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APPLICATION OF YEAST AND OR HYDROGEN CYANAMIDE FOR IMPROVING VINE VIGOR, BUD BEHAVIOR AND FRUIT QUALITY OF THOMPSON SEEDLESS GRAPEVINES

Omran, Y. A. M. M.[1]; T. Ramadan[2] and F.M. El-Borai[3]

1- **Viticulture Department, Horticultural Research Institute, Agriculture Research Center, Giza, Egypt,**

2- **Botany Department, Faculty of Science, Assiut University, Egypt**

3- **Horticulture Department, Faculty of Agriculture, Assiut Univ., Egypt**

ABSTRACT

Field experiment was carried out at the Orchard faculty of Agriculture, Assiut University during three consecutive seasons 2001, 2002 and 2003 on 6-years-old Thompson Seedless grapevines, planted at 1.5x 3 meter in a loamy clay soil. Yeast and /or hydrogen cyanamide (HC) and the time of application affected on the percentage of bud fertility and the results were greatly variable with no trend from season to another; but the highest percentage showed by application of yeast at May. Yeast or HC significantly inhibited catalase activity and increased bud burst. Application of HC at January advanced the timing of bud burst, and gave earliness by about one month compared to control or application of yeast. In the same trend, yeast earlier period from bud burst to full bloom by one - three weeks; hence the vines of all treatments reached full bloom on 10-18 April. Application of yeast at April and May significantly increased fruitful buds. Most treatments improved vine vigor indicated by increasing the shoot length and thickness, number of leaves and leaf area. All vegetative parameters have reflected on the metabolism and parallelly increased the mass of pruning wood, total yield and contents of total carbohydrates and nitrogen in canes. Berries number per bunch and bunch weight were correlated with the weight of rachis. The best juice quality with decreased total acidity and increased TSS/acid ratio was obtained by application of yeast at April and May, or when used in combination with HC.

INTRODUCTION

Numerous studies have been delt with the termination of bud dormancy in grapevines by treatments with chemicals, especially plant growth regulators (Spiers *et al.* (1961 & 1968). In this respect, spraying dormex (hydrogen cyanamide) has been used to induce leaf and flower bud break on many deciduous fruit crops around the world. It has been used on fruit crops to replace inadequate or lack of chilling and stimulate uniform bud break. Physiologically, dormex acts as a plant growth regulator to force the plant to break its dormancy. Dormex causes bud scale removal, terminates rest in grapevines and advances maturity (Omran 2000). In addition, it enhances vegetative growth, yield and fruit quality of Thompson Seedless grapevines (Anon, 1985, Tourky et al, 1995). It is suggested that H_2CN_2 is involved in breaking dormancy by reducing catalase activity in the buds. Accordingly, Shulman *et al.* (1983 & 1986) found that application of hydrogen cyanamide caused a reduction in catalase enzyme activity, while peroxidase activity unchanged in grapevine buds. Boozer and Pitts (2002) found that Dormex exceeded Drop (thidiazuron) as a compound for breaking rest in fruit buds of peach, and the time of application was shown to be important for the use of Dormex.

Hydrogen cyanamide is classified in the European Union as "toxic" if swallowed, "harmful" in contact with skin, "irritating" to eyes and skin, and capable to produce sensitization after skin contact. The US-EPA places Hydrogen cyanamide (the active ingredient) and Dormex (the product which contains 50% HC) into the acute toxicity category I (danger).

Environmentally, yeast (*Saccharomyces cerevisiae*) is safe and non-pollutant compared to H_2CN_2. According to the U.S. Environmental Protection Agency (EPA, 2003), dormex may cause cancer. Many farm workers have suffered from severe skin problems such as burning sensations and cheeks swollen (sometimes to the size of baseballs) from overexposure to dormex. In comparison, yeast contains a considerable amount of amino acids (Abou-Zaid, 1984), mineral elements, carbohydrates, reducing sugars, enzymes and vitamins B1, 2, 6, 12 (Somer, 1987 and Mahmoud, 2001). Also, it is a cheap source of cytokinins and protein that enhance division and enlargement of cells (Ferguson *et al.*, 1987), activate net photosynthesis process and facilitate the opening of stomata (Abd-El-Ghany *et al.*, 2001 and Hassan, 2002). Omran (2000) found that H_2CN_2 or yeast applications enhanced productivity and fruit quality via earlierness, regulation and increase in the rate of bud burst, homogeneity in vegetative growth and bunches, reduction in the phenomena of run-out and millerandage of bunches and homogeneity of harvest date. In addition, yeast increased the bud fertility compared to HC.

The objective of the present study was to compare between the effects of dormex and yeast on buds fertility, burst and behavior, vine vigor and fruit quality of Thompson Seedless grapevines for replacement natural and by using safe material (*Saccharomyces cerevisiae*) instead of chemical material (hydrogen cyanamide).

