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(Original Article)

Effect of Natural Moringa Leaf Extract, Dry Yeast, and Potassium Thiosulfate on Thompson Seedless Grape cv. under Assiut Climatic Conditions

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Abstract

Numerous dual challenges place major limits on crop productivity and the long-term viability of farming systems in semi-arid regions. It is necessary to explore and implement agricultural strategies capable of enhancing the plant's ability to tolerate abiotic stresses. This study was conducted in the 2022 and 2023 seasons to investigate the effects of natural moringa leaf extract (MLE), dry yeast, and potassium thiosulfate on the vegetative growth, yield, and berry quality of grape cv. Thompson Seedless under Assiut climatic conditions, Egypt. Sprays consisting of water only (control), MLE at 3%, dry yeast at 1%, potassium thiosulfate at 1%, and MLE at 1.5% + dry yeast at 0.5% + potassium thiosulfate at 0.5% were sprayed three times on Thompson Seedless vines at growth beginning, after fruit set, and one month later. The results showed that foliar spraying of all studied treatments enhanced leaf area, chlorophyll content, pruning wood weight, yield, berry quality, and tolerance to abiotic stresses. The highest percentage of berry set (17.21%) and yield per vine (14.69 kg) were recorded by the combined treatment compared to the control, which produced the lowest value for berry set (15.6%) and yield (9.94 kg/vine). The combined treatment achieved the highest percentage of TSS at 21.47%, reducing sugar at 16.26%, and the lowest acidity at 0.301%, while the percentages in the control treatment were 19.07%, 14.84%, and 0.481%, respectively. Overall, the MLE 1.5% + dry yeast 0.5% + potassium thiosulfate 0.5% treatment was found to be the most effective that should be advocated, as it involves economical materials and eco-friendly practices.

Keywords: *Abiotic stress, Berry quality, Moringa, Potassium, Yield.*

Introduction

Grape (*Vitis vinifera* L.) is regarded as one of Egypt's most consumed and well-liked fruit crops after citrus and mango. The grape acreage in Egypt has witnessed a tremendous increase over the past ten years. The total grape area reached 186,735 feddans (1 feddan =4200m^2), producing about 1,715,410 tons with an average yield of 9.789 tons per feddan (M.A.L.R. 2023).

The high concentration of phenolic compounds in grapes makes them an excellent source of natural antioxidants (Drosou *et al.,* 2015). It was reported by Torres-Urrutia

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et al. (2011) that grapes are a significant source of manganese and potassium and a good source of short-chain carbs (Gibson, 2017) and vitamin C (Carr and Maggini, 2017), which is important for immunology. In Egypt, the Thompson Seedless grape is the most widely grown grape cultivar. Because of its high total soluble solids, thin peel, and ideal shape, Thompson Seedless is becoming more and more popular as a table fruit and for raisins made.

Under semi-arid conditions, Thompson Seedless has common problems such as smaller berry sizes, higher shot berries, and poorer cluster traits. Numerous internal and external elements, such as cultivar, exposure to sunlight, temperature, humidity, soil, nutrition, and hormonal regulation, influence the growth and development of grapevines as well as on the quality of the berries they produce (Conde *et al.,* 2007). The excessive utilization of agrochemicals might result in many adverse consequences, such as pollution of water and the emergence of strains resistant to fungicides and bactericides (Fernández-Ortuño *et al.,* 2014). Even worse, as one of the mechanisms contributing to global climate change, these issues not only directly affect the availability of food for humans but also negatively impact all species on Earth (Arunanondchai *et al.,* 2018). New environmentally acceptable alternatives to agrochemicals have been found and employed to prevent their overuse. Among these are bio-stimulants, which are substances found in nature or microbes that help plants grow, develop, and become more resilient to biotic and abiotic challenges (Du Jardin, 2015).

It is essential to use natural extracts that are inexpensive, organic, safe for the environment, and non-toxic. The highly prized nutritious herb *Moringa oleifera* is also used medicinally (Moyo *et al.,* 2011). MLE has been suggested as an effective bio-stimulant for enhancing the growth, production, and quality of Thompson Seedless grapes (Aly *et al*., 2020). Applying MLEs (MLE) to plants helps them withstand abiotic stresses by boosting antioxidant enzyme activity and raising phenol, flavonoid, and sugar content. This lowers reactive oxygen species, lipid peroxidation, and electrolyte leakage levels (Arif *et al.,* 2023). Furthermore, it makes crops more resistant to adverse weather conditions (Aljabary *et al.,* 2024). Zeatin concentrations in moringa leaves can reach 1 mg/g, together with antioxidants, macro- and micronutrients (Islam *et al.,* 2022). Additionally, moringa leaves are a great source of β-carotene, Fe, Ca, and vitamins A and C. It contains a lot of purine and adenine, which are products of the cytokinin hormone family of plants (Nambiar *et al.,* 2005). According to MLE analysis, IAA, GA_3, and ABA plant growth regulators are present in MLE (Rady and Mohamed, 2015). A higher concentration of antioxidant molecules is the outcome of MLE's ability to alter primary and secondary metabolism (Latif and Mohamed, 2016). MLE should be taken into consideration as an alternative to synthetic agrochemicals for increasing crop yield due to evidence of its growth and yield enhancing effects as well as its ability to improve plant physiology under abiotic stresses (Merwad, 2018).

