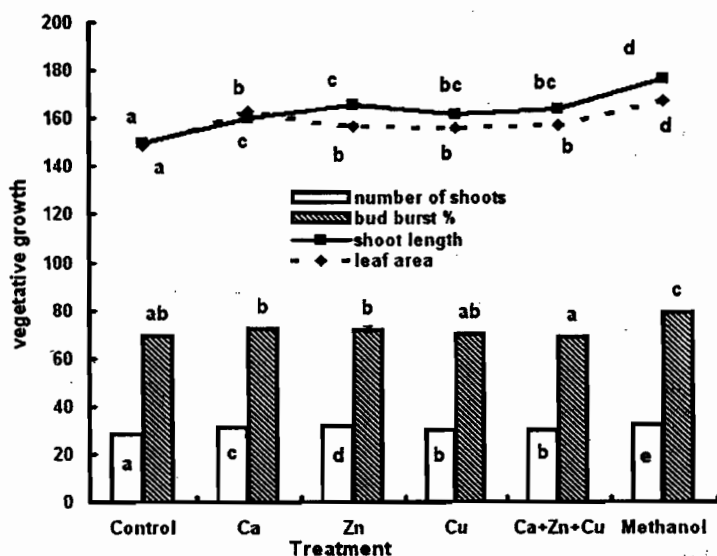
The concentration of NPK, Zn and Cu in leaf petiole significantly increased by foliar application of Zn, Cu, (Ca+ Zn +Cu) or methanol. (Table, 1) Ca did not found to have no effect on the concentration of nitrogen as well as all the treatments which showed insignificant effect in this respect except methanol application. The highest concentrations of elements were obtained form methanol application. This may be due to increasing the activity of photosynthesis by these elements .The positive effect of methanol application on petiole composition may be due to abundant CO<sub>2</sub> supply from methanol as suggested by (Hemming *et al.* 1995), and which may have reduced photorespiration in favor of photosynthesis.

**Table 1: Influence of foliar application with Ca, Zn, Cu and their combination and methanol on leaf petiole mineral composition of Redglobe grapevines. Different superscript letters are significantly different at  $p < 0.05$ .**

Treatment	Petiole composition(%)			petiole composition (mg/kg)		
	N	P	K	Ca	Zn	Cu
1:Untrated control	0.70 <sup>a</sup>	0.145 <sup>a</sup>	2.20 <sup>a</sup>	1.06 <sup>a</sup>	42.8 <sup>a</sup>	2.11 <sup>a</sup>
2: Ca	0.72 <sup>a</sup>	0.167 <sup>b</sup>	2.71 <sup>b</sup>	1.21 <sup>a</sup>	59.4 <sup>b</sup>	2.42 <sup>b</sup>
3: Zn	0.75 <sup>b</sup>	0.186 <sup>b</sup>	2.74 <sup>b</sup>	1.20 <sup>a</sup>	70.1 <sup>c</sup>	2.51 <sup>b</sup>
4: Cu	0.76 <sup>b</sup>	0.190 <sup>b</sup>	2.73 <sup>b</sup>	1.15 <sup>a</sup>	58.8 <sup>b</sup>	3.04 <sup>c</sup>
5: Ca +Zn + Cu	0.78 <sup>b</sup>	0.213 <sup>c</sup>	2.74 <sup>b</sup>	1.18 <sup>a</sup>	52.0 <sup>c</sup>	3.11 <sup>c</sup>
6: Methanol	0.81 <sup>c</sup>	0.229 <sup>c</sup>	2.94 <sup>b</sup>	1.41 <sup>b</sup>	71.3 <sup>c</sup>	3.07 <sup>c</sup>

**Vegetative growth:**

Data in fig 2. Show that all treatments had no effect on bud burst %, with the exception of methanol that significantly affected this estimate. All treatments significantly increased leaf area (cm<sup>2</sup>), vine leaf area (m<sup>2</sup>), number of leaves per shoot, length and diameter of the shoots and number of shoot vine Table (2). The highest value was observed after using 20% methanol.