MATERIALS AND METHODS

This study was carried out during three successive seasons 2001, 2002 and 2003 on 75 Thompson Seedless grapevines at the Orchard of Assiut University. The vigor of selected vines was almost uniform. The vines were 6 years old, grown in clay loam soil at 1.5x3 meters and pruned to canes, leaving 80-buds per vine, at the first week of January of each experimental season. The 75 vines were divided into 15 groups, five each. One group was used as control; two were treated with 2.5% hydrogen cyanamide (HC) at the 1st week of January (65 days before anticipated bud burst) or 2nd week of February (3-weeks before anticipated bud burst); and another four groups were treated with commercial yeast (*Saccharomyces cerevisiae*; 5 g per vine as soil drench) at 1st week of January, 2nd week of February, April (2 weeks pre bloom) or at May (3-4 weeks after anthesis). The rest 8 groups were treated with yeast + HC in combination between each date of applying yeast and the two dates of spraying HC.

The canes of each vine were sprayed with half liter of HC solution after pruning at the first week of January or at the second week of February. The commercial yeast used in this study was active wetted containing 28% dry matter and 75% of yeast fungus cells. Each vine received, base, three liters solution containing 25 g sugar and 5 g suspended yeast as soil drench around the trunk

The following parameters were estimated:

Total catalases

Samples of 4-buds/ vine were collected at one week before bud burst and the scales and woody base were removed. Four buds were weight and then homogenized, using an ice-cooled mortar, in 1 ml of 100 Mm HEPES/ KOH buffer (pH 7.4) and centrifuged at 10000 xg for 10 minutes at 4 °C. The supernatant was used for determination of catalase activity. The activity was measured by estimating O_2 evolution from H_2O_2 conversion (Del Rio *et al.*, 1977) using Clark type oxygen electrode computerized to an oxygen monitoring system (Hansatech Instruments).

Bud fertility%

Sixty buds on five canes (from the first bud at base to 12^{th} one) were taken every season from each treatment at December and examined microscopically as described by Prasad and Pandey (1969). This parameter was recorded on three seasons, where the results of the second and third seasons were compared relative to that in the first season.

$$\text{Percentage of fruitful buds} = \frac{\text{Number of fruitful buds}}{\text{Number of burst buds per vine}} \times 100$$

Bud behavior

Dates of bud burst occurrence, bursted bud number and fruitful buds% were recorded. Number of clusters originating from the bud at different positions on the canes was recorded. The obtained data were calculated using the equations pointed out by Bessis (1960).

$$\text{Percentage of bud burst} = \frac{\text{Number of bursted buds}}{\text{Bud load per vine}} \times 100$$

Also, fertility coefficient was calculated by dividing number of bunches per vine by total number of buds load at winter pruning as mentioned by Bessis (1960). Date of full-bloom was recorded when 90% of flowers was opened (anthesis stage)

Vegetative growth

Length and thickness of five shoots per vine were recorded in November. The diameter was measured in the intermediate internode under the first cluster. Leaf area was measured, according to Sourial *et al.* (1985), in twenty leaves per vine opposite to the first cluster on each shoot at the first week of July in both seasons. Also, the number of leaves per shoot was counted.

Yield and fruit quality

At harvest time: total yield per vine, bunch weight, berry weight and rachis weight were estimated in both 2002 and 2003 seasons.

Total soluble solids (TSS%) using hand refractometer and total acidity % were determined in each season according to A.O.A.C. (1995). Also, TSS/ acid ratio was calculated.

Chemical contents of one-year old wood

Samples of bunches-born canes that were collected at the pruning time in winter, and the following parameters were estimated in triplicates:

- Pruning wood weight (kg): It was recorded per vine by weighting all one-year old shoots as a parameter of vegetative growth vigor.
- Total carbohydrates % was determined according to Dubois *et al.*, (1956).
- Total nitrogen contents according to A.O.A.C. (1995).

Statistical analysis

The experiment was conducted in a split- plot arrangement of randomized complete block design (RCBD) with 5 replicates. Dormex spraying and yeast as soil drench were assigned whole plot, while application times were considered as sub-plot (Mead *et al.*, 1993). The results were tabulated and statistically analyzed using SPSS. Analysis of variance was carried using a general one-way model, and S-N-K was used for comparison between particular means.