For people, animals, and the environment, bio-fertilization is extremely safe. It also reduces the expense of fertilization (Sarker *et al.,* 2012). Yeast (*Saccharomyces cerevisiae*) performs a variety of tasks, such as producing carbon dioxide, alcohol, acids, and esters; it is a natural bio-stimulant that increases the development and productivity of many crops (Martinez-Anaya *et al.,* 1990). Proteins, vitamin B, natural hormones, and minerals with a high concentration of N, P, and K can all be found in yeast. It might be crucial to the movement and synthesis of proteins, carbohydrates, and nucleic acids, as well as the translocation of metabolites from leaves to the organs of production (Ferguson *et al.,* 1995). Treatments with dry yeast extract may improve photosynthesis while releasing CO_2, a crucial component of this process (Shaaban *et al.,* 2015). Yeast extracts can effectively be used as a foliar fertilizer to encourage plant development and improve ultimate output (Marzauk *et al.,* 2014). Yeast extract's delay the breakdown of chlorophyll and boost protein synthesis. Cytokinin and auxin concentrations also delay the aging of leaves in addition to directly modifying nutrients (Abou El-Yazied and Maday, 2012).

Potassium (K) is a crucial nutrient for plant growth and development, and it plays a significant role in several other processes. Research showed that K plays several physiological metabolic roles in plants throughout their life cycle (Villette *et al.,* 2020). These roles include stimulating the activity of multiple enzymes (Oosterhuis *et al.,* 2014), taking part in osmoregulation (Chérel and Gaillard, 2019), preserving the equilibrium of anions and cations (Nieves-Cordones *et al.,* 2019), improving plant photosynthesis and assimilation of product transport (Zahoor *et al.,* 2017), metabolizing reactive oxygen species (Zhang *et al.,* 2017), enhancing the plant's resistance to disease and stress tolerance (Wang and Wu, 2013), and enhancing resistance to external abiotic stresses (Hasanuzzaman *et al.,* 2018). As per Villette *et al.* (2020), the enhancement of fruit yield and intrinsic and extrinsic fruit quality is the outcome of all these aspects. Enzymes involved in water and nutrient transport, root development, resistance to salinity and drought, sugar production and translocation, and stomata opening and closing are all triggered by potassium (Dhillon *et al.,* 1999). For example, K can improve crop photosynthetic physiology by encouraging stomata to open and increase chlorophyll levels, which in turn increases the activity of enzymes involved in photosynthesis. Ultimately, this improves crop photosynthetic processes (Sun *et al.,* 2006).

The primary objective of this study was to assess how foliar applications of MLE, dry yeast, potassium thiosulfate, and their combinations affects the vegetative growth, yield, berry quality, and abiotic stress tolerance of Thompson Seedless grapevines grown under Assiut, Egypt hot climate conditions.

Materials and Methods

1-Experimental Site and Field Treatments

The research was conducted during 2022–2023 seasons to examine the impact of the foliar spraying of natural MLE, dry yeast, and potassium thiosulfate on eighteen-year-old "Thompson Seedless" grapevines. The vines were grown in clay loam soil in an experimental orchard located at the Faculty of Agriculture, Assiut University, Egypt, at 2 × 2.5 meters apart, irrigated using surface irrigation, and exposed to the same horticulture techniques used in the orchard as untreated vines. The weather conditions at the experimental site are presented in Fig. 1. A head pruning strategy was used to leave a total of 48 buds for all chosen vines, based on each vine having 11 fruiting spurs with 4 buds each and 2 replacement spurs with 2 buds. The soil analysis results are shown in Table 1. The trial was composed of five treatments, set up in a randomized complete block design where each treatment was applied to three replicates (three vines). The vines were sprayed with a back-held sprayer three times: the first was in March (at the beginning of growth, 10 cm of shoot length), and the subsequent sprays were carried out after fruit set, and one month later. The vines were completely covered by the solution of MLE at 3%, dry yeast at 1%, potassium thiosulfate at 1%, MLE at 1.5% + dry yeast at 0.5% + potassium thiosulfate at 0.5%, and water only (control), and each vine was sprayed with 3 liters. Tween-20 surfactant was added to all treatments (2 mL/L) to assist in spray solution absorption.