**Figure 2: Effect of calcium,zinc,copper and methanol on vegetative growth of Redglobe grapevines. Values are means  $\pm$  SE, n=30, means with different letters are significantly different at  $p < 0.05$  according to the S-N-K test.**

The stimulatory effect of Ca, Cu, Zn and methanol application on canopy could be due to the important role of these elements and methanol in facilitating the availability of mineral or organic nutrients to the vines and utilization of methanol as a carbon source Nonomura & Benson (1992 a) ,

Brown *et al.*(1993), Poovaiah & Reddy (1993) & Raven *et al.*(1999).CO<sub>2</sub> increased in the ambient air as a result of methanol oxidation (Gout *et al.* 2000) and depressing effect of CO<sub>2</sub> may be partially indirect, this effect can cause stomatal closure, thus limiting respiration and increasing photosynthetic efficiency of leaves (Lieberman and Wang, 1982 ) A highly significant positive correlation was found between shoot length and leaf area, shoot number or shoot diameter ( $r^2 = 0.705$  0.812 and 0.956).In addition, there is a positive correlation between shoot density and total carbohydrates, yield weight, TSS% and anthocyanins ( $r^2 = 0.941, 0.787, 0.909$  and 0.779).

These results are supported by (Zbiec' *et al.* 2003) who found that spraying methanol 10-40% solutions on some plants significantly improved vegetative growth. The positive effect of methanol application on leaf area may be due to abundant CO<sub>2</sub> supply from methanol as supposed by (Hemming *et al.* 1995), this may cause photorespiration in favour of balancing the nutritional status of leaves by acting as a carbon source (Benson & Nunomura, (1992) or by enhancing the engendered root activity ( Makhdum *et al.*2002).These results are in agreement with those obtained by (Ramadan & Omran, 2005) who found that methanol had a significant effect on canopy of Flame Seedless grapevines.

**Table 2: Influence of foliar application of Ca, Zn, Cu and their combination or methanol on vine growth, yield and total carbohydrates of Redglobe grapevines. Values are mean, n = 30 for each treatment with different superscript letters are significantly different at p < 0.05 .**

Parameter	Treatment					
	Control	Ca	Zn	Cu	Ca+ Zn+ Cu	Methanol
Weight of Pruning (kg/vine)	2.03 <sup>c</sup>	2.66 <sup>b</sup>	2.64 <sup>b</sup>	2.74 <sup>b</sup>	2.89 <sup>c</sup>	3.27 <sup>d</sup>
Shoot diameter(cm)	0.95 <sup>a</sup>	0.99 <sup>b</sup>	1.05 <sup>c</sup>	1.02 <sup>b</sup>	1.01 <sup>b</sup>	1.09 <sup>d</sup>
Shoot density	40.43 <sup>a</sup>	47.50 <sup>b</sup>	47.31 <sup>b</sup>	45.9 <sup>b</sup>	46.95 <sup>b</sup>	53.73 <sup>c</sup>
Crop Weight of Pruning	5.43 <sup>c</sup>	4.44 <sup>a</sup>	4.68 <sup>b</sup>	4.31 <sup>a</sup>	4.33 <sup>a</sup>	4.23 <sup>a</sup>
Total leaf area /vine(m <sup>2</sup> )	11.63 <sup>a</sup>	14.78 <sup>c</sup>	15.07 <sup>c</sup>	13.84 <sup>b</sup>	14.19 <sup>b</sup>	17.57 <sup>d</sup>
Leaf area/fruit wt (cm <sup>2</sup> /g)	13.83 <sup>a</sup>	13.92 <sup>ab</sup>	14.59 <sup>c</sup>	14.24 <sup>b</sup>	14.20 <sup>b</sup>	14.79 <sup>c</sup>
Leaf area/yield weight(m <sup>2</sup> /kg)	1.07 <sup>ns</sup>	1.28 <sup>ns</sup>	1.24 <sup>ns</sup>	1.35 <sup>ns</sup>	1.16 <sup>ns</sup>	1.29 <sup>ns</sup>
Total carbohydrate content of canes	15.33 <sup>a</sup>	16.58 <sup>c</sup>	16.85 <sup>d</sup>	16.08 <sup>b</sup>	16.95 <sup>d</sup>	18.45 <sup>e</sup>

#### Yield components:

As shown in fig 3. Yield per vine was significantly increased by all treatments in comparison with control. Foliar application of Zn or in combination with Ca, and Cu increased yield by 12.5% more than the control. This may be attributed to the positive role copper nutrition the redistribution of zinc within plants. Spraying methanol at 20% increased yield by 25.3% over control. A highly positive correlation was found between yield, leaf area and number of leaves ( $r^2=0.898$  and 0.952). Bunch weight and size and number of berries increased significantly by all treatments. Spraying Ca, Zn and Cu or their combination caused increases in bunch weight by about (7.4: 12.6 %) whereas methanol application increased this parameters by (25.8%) over the control. Furthermore, there is a relationship between berry size and leaf area ( $r^2 = 0.716$ ).Maximum bunch compactness coefficient resulted from