RESULTS

Bud behavior

The percentage of bud fertility per cane of Thompson Seedless grapevine cultivars was anatomically estimated on three seasons and data represented in Fig. 1. The results were greatly variable with no general trend or significant differences neither between seasons nor among different treatments. On the first season, the percentages were less than 50% of cane buds (except treatment with HC at February combined with yeast at May; 54.5%). Bud fertility was increased (more than 50%) with seven treatments on the second and three on third seasons, but Treatment with HC only was not one of them. Generally, the lowest fertility recorded when HC was applied at January and regardless treatment with yeast. The highest percentage of bud fertility was recorded on the second season by applying yeast at May. Some treatments have increased the percentage of fertility in the second season then decreased again in the third one (Fig. 1). Only, applying yeast at April and HC at February increased the fertility from season to another.

Figure (2) shows that Dormex only or when combined with yeast significantly increased the percentage of bud burst. Applications of yeast at any date have no significant effect on the percentage of bud burst. The highest percentage was approximately 80% of total buds and resulted by using HC and yeast in combination at February (2- to 3-weeks before anticipated bud burst).

Also, the results in Figure 2 shows that the untreated buds (control) had the highest catalase activity compared with those treated with HC and/or yeast. Both of HC and yeast, in comparison to control, reduced the catalase activity during bud dormancy.

1-2

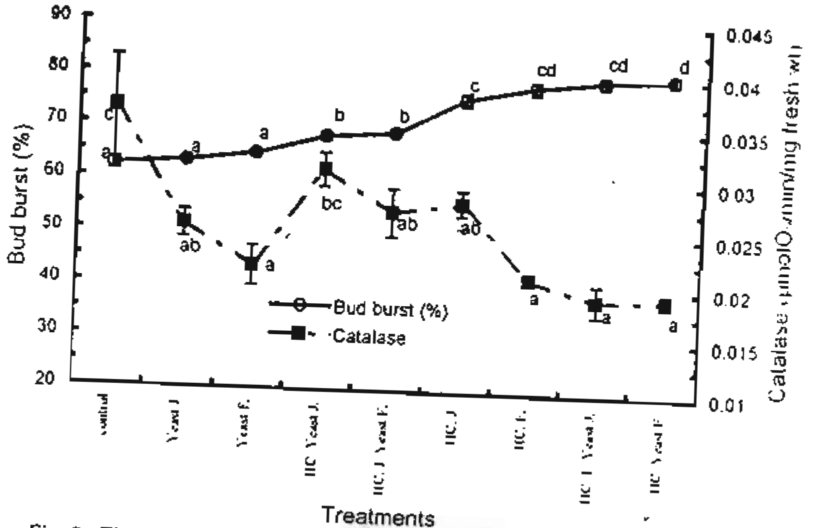


Fig. 2: The rlationship between bud burst (%) of bud load per vine and catalase activity in buds as affected by application of HC or/and yeast at different dates. Values are means ±SE. n = 10 for bud burst and 3 for catalase activity. For each line, means with different letters are significantly different at $p < 0.05$ according to S-N-K test

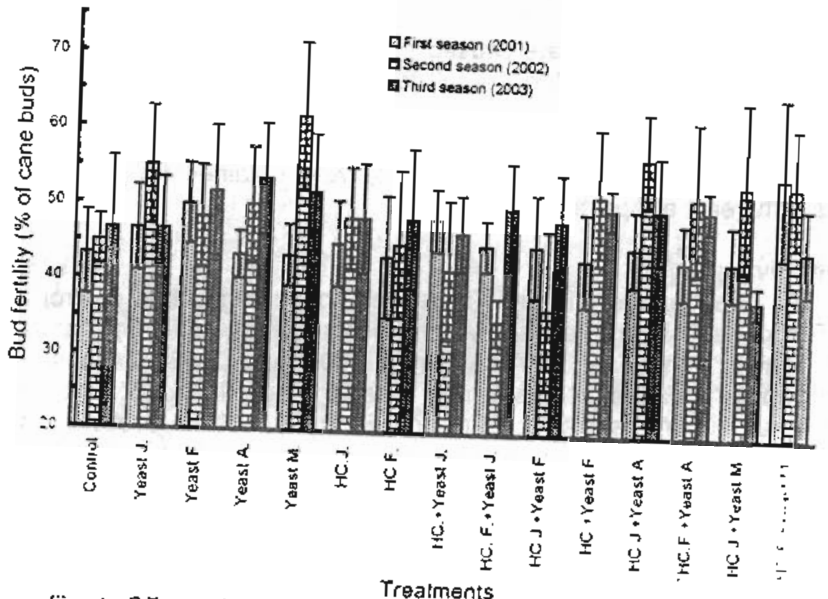


Fig. 1. Effects of spraying hydrogen cyanamide (HC).soil drench yeast and its combination at different dates on bud fertility as a percentage of cane-buds. Values are means ±SE. n = 5 canes (12 buds per cane)