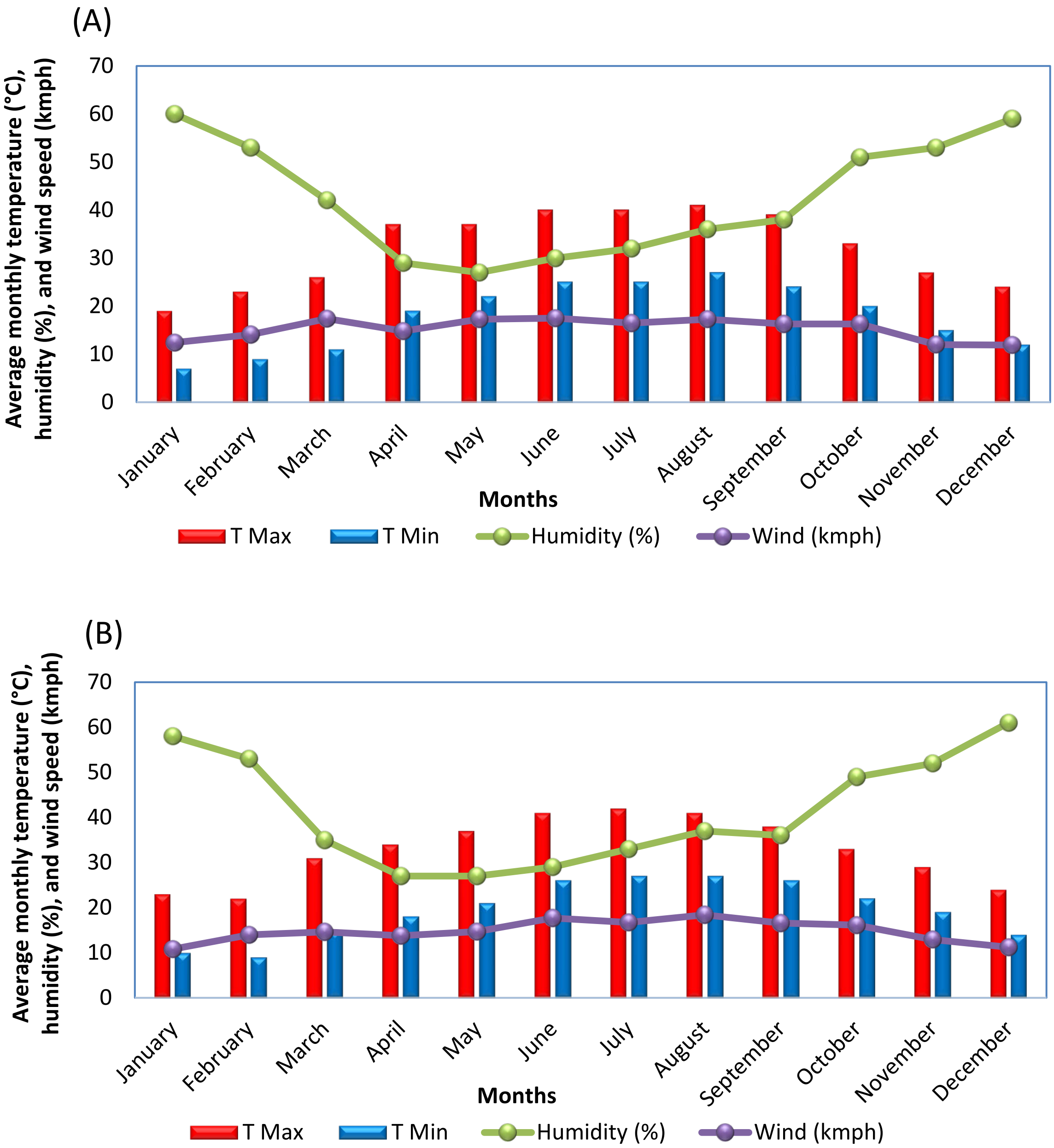

Fig 1. The weather chart represents the climatic condition of the experimental site during (A) 2022 and (B) 2023 seasons. Source: worldweatheronline.com; T Max = maximum temperature, T Min = minimum temperature

Table 1 Physical and chemical soil properties of the experimental site (0-50 cm).

Properties	0-50 cm
Sand (%)	22.52
Silt (%)	12
Clay (%)	66.48
Texture	Clay loam
pH (1:2.5 suspension)	7.77
ECe (dS m^{-1})	1.01
Organic matter (g kg^{-1})	5.41
Total N (mg kg^{-1})	400
Available Olsen P (mg kg^{-1})	12
Available K (mg kg^{-1})	300

Each value represents a mean of three replicates.
EC_e: the Electric Conductivity of the saturated soil extract.

MLE Preparation

Moringa plant leaves were cleaned with distilled water and allowed to dry in the shade. Then they were powdered and processed to fine consistency. A laboratory mixer was used to blend 30 grams of powdered moringa leaves with 200 mL of methanol (96%) and distilled water (20:80 *v/v*) for 10 minutes. The mixture was placed in dark glass bottles for 72 hours to finish the extraction process. The extracts were filtered using thin sheets of cotton cloth. The final extracts were collected separately in additional opaque glass vials and allowed to evaporate from methanol at 60 °C in a water bath for 30 minutes. When not in use, the extract was kept in the refrigerator at a temperature of 5 °C (Ezz *et al.,* 2015).

Preparation of Dry Yeast Extract

To activate the reproduction of yeast, active dry yeast solutions at two concentrations (1% and 0.5%) were used and prepared before spraying by dissolving the specified amount of active dry yeast in warm water (38 °C), adding 0.3% Egyptian treacle (as a source of sugar), and waiting for one hour before spraying (Hegab *et al.,* 1997).

The chemical analyses of MLE and yeast extract are shown in Tables 2 and 3, according to Islam *et al.* (2022) and Abou El-Yazied and Mady (2012), respectively.

Table 2 Chemical composition of MLE.

Moringa leaves					
Minerals composition (mg/100 g DW)		**Antioxidants (mg/g DW)**		**Phytochemicals and anti-nutrients (g/100g DW)**	
Nitrogen	1070	Ascorbic acid	549.5	Anthraquinone	11.7
Calcium	364.5	Glutathione	301.2	Tannins	9.4
Potassium	1500	α-Tocopherol	0.04	Terpenoids	4.8
Sulphur	630	**Osmolytes (mg/g DW)**		Steroids	3.2
Magnesium	76.6	Total soluble sugars	198.6	Alkaloids	3.1
Phosphorous	70.0	Amino acids	142.2	Phytates	2.6
Manganese	9.6	Proline	32.1	Saponins	1.5
Iron	7.0	**Phytohormones (mg/g DW)**		Carotenoids	1.2
Copper	4.4	Zeatin	1.0	Oxalates	0.5
Zinc	1.8	Indole-3-acetic acid	0.8	Hydrogen cyanide	0.1
Sodium	1929.5	Gibberellins	0.7		
		Abscisic acid	0.3		
		Salicylic acid	0.08		

DW: Dry weight.

Table 3 Chemical composition of yeast extract.

Amino acid (%)		Vitamins (mg/100 g DW)		Growth regulators (ppm)	
Alanine	1.69	Vit.B1	23.33	Adenine	31
Arginine	1.49	Vit.B2	21.04	Betaines	56
Aspartic acid	2.32	Vit.B6	20.67	Minerals	
Cystine	0.63	Vit.B12	19.17	Nitrogen	6.88 %
Glutamic acid	3.76	Thimain	23.21	Phosphorus	0.66 %
Glycine	1.45	Riboflavin	27.29	Potassium	0.95 %
Histidine	0.71	Insitol	20.43	Magnesium	0.19 %
Isoleucine	0.85	Biotin	20.04	Calcium	0.17 %
Leucine	1.91	Nicotinic acid	73.92	Sulfur	0.48 %
Lysine	1.13	Panthothenic acid	38.43	Iron	107 ppm
Phenylalanine	1.18	Paminobenzoic acid	29.49	Zinc	77 ppm
Proline	1.29	Folic acid	26.22	Copper	5 ppm
Serine	1.98	Pyridoxine	22.09	Manganese	13 ppm
Threonine	1.54			Others	
Tryptophan	0.25			Crude Protein	43.00 %
Tyrosine	0.99			Crude Fat	2.20 %
Valine	1.4			Carbohydrates	33.21 %
Methionine	0.4			Crude Fiber	7.20 %
				Ash	3.80 %

DW: Dry weight.