Zn and methanol application. The leaf area: Fruit weight ratio (expressed as  $m^2/kg$ ) has been used as a measure of crop load and vine balance, and approximately 1.15 to 1.30  $m^2/kg$  is generally required to fully ripen Redglobe grapes on double cordon training system. In addition, a fruit: weight of prunings ratio of 4.2 to 4.4 has been used as an indicator of balance for vines capable of producing high quality fruit (Table.2). Generally, the heaviest bunch components were obtained by spraying 20 % methanol. These increments in the yield and yield components may be due to the enhancement of canopy development, and vegetative growth. Ca regulates the absorption of nutrients across plasma cell membranes, and vegetative growth. Ca regulates the absorption of nutrients across plasma cell membranes, has a special function in plant cell elongation and division, structure and permeability of cell membranes, nitrogen metabolism, and carbohydrate translocation (Poovaiah & Reddy, 1993). Zinc induces the synthesis of auxin that positively influences cell enlargement, bud formation and root initiation (Daphne *et al.* 2005).

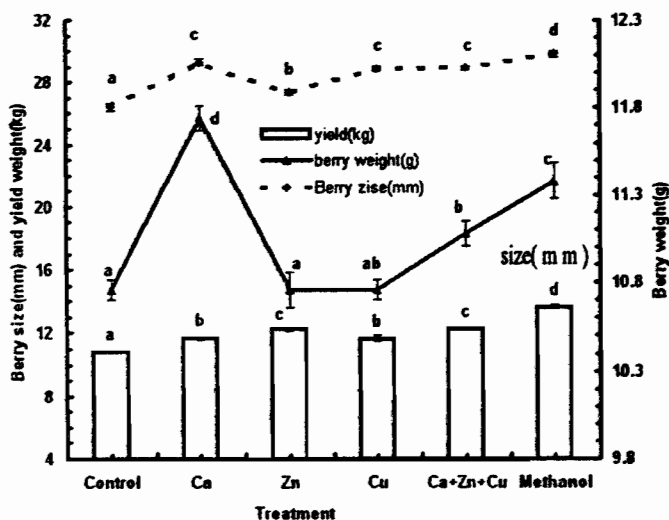


Figure 3: Effect of calcium, zinc, copper and methanol application on yield components of Redglobe grapevines. Values are average of the two seasons  $\pm$  SE $n=30$ , values with different letters are significant different at  $p < 0.05$  according to the S-N-K test.

**Infected berries:**

Fig (4) Shows that untreated vines gave the highest percentage of infected berries either with sunburn or bunch rot (16.24 % per vine), this may be due to the limited of the vegetative growth and sensitivity of this cv to rots under humidity and high temperatures of the climatic. Spraying Ca, Zn, Cu and their combination or methanol reduced percentage of infected berries by about (84, 88, 90, 82.4 or 88% respectively), the effect of these treatments may be due to the direct enhancing effect on foliage canopy and vine vigor. Furthermore, exogenous application of methanol, Ca and Cu affected directly the metabolic pathways related to plant growth and development. In addition, to the pathways related to plant defence mechanisms and produced "alarm

substances" such as ethylene, jasmonic acid, ABA, hydrogen peroxide, a polypeptide systemin, and others, these substances are known to promote the expression of some genes (Ryan, 2001). Furthermore, zinc and other minerals appear to reduce the disease by exerting an indirect beneficial effect on indigenous and introduced antagonistic microorganisms (Elmer, 1995 and Huber, 1989).Cu induced the generation of hydrogen peroxide (Wang *et al.* 2004).

Strong significant positive correlations were found between using Ca and Zn and the reduction of infected berries percentage ( $r^2= 0.827$  and  $0.950$ ). Calcium serves as a detoxifying agent by tying up toxic compounds and maintaining the cation-anion balance in the vacuole. Since calcium is a part of the cell wall it is an important factor for increasing berry firmness and prolonging storage life of fruit. (Tähtiharju *et al.* 1997).