The lowest catalase activity was recorded in buds of vines treated with yeast as drench-soil at January and February and when combined with spraying HC at February only, where the activity reduced by about 42% from that in control buds. Applying HC at February inhibited catalase activity in the dormant buds more than that at January (Fig. 2). The bud burst increased as the catalase activity decreased by spraying with HC (R^2= -0.950), treatment with yeast (R^2= -0.972) or using HC plus yeast (R^2= -0.986**). These data reflect the enhancement effects of HC and yeast application on the percentage of bud burst via a temporary rise in H_2O_2. Moreover, HC and yeast induced earlier opening of buds (Fig. 3). Buds of vines treated by HC at January and February tended to burst earlier than control by about 35 and 10 days, respectively (only 4–7 days in case of yeast). Despite that, vines of different treatments reached to full bloom between 10 and 18 April. However, the period from bud burst to full bloom increased by application of HC but decreased by yeast. Vines treated with HC or yeast at January harvested 11-13 days (22-24 June) earlier than control (4 July). Harvesting date of other treatments was more close to control.

Data in Fig. (4) indicate that applying yeast at April and May, and HC at January combined with yeast at January or February induced a significant increase in fruitful buds compared to control. Also, HC+ yeast applied at January, as well as yeast applied at April and May exhibited the highest percentage of fruitful buds during the two study seasons; but the increase was more pronounced in the next season. In general, all treatments significantly increased fruitful bud in season 2002; while in season 2003 some treatment such as spraying Dormex at January, at February or at February + yeast at any date caused a decrease in fruitful buds percentage. This decrease may be a direct result for increasing the number of bud burst per vine.

It was found that all treatments significantly increased fertility coefficient, and the highest percentages were obtained from treatment of vines with yeast at April and May.

Vegetative growth

All treatments significantly increased shoot length than control (Fig. 5 A). The longest shoots were shown on vines sprayed with Dormex at January and treated with yeast at April during 2002 and 2003 seasons. Non-significant differences were obtained between shoot length of vines treated with either HC or yeast at January and February, but the best results were obtained, from those treated with HC at January and yeast at any date. The effect of application date was more pronounced on the shoot diameter, Treatments applied at January yielded thick shoots than at February.

Number of leaves per shoot and leaf area were increased by most treatments (Fig. 5 B). The highest number of leaves and leaf area were found by applying yeast at April, but neither yeast nor HC applied at January and February have any significant effect on the leaf area.

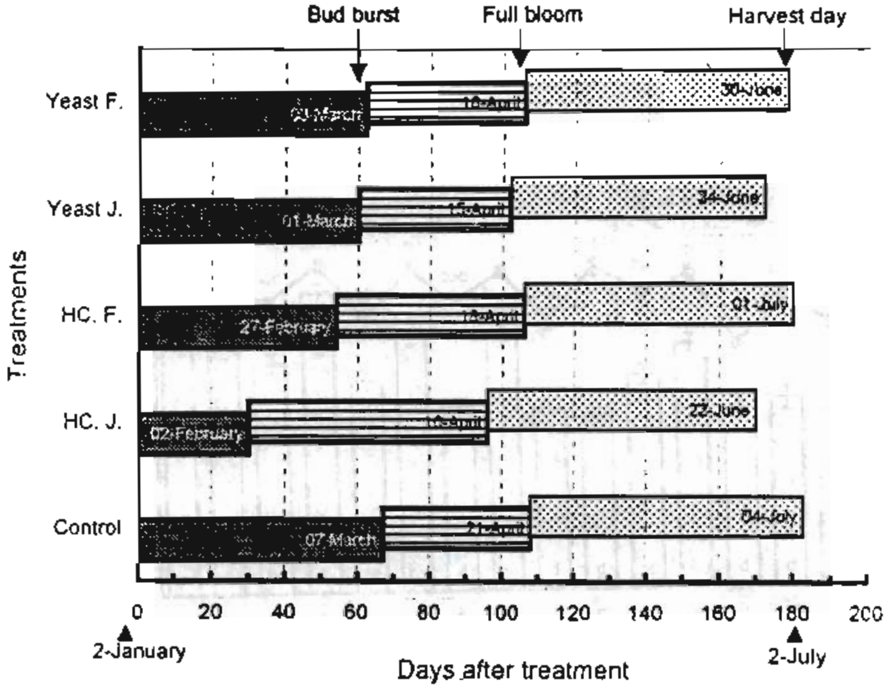


Fig. 3: Effects of applying HC or yeast at January and February on the longevity and timing of different growth stages and buds development of Thompson Seedless grapevines.