2-Vegetative Growth Parameters

The dimensions of five mature leaves (for each replicate) were measured from those on the main stems opposite the basal clusters, and leaf area (cm^2) was calculated (Ahmed and Morsy, 1999) according to this formula:

$$\textbf{Leaf area } (\mathbf{cm^2}) = \mathbf{0.44}\ (\textbf{length of leaf x width of leaf}) + \mathbf{18.13}$$

Measurements of the chlorophyll amount in leaves were carried out by a portable chlorophyll meter (SPAD 502 Plus), according to Wood *et al.* (1992).

At pruning time (15 January), the weight of pruning wood (one-year-old wood) was determined and expressed as kg per vine.

3-Yield and Its Components

Three flower clusters were bagged on previously tagged shoots of each vine in perforated white paper bags prior to bloom. The berry set (%) was calculated according to the next equation:

$$\textbf{Berry set } (\%) = \frac{\textbf{No. of berries/cluster}}{\textbf{total No. of flowers/cluster}} \times \mathbf{100}$$

At harvest time (the end of July), the total yield weight (kg/vine) was calculated by multiplying the number of clusters per vine by the average cluster weight. Four clusters were randomly selected from each vine and transferred straight to the Pomology Department Laboratory to assess their physical and chemical characteristics.

4-Physical Cluster Characteristics

The cluster weight (g), cluster length (cm), 25 berries weight (g), number of shot berries, and number of berries/cluster were determined. To calculate the compactness coefficient (Winkler *et al.,* 1974), the following formula was used:

$$\textbf{Compactness coefficient } = \frac{\textbf{No. of berries/cluster}}{\textbf{cluster length } (\mathbf{cm})}$$

5-Chemical Cluster Characteristics

From each treatment replication, twenty-five berries were homogenized. After centrifugation, the clear supernatant of the homogenate was utilized for further analysis. The total soluble solids (TSS%) in the berry juice was measured by a digital refractometer (Model MA-871, Milwaukee, Romania). Titratable acidity (%) was identified by direct titration of sodium hydroxide 0.1 N utilizing phenolphthalein 1% as an indicator and was expressed as tartaric acid percentage (A.O.A.C. 1995). The following equation was used to calculate the acidity:

$$\textbf{Acidity}(\%) = \frac{\textbf{NaOH volume used in titration} \times \textbf{NaOH molarity} \times \textbf{equivalent weight of tartaric acid}}{\textbf{1000} \times \textbf{sample volume}} \times \textbf{100}$$

Where: Equivalent weight of tartaric acid = 75

NaOH molarity = 0.1 N

Sample Vol. = 5 ml

The reducing sugar (%) was determined according to the volumetric method of Lane and Eynon that was outlined in A.O.A.C. (1995).

6-Activities of Peroxidase, Polyphenol Oxidase, Catalase, and Phenylalanine Ammonia-lyase

Grapevine leaves have been used to determine enzyme activity. The activity of peroxidase (POD) was determined as mg g protein^{-1} min^{-1} using a spectrophotometer at 470 nm in accordance with Zaharieva *et al.* (1999). Activity of polyphenol oxidase (PPO) was measured at 495 nm as mg g protein^{-1} min^{-1} using the method of Kumar and Khan (1982). Catalase (CAT) activity was measured according to the method of Aebi (1984), using a spectrophotometer at 240 nm based on the H_2O_2 rate consumption as mg g protein^{-1} min^{-1}. The phenylalanine ammonia-lyase (PAL) activity was identified by measuring the absorbance at 290 nm using a spectrophotometer. The PAL activity was expressed as mg g protein^{-1} min^{-1}, using the method described by Havir and Hanson (1968).

7-**Statistical Analysis**

Five treatments and three replicates for each treatment were included in the completely randomized design (RCBD) of the trial. Analysis of variances to the collected data according to Snedecor and Cochran (1980). The Least Significant Difference (LSD) at the 5% level of probability was used to determine means difference.

Results

1-Vegetative Growth Parameters

Vegetative growth parameters such as leaf area, chlorophyll content, and pruning wood weight under different treatments are summarized in Fig. 2. MLE, dry yeast, potassium thiosulfate, and their combined application increased leaf area, chlorophyll content, and pruning wood weight compared to the control. The highest values were obtained by the combined application, followed by dry yeast and MLE. Moreover, MLE + dry yeast + potassium thiosulfate considerably increased the leaf area, chlorophyll content, and pruning wood weight by 7, 11, and 23%, respectively, compared to the control, while these increases were 7, 10, and 17% in the case of dry yeast. Hence, using MLE, dry yeast, and potassium thiosulfate, separately or in combination, can enhance plant development.