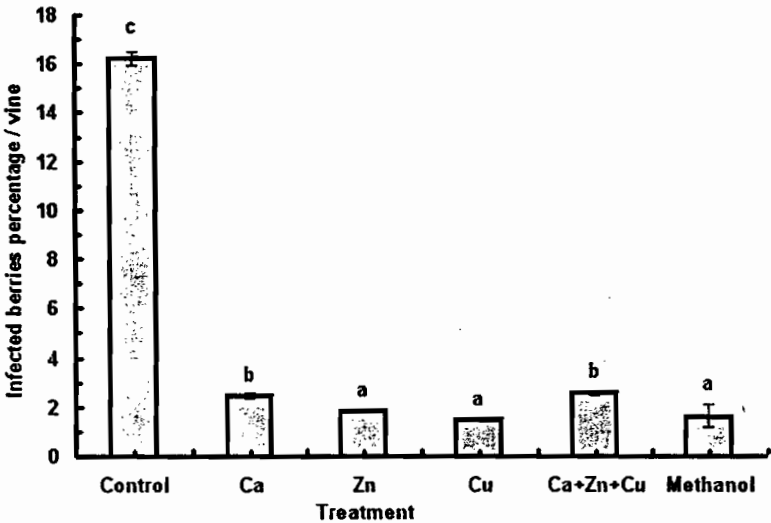


Figure 4: Effect of spraying Ca,Zn,Cu and their combination or methanol on percentage of infected berries with sunburn and rots of Redglobe grapevines.Values are means  $\pm$  SEwith different letters are significantly different at  $p<0.05$ .

#### Fruit quality:

All treatments were found to increase significantly TSS %, TSS / acid ratio of berry fruit, and decreased total acidity percentage (Fig 5). All treatments significantly increased the anthocyanin content in the skin of berries with the exception of spraying with Ca. The highest values of anthocyanins were obtained by 20 % methanol. The results in this concern are in agreement with (Nikolaos *et al.* 2003) who found that spraying vines with methanol advanced maturity and increased anthocyanins in berry skin via



induction of ethylene synthesis. There was a highly significant positive correlation between leaf area / fruit weight and anthocyanins ( $r^2 = 0.837$ ). These results are supported by (Ramadan & Omran, 2005) who found that foliar application of 10-50% methanol caused a significant improvement of Flame Seedless fruit quality. However, the increments in TSS% and anthocyanins may be ascribed to the increase in ethylene production when calcium and copper were applied together (Jones *et al.* 1967) calcium treatment reduced respiration rate and ethylene evolution which may have extended the potential storage life of the fruit (Gregory *et al.* 1988).

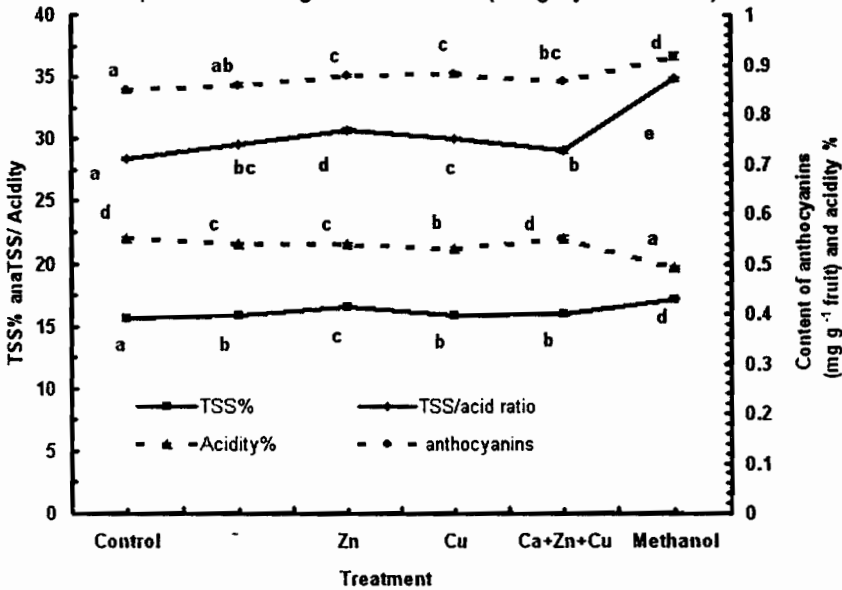


Figure 5: Effect of calcium, zinc, copper and methanol application on fruit quality of Redglobe grapevines. Values are averages of the two seasons  $\pm$  SE n=30, values with different letters are significantly different at  $p < 0.05$  according to the S-N-K test.

**Total carbohydrate content of canes and pruning weight:**

All treatments significantly increased the content of total carbohydrates in the canes. Spraying methanol resulted in the highest increase in this parameter by about (20.4 %) over the control. (Ramadan & Omran, 2005) found that foliar application of 20-30 % methanol significantly increased total carbohydrates and pruning weight of Flame seedless grapevines. Zinc exerts an effect on carbohydrate metabolism through its effects on photosynthesis and sugar transformation to starch and its role in the metabolism of starch could be due to the role of zinc in the integrity of biomembranes (Brown, 1993).