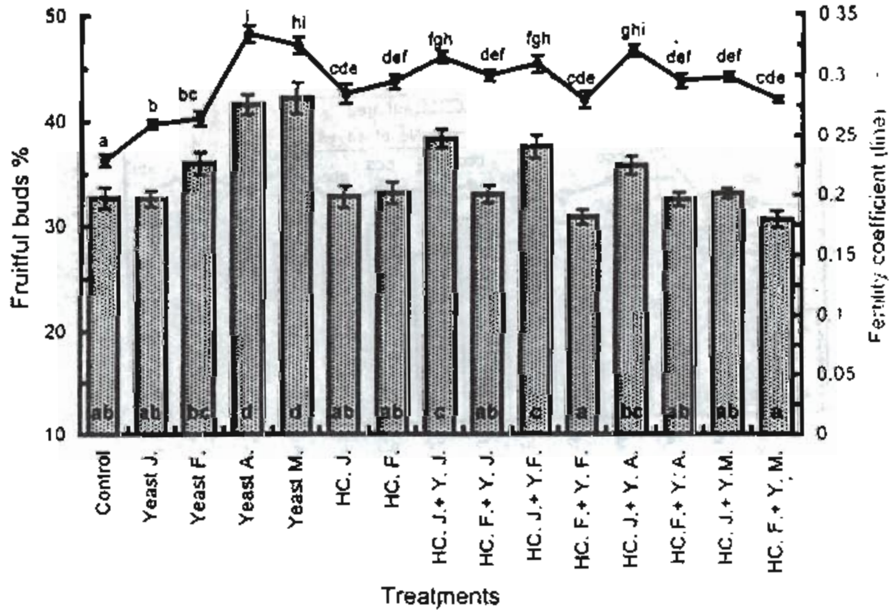
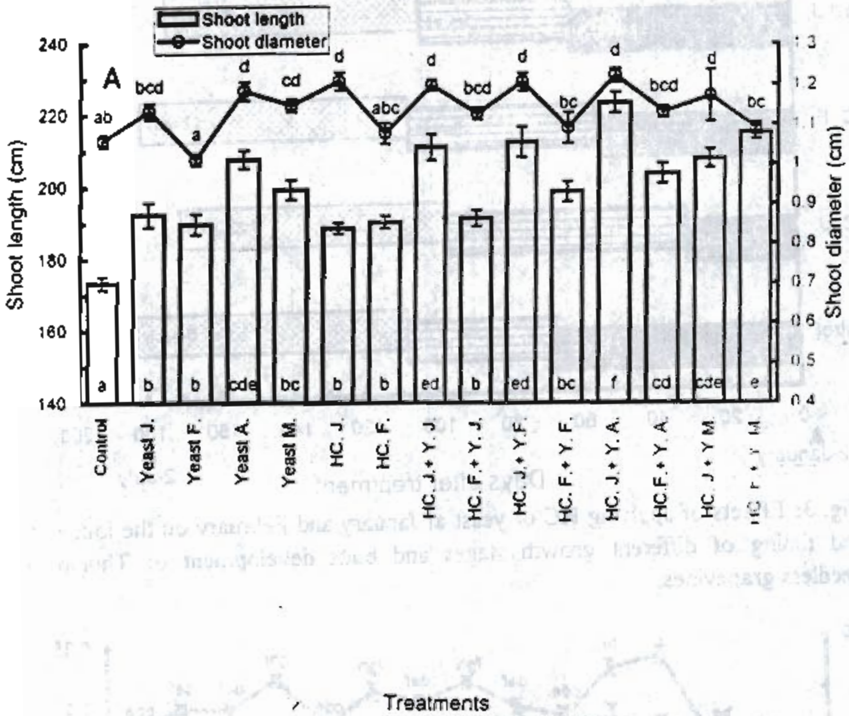


Fig. 4 : Effects of applying HC. yeast or their combinations at differen dates on the percentage of fruitful bud and fertility coeffelcient.Values are means ± SE. n = 10. For coulumns or line, means with different letters are significantly different at $p < 0.05$ according to S-N-K test.



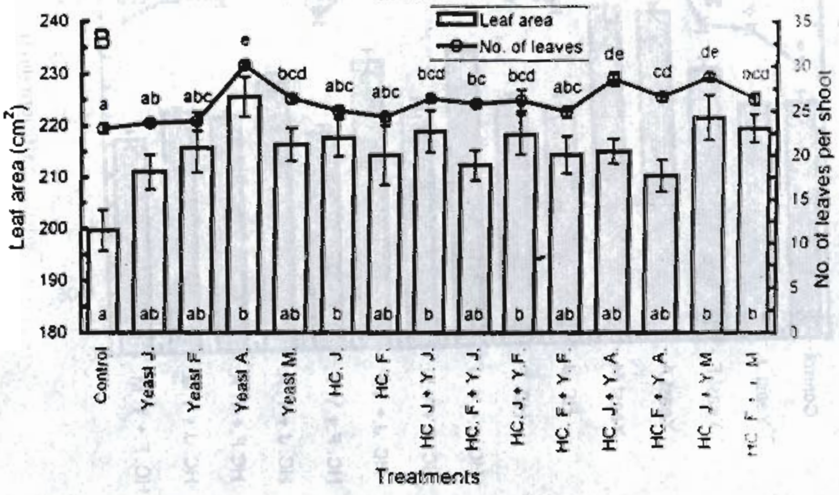


Fig 5 : Vegetative growth as a affected by applying HC and / or yeast at different dates. A, shoot length and diameter ; B, leaf number per shoot and leaf area. For statistics see Fig. 4