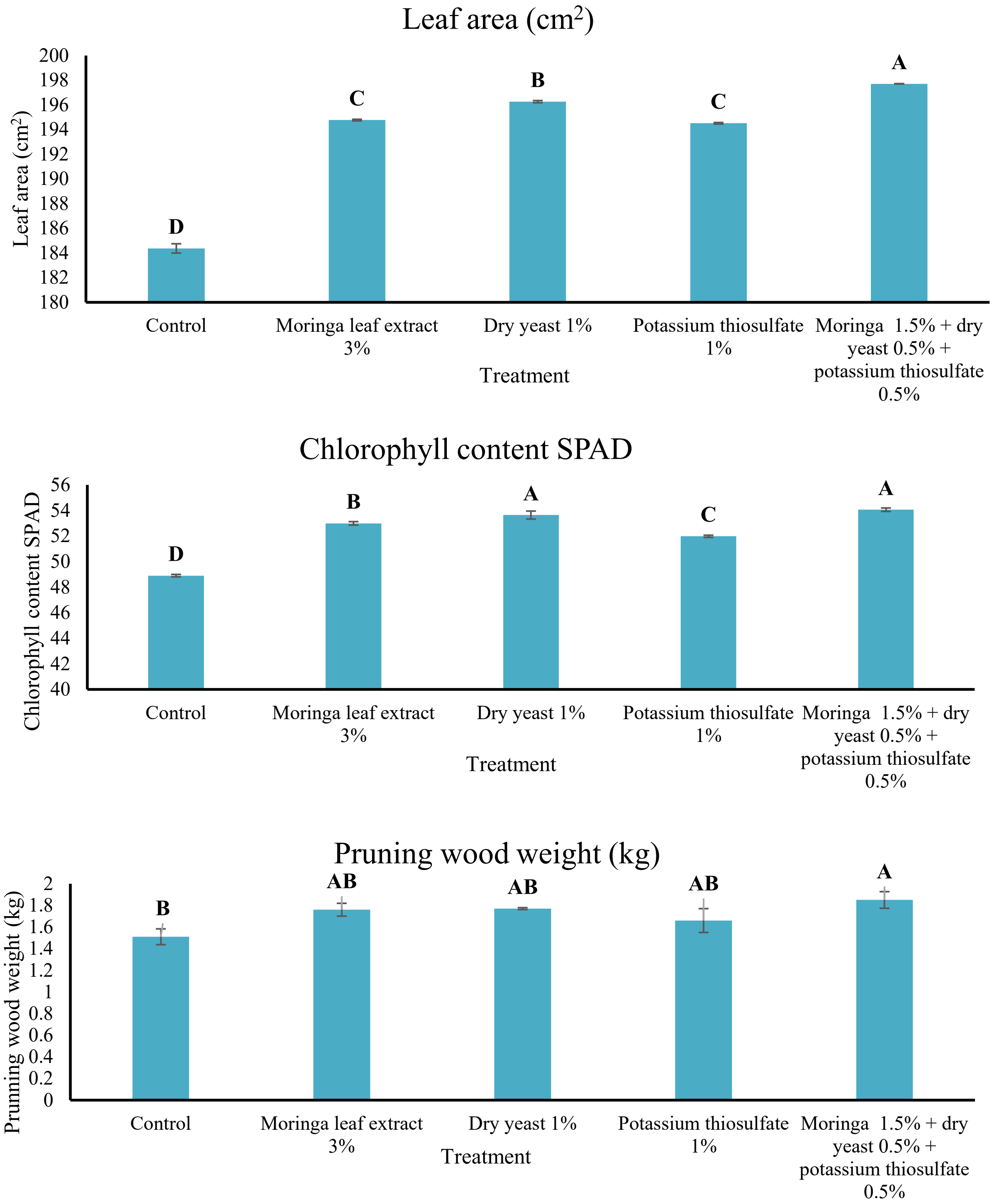

Fig. 2. The effect of the foliar application of MLE, dry yeast, and potassium thiosulfate on the leaf area (cm^2), chlorophyll content (SPAD), and pruning wood weight (kg) of the Thompson Seedless grapevines (two-season average data). The letter on each bar refers to the results of the LSD test at the 0.5 level; the differences among means with different letters are significant.

Yield and Its Components

According to the results in Table 4, foliar spraying of all tested treatments improved berry set, cluster weight, and yield. The treatment of MLE + dry yeast + potassium thiosulfate surpassed the MLE in enhancing the berry set, cluster weight, and yield of Thompson Seedless grapevines. Moreover, the combined application of MLE, dry yeast, and potassium thiosulfate significantly increased the berry set, cluster weight, and yield by 10, 41, and 48%, respectively, compared to the control, while these increases were 8, 34, and 40% in the case of MLE.

Table 4. The effect of the foliar application of MLE, dry yeast, and potassium thiosulfate on the berry set (%), cluster weight (g), and yield (kg/vine) of the Thompson Seedless grapevines during the 2022 and 2023 seasons.

Treatment	Berry set%			Cluster weight (g)			Yield (kg/vine)		
	2022	2023	Mean	2022	2023	Mean	2022	2023	Mean
Control	15.82b	15.38b	15.6	338.52c	372.91c	355.72	9.73c	10.15d	9.94
MLE 3%	17.11a	16.71a	16.91	461.80a	489.62a	475.71	12.88ab	14.42b	13.90
Dry yeast 1%	16.73ab	16.42ab	16.58	421.35b	443.83b	432.59	11.76b	13.69bc	12.73
Potassium thiosulfate 1%	15.67b	15.63b	15.65	416.78b	439.69b	428.24	12.35b	12.95c	12.65
MLE 1.5% + dry yeast 0.5% + potassium thiosulfate 0.5%	17.30a	17.11a	17.21	485.64a	513.81a	499.73	13.56a	15.82a	14.69
LSD 5%	0.95	0.91		27.14	25.84		1.12	1.28	

The same superscript letters make it clear that there are no significant differences (LSD) at the 0.05 level among treatments in the same column.