A highly positive correlation was found between total carbohydrates and either vine leaf area or yield weight ( $r^2 = 0.931$  and  $0.976$ ). Pruning weight as a measure of vine vigour and vine capacity, was significantly increased

after spraying either by Ca, Zn, Cu and their combination or especially by methanol. Moreover, yield weight: pruning weight ratio was significantly decreased by all treatments as, there was a strong significant positive correlation between yield weight: pruning weight and infected berries percentage ( $r^2 = 0.930$ ) as well Fig (6).

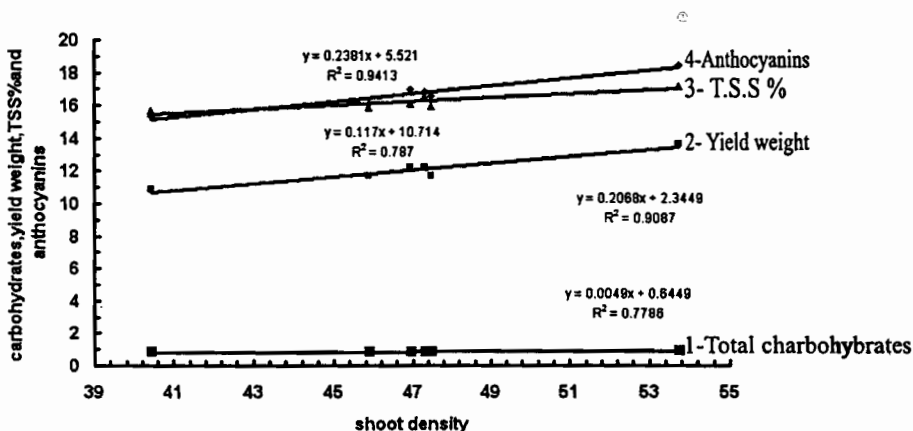


Figure.6: Relationship between shoot density (m/canopy) total carbohydrates, yield weight, TSS% and anyhocyanins of Redglobe grapevines.

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## تأثير الرش بالعناصر الصغرى والميثانول على لفحة الشمس بالحببات وعفن العنقود فى صنف العنب البذرى روجلوب

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أجرى هذا البحث عامى ٢٠٠٥ & ٢٠٠٦ على كرمات عنب صنف روجلوب عمر سبع سنوات لإختبار تأثير الرش بالعناصر الصغرى والميثانول على لفحة الشمس بالحببات وعفن العنقود (الحببات) .

و تتلخص أهم النتائج التي تم الحصول عليها فى الآتى:-

- زاد محتوى الأوراق من كلوروفيل أ ، ب والكاروتينات نتيجة المعاملة بكل من الكالسيوم والزنك والنحاس والمعاملة المشتركة بينهم وكذا الميثانول
- كان الرش بالميثانول بمفرده أكثر تأثيراً على نسبة تفتح البراعم
- أعطت كل المعاملات زيادة معنوية فى النمو الخضرى و المحصول
- أعطت الكروم الغير معاملة أعلى نسبة من العناقيد المصابة بلفحة الشمس والأعفان(١٦,٢٤ % للكرمة)
- أدى الرش بالكالسيوم والزنك والنحاس أو المعاملة المشتركة بينهم وكذلك الميثانول إلى نقص نسبة الحببات المصابة بحوالى (٨٤ ، ٨٨ ، ٩٠ ، ٨٢,٤ ، ٨٨ % على التوالى)
- أدت كل المعاملات إلى زيادة نسبة المواد الصلبة الذاتية ، نسبة المواد الصلبة / الحموضة بعصير الحببات بينما انخفضت النسبة المئوية للحموضة الكلية
- لم يكن للمعاملة بالكالسيوم أي تأثير على محتوى جلد الحبة من الأنثوسيانين بينما زاد هذا المحتوى فى باقى المعاملات
- أدت كل المعاملات إلى زيادة محتوى الكربوهيدرات فى القصبات وكذلك وزن القصاصه للكرمة
- كان هناك ارتباط معنوى قوى بين وزن المحصول ووزن نواتج التقليم وبين نسبة الحببات المصابة وكانت أكثر التأثيرات ايجابية نتيجة للرش بالميثانول بتركيز ٢٠ % ميثانول