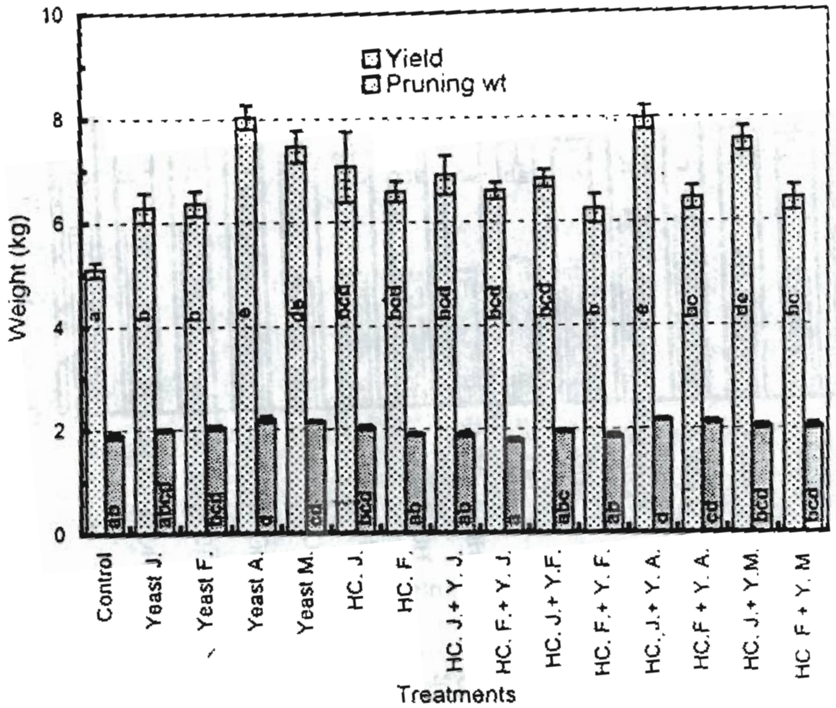


Fig. 6: Effects of HC, yeast and their combination on pruning wt and yield of Thompson Seedless grapevines. Values are means ± SE n= 10 vines. Means with different letters are significantly different at $p < 0.05$ according to S-N-K test

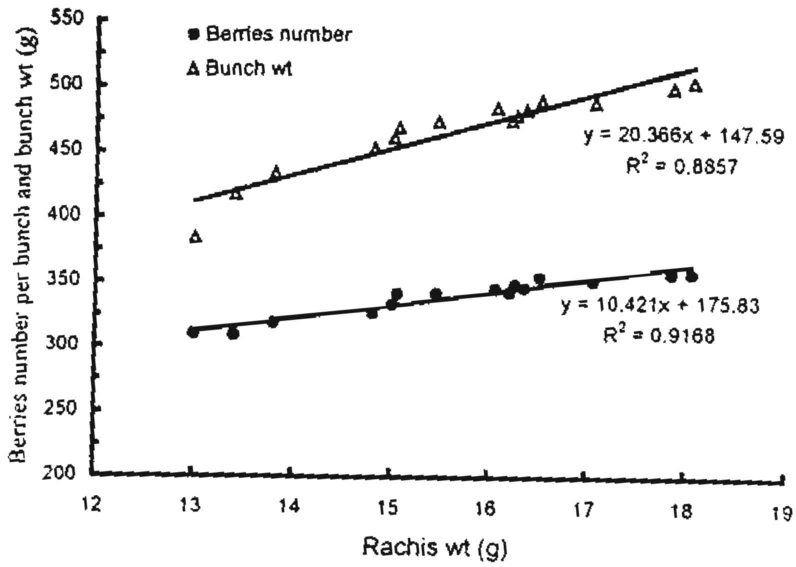


Fig. 7: Regression lines for rachis weiht (g) and either berries number per bunch or bunch weight (g).