2-Physical Cluster Characteristics

The results in Tables 5 and 6 made it clear that all treatments enhanced the cluster length and weight of 25 berries, with a significant reduction in compactness coefficient and shot berries in comparison to the control. MLE + dry yeast + potassium thiosulfate increased significantly ($p < 0.05$) the number of berries, weight of 25 berries, and cluster length by 10, 26, and 14%, respectively, compared to the control, while these increases were 7, 22, and 14% in the case of MLE. Moreover, MLE + dry yeast + potassium thiosulfate and dry yeast treatments showed superiority in reducing the shot berries by 73% over the control. Dry yeast treatment significantly decreased the compactness coefficient by 5% while increasing the number of berries by 5% compared to the control. Additionally, MLE treatment significantly decreased the compactness coefficient and shot berries by 6 and 72%, respectively, compared with the control.

Table 5 The effect of the foliar application of MLE, dry yeast, and potassium thiosulfate on the cluster length (cm), number of berries, compactness coefficient, shot berries (%), and weight of 25 berries of the Thompson Seedless grapes during the 2022 and 2023 seasons.

Treatment	Cluster length (cm)			Number of berries			Compactness coefficient		
	2022	2023	Mean	2022	2023	Mean	2022	2023	Mean
Control	20.3c	20.6c	20.45	231.8b	243.8b	237.8	11.43a	11.73a	11.58
MLE 3%	22.6a	23.9a	23.25	248.9a	261.3a	255.1	10.97b	10.91b	10.94
Dry yeast 1%	22.4a	22.5b	22.45	241.3a	256.2a	248.7	10.74b	11.20b	10.97
Potassium thiosulfate 1%	21.6b	21.9b	21.75	233.3b	245.3b	239.3	10.80b	11.20b	11.11
MLE 1.5% + dry yeast 0.5% + potassium thiosulfate 0.5%	22.8a	23.6a	23.2	253.1a	268.8a	260.9	11.01b	11.23b	11.12
LSD 5%	1.08	1.23		14.11	13.68		0.41	0.46	

The same superscript letters make it clear that there are no significant differences (LSD) at the 0.05 level among treatments in the same column.

Table 6 The effect of the foliar application of MLE, dry yeast, and potassium thiosulfate on shot berries (%), and weight of 25 berries of the Thompson Seedless grapes during the 2022 and 2023 seasons.

Treatment	Shot berries%			Weight of 25 berries (g)		
	2022	2023	Mean	2022	2023	Mean
Control	8.11a	8.43a	8.27	35.16c	36.38c	35.7
MLE 3%	2.31b	2.33b	2.32	42.91a	44.16a	43.5
Dry yeast 1%	2.27b	2.25b	2.26	41.13b	41.73b	41.4
Potassium thiosulfate 1%	2.38b	2.43b	2.41	42.25b	42.18b	42.22
MLE 1.5% + dry yeast 0.5% + potassium thiosulfate 0.5%	2.15b	2.30b	2.23	44.51a	45.18a	44.9
LSD 5%	0.28	0.31		1.82	1.89	

The same superscript letters make it clear that there are no significant differences (LSD) at the 0.05 level among treatments in the same column.

3-Chemical Cluster Characteristics

Cluster chemical quality characteristics (TSS, reducing sugars, and titratable acidity) under different treatments are summarized in Fig. 3. All investigated treatments caused an increase in total soluble solids and sugars while significantly reducing titratable acidity as compared with the control. MLE + dry yeast + potassium thiosulfate proved to be a superior treatment in this respect. The treatment of MLE + dry yeast + potassium thiosulfate significantly increased the TSS and percentage of reducing sugars by 13 and 10%, respectively, while reducing the titratable acidity by 37% over the control. In addition, the application of MLE resulted in a 35% decrease in titratable acidity and dramatically raised the TSS and percentage of reducing sugars by 12 and 7%, respectively, as compared with the control groups.