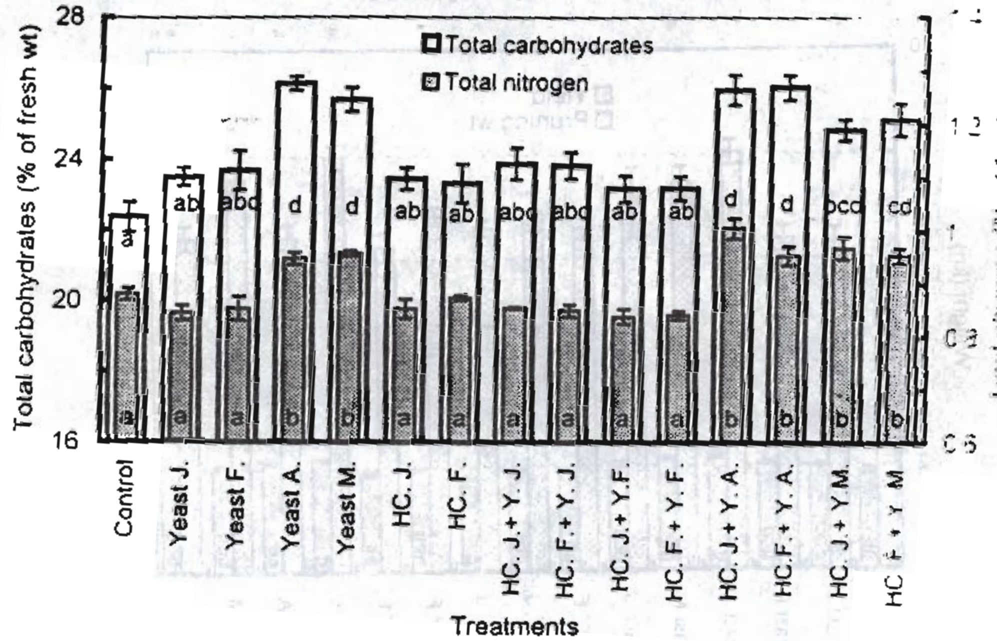


Fig. 8: Contents of total carbohydrates and total nitrogen in canes of Thompson seedless grapevines treated with HC and /or yeast.For statistics, see Fig 6

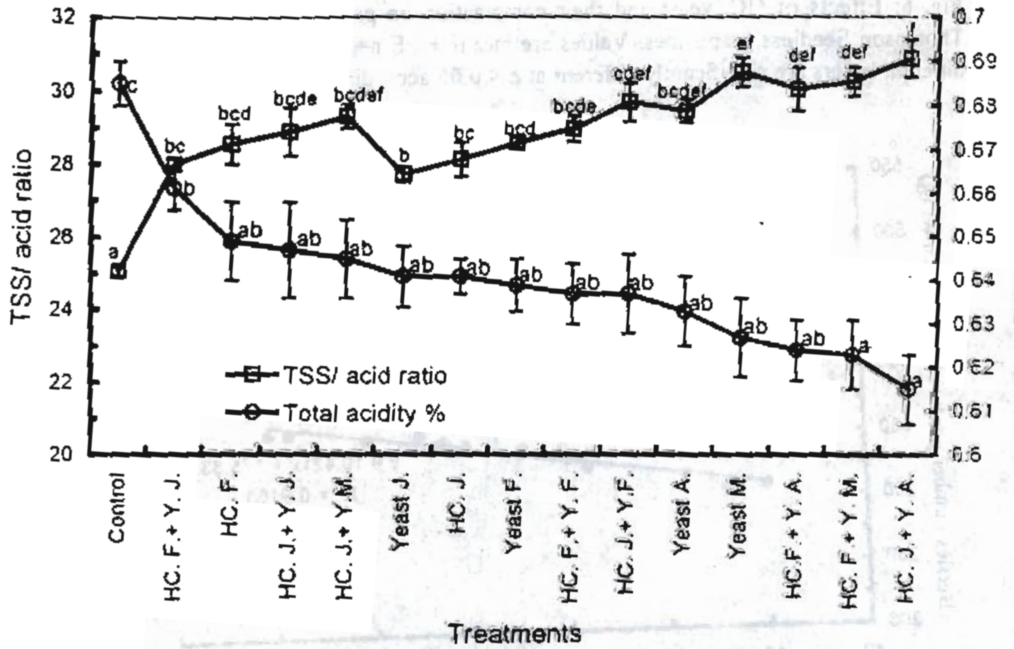


Fig 9 : Effects of HC, yeast, their combination and their application date on the percentage of total acidity and total soluble solids (TSS)/acid ratio in berries juice Treatments arranged descindingly according to total acidity. For statistics, see Fig 4

Yield component

As shown in Fig. 6, yield per vine significantly increased by application of HC and/or yeast in comparison to control. Generally, applying yeast at April and May and when combined with HC at January resulted in the highest yield compared to control and other treatments.

Application of yeast at April (and when combined with spraying HC at January or February) and at May significantly increased the weight of pruning wood. The lowest weight of pruning wood was obtained by applying yeast at January + HC at February and the differences were not significant.

Fig. (7) shows the linear regression relationship between the rachis weight and berries number per bunch or the bunch weight. Both of the linear regression equations indicate a proportional relationship between the weight of rachis and the berries number and bunch weight. A highly positive correlation was resulted between the rachis weight and number of berries per bunch (r= 0.958**) or the bunch weight (r= 0.941**). The highest values of rachis weights, bunch weights and number of berries from resulted application of yeast at April and May, HC + yeast at January and by HC at February + yeast at April.

Total carbohydrates and nitrogen

All treatments increased the content of total carbohydrates in canes. However, Total nitrogen content in the canes did not change by all treatments applied at January and February (Fig. 8). Vines sprayed with Dormex at January when treated with yeast at April showed the highest percentage of total nitrogen in the two seasons in comparison with other treatments.

TSS and acidity

Data in Fig. (9) showed that all treatments significantly decreased total acidity % while increased TSS% and TSS/ acid ratios were increased. The lowest percentages of total acidity were estimated in berries of vines treated with HC at February + yeast at May and HC at January + yeast at April. The TSS in berries of control vines was 17.2%, while the values for treatments increased to be ranging between 18.0% and 19.1%.

DISCUSSION

The results revealed that the application date of yeast or HC is effective in improving the growth and yield characteristics of Thompson Seedless grapevines. The best date for yeast application was at April that represents the date before starting flower initiation (El-Mogy, 1982). Yeast is a rich source of cytokinins (Ferguson et al, 1987), IAA (Moor, 1979), B-complex vitamins, protein and minerals (Somer, 1987; Mahmoud,2001). These substances may eliminate the polarity phenomenon known in *Vitis venifera*. All yeast treatments increased shoot length and thickness, number of leaves and leaf area. According to Walker and Weinstein (1991), the increase in the vines biomass may be due to enrichment of yeast with amino acids, vitamins, CO_2 released from the yeast fermentation and activation of magnesium dechelatase enzymes that improve net photosynthesis. In comparison, berry juice were obtained when Sultana and cardinal grapevines received a single spray of HC 8-4 weeks before the usual time of bud burst.

This study indicates that, all estimated parameters were advanced by yeast in favour of growth and yield of grapevines. In some parameters, yeast was having an advantage over HC. However, application of yeast did not show any negative effect on growth and yield of grapevines. Beside that, it is environmentally more save than HC.

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إضافة الخميرة و سيناميد الهيدروجين لتحسين قوة النمو، سلوك البراعم وجودة الثمار لعنب الطومسون سيدلس.

ياسر أنور محمود محمد عمران[١] ، طه رمضان محمد[٢] وفاروق مصطفى البرعى[٣]

١- قسم بحوث العنب- معهد بحوث البساتين- مركز البحوث الزراعية- الجيزة - مصر

٢- قسم النبات- كلية العلوم- جامعة أسيوط- مصر

٣- قسم البساتين- كلية الزراعة - جامعة أسيوط - مصر

أجريت هذه الدراسة خلال مواسم ٢٠٠١ ، ٢٠٠٢، ٢٠٠٣ على كرمات عنب طومسون سيدلس عمرها ٦ سنوات مزروعة في أرض طينية على أبعاد ٥ و ١ في ٣ م.

أوضحت النتائج أن كلا من معاملة الخميرة و أو سيناميد الهيدروجين اثرت كثيرا على النسبة المئوية لخصوبة البراعم بدون اتجاه محدد من موسم إلى أخر، أعلى نسبة مئوية لخصوبة البراعم كانت عن طريق المعاملة بالخميرة في شهر مايو. الخميرة أو سيناميد الهيدروجين ثبطا معنويا نشاط إنزيم الكتاليز وعملا على زيادة النسبة المئوية لتفتح البراعم. المعاملة بسيناميد الهيدروجين في أول شهر يناير بكرت من موعد تفتح البراعم بحوالي شهر مقارنة بالكنترول .في نفس اتجاه الهيدروجين سيناميد، بكرت الخميرة من الفترة من تفتح البراعم إلى التزهير بأسبوع إلي ثلاثة أسابيع ولذلك كل المعاملات وصلت إلي مرحلة قمة التزهير في ١٠ - ١٨ أبريل. معاملة الخميرة عند أبريل ومايو زودت معنويا النسبة المئوية للبراعم الثمرية. معظم المعاملات حسنت قوة النمو (طول وسمك الأفرخ، عدد الأوراق والمساحة الورقية) وزن خشب التقليم ، وزن المحصول ومحتوى القصبات من الكربوهيدرات الكلية والنيتروجين. ارتبط عدد الثمار ووزن العنقود بوزن الحامل النورى ، أفضل خصائص جودة كانت لكرمات معاملة بالخميرة في شهر أبريل ومايو أو لكرمات مضاف لها خميرة في أبريل ومايو ومرشوشة بسيناميد الهيدروجين.