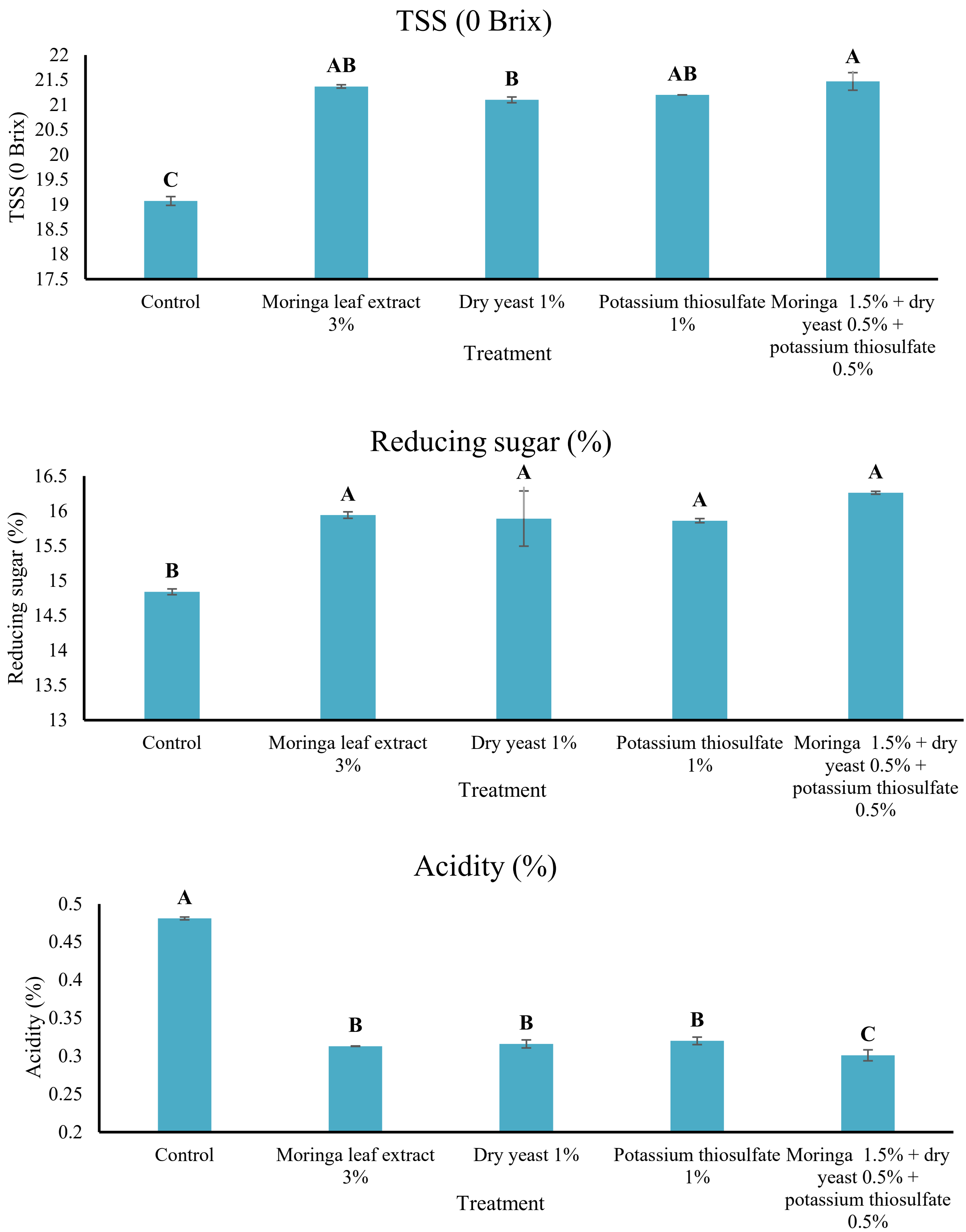

Fig. 3. The effect of the foliar application of MLE, dry yeast, and potassium thiosulfate on the total soluble solids (TSS), reducing sugar (%), and acidity (%) of the Thompson Seedless grapes (two-season average data). The letter on each bar refers to the results of the LSD test at the 0.5 level; the differences among means with different letters are significant.

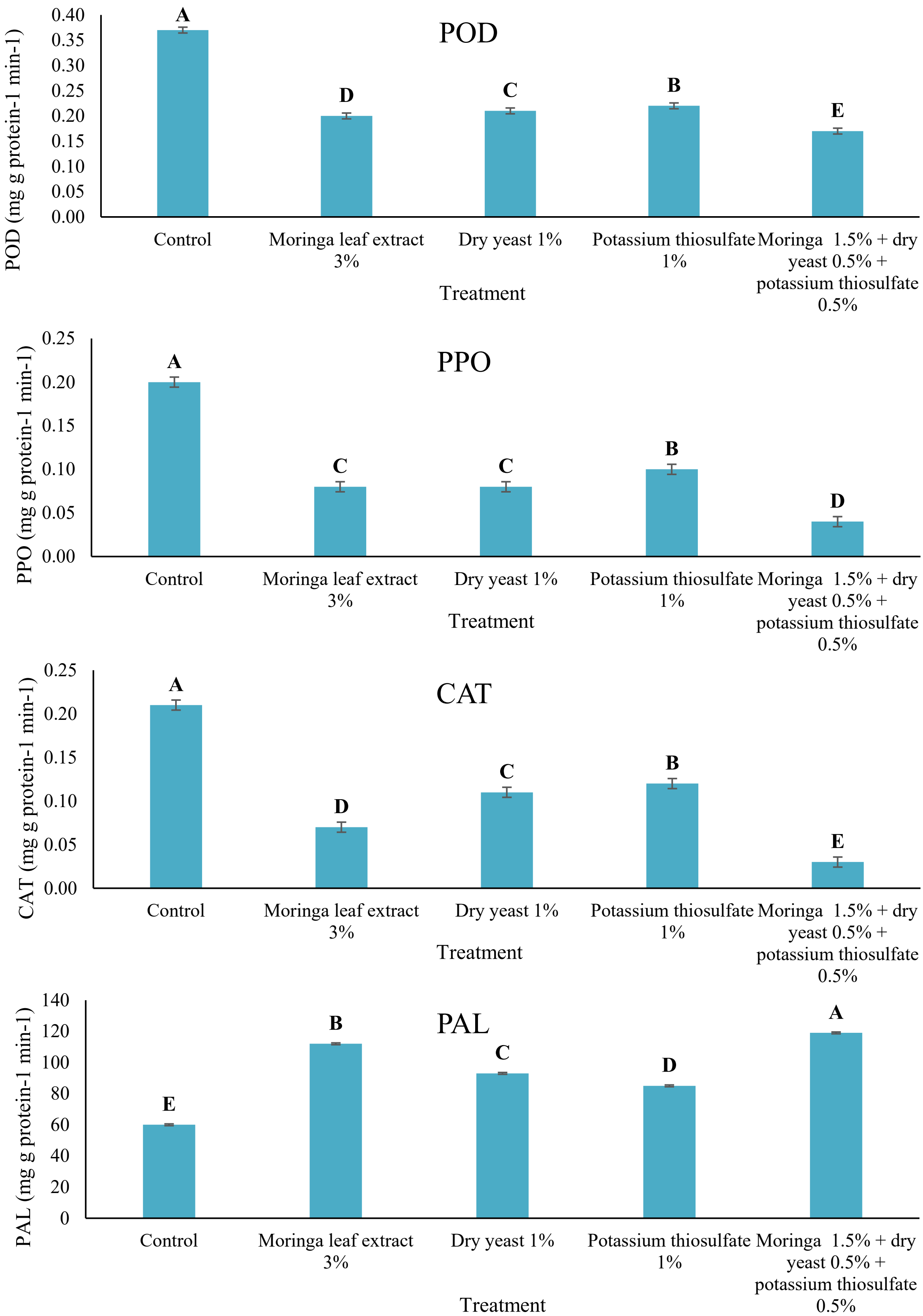

Fig. 4. The effect of the foliar application of MLE, dry yeast, and potassium thiosulfate on the POD activity, PPO activity, CAT activity, and PAL activity of the Thompson Seedless grapes (2023 season data). The letter on each bar refers to the results of the LSD test at the 0.5 level; the differences among means with different letters are significant.

4-Activities of Peroxidase, Polyphenol Oxidase, Catalase, and Phenylalanine Ammonia-lyase

As shown in Figure 4, abiotic stress on untreated grapevines resulted in higher levels of peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT) compared to treated grapevines, indicating that the treated grapevines were tolerant to abiotic stress by regulating enzyme activity. The MLE 1.5% + dry yeast 0.5% + potassium thiosulfate 0.5% treatment caused the most significant decrease in enzyme activity. POD activity decreased by 54%, PPO activity decreased by 80%, and CAT activity decreased by 86% over the control groups.

Moreover, the activity of phenylalanine ammonia-lyase (PAL) substantially increased in response to all studied treatments compared with the control treatment. The PAL activity in the control group was 60 mg g protein^{-1} min^{-1}. In the treated grapevines with MLE, dry yeast, potassium thiosulfate, and combined application, the PAL activity was 1.87, 1.55, 1.42, and 1.98 times that of the control, respectively.

Discussion

The potential effect of MLE, yeast, potassium thiosulfate and their mix in tolerating abiotic stress on Thompson Seedless grapevines under field conditions is discussed herein. Except for the effect of dry yeast and potassium thiosulfate on the berry set, both the single application of MLE and the combination of MLE, dry yeast, and potassium thiosulfate significantly increased the berry set, cluster weight, and yield when compared to control groups. Out of all the treatments, the combined application of MLE, dry yeast, and potassium thiosulfate together demonstrated the greatest improvement impact. Previous studies indicate that MLE can improve various cluster characteristics, including berry set, cluster weight, and yield (Aly *et al*., 2020; Mohamed *et al.,* 2022; Abou-Zaid *et al.,* 2022). The application of MLE, particularly in combination with potassium sulfate and other treatments, has shown significant improvements in yield (Aly *et al.,* 2020). *Moringa oleifera* leaf extract is a natural bio-stimulant that has been shown to improve plant growth and yield in various crops, including grapes. The extract is rich in vitamins, minerals, amino acids, and plant hormones that can enhance physiological processes in plants (Makkar *et al.,* 2007). The integrated use of MLE, dry yeast, and potassium thiosulfate can significantly enhance grape berry yield and quality through their synergistic effects on plant physiology and nutrient status (Abou-Zaid *et al.,* 2022; Soliman *et al.,* 2022). Moringa is found to be rich in minerals, including K, Fe, and Zn (Nasir *et al.,* 2016). Zeatin (cytokinin) levels in *Moringa oleifera* leaves are high (Ashfaq *et al.,* 2012). Zeatin boosts the antioxidant abilities of numerous enzymes and guards against the cell-aging effects of various reactive oxygen species (Zhang and Ervin, 2004). The action of MLE supports the idea that cell proliferation and cell enlargement lead to an increase in fruit size, which may be a result of those cytokinin (Santner *et al.,* 2009). This could be because of the substantial nitrogen input seen in MLE, which led to cell division, cell enlargement, and general plant growth (Kanchani and Harris, 2019). There have been significant levels of indole-3-acetic acid, gibberellins, zeatin (a natural cytokinin), and abscisic acid discovered in moringa extract (Rady and Mohamed, 2015). These growth hormones have a significant impact on yield. Similarly, it has been claimed that moringa extract enhanced the productivity of crops like Thompson Seedless grape cv. (Aly *et al.,* 2020), Red Roomy grape cv. (Mohamed *et al*., 2022), and Salakhani pomegranate (Aljabary *et al.,* 2024). The TSS and sugar levels of grape berry juice were also enhanced by moringa extract, while the TA% was proportionally decreased. As a result, when Hollywood plum trees were foliar sprayed with moringa extract as opposed to untreated trees, the TA was dramatically lowered (Mohamed *et al*., 2022). MLE has a simulative influence on plant growth and is crucial for the metabolic processes of photosynthesis and carbohydrate synthesis because of the high concentration of micro- and macronutrient components in it (Sakr *et al.,* 2018). Our findings showed that the Thompson Seedless grape cultivar's vegetative growth, production, and berry quality were improved by the application of MLE.

The current study found that the growth of Thompson Seedless grapevines under abiotic stress was significantly enhanced by dry yeast application, with significant increases in yield and berry quality compared to the control. The positive effects of dry yeast on plant growth were similarly obtained in studies on Flame Seedless grapevines (Omar *et al.,* 2020) and Early Sweet grapevines (Soliman *et al.,* 2022). This is due to the ability of the yeast to increase water content and promote development by lowering oxidative stress in salinity and drought conditions (Nimsi *et al.,* 2023). The minerals, proteins, vitamin B, and natural hormones (IAA, GA_3, and cytokinin) present in activated dry yeast contribute to its wide range of benefits (Shaaban *et al.,* 2015). The benefits of applying activated dry yeast could be attributed to its role in increasing endogenous hormones like IAA and GA_3 in treated plants, which stimulates cell division and enlargement. Additionally, the role of amino acids and vitamins in promoting metabolic processes and their effects on activating photosynthesis by activating CO_2 in treated plants could be considered positive effects of activated dry yeast application (Ferguson *et al.,* 1995). Dry yeast may have these effects because it contains a significant amount of nutrients, including cytokinin, amino acids, minerals, carbohydrates, reducing sugars, enzymes, and vitamins (B1, B2, B3, and B12), that can enhance the fruit's physical and chemical properties (Khedr and Farid, 2000).

In this study, it was found that potassium thiosulfate application also affected the growth and fruiting attributes of the Thompson Seedless cultivar grown under abiotic stress conditions. Significant direct effects of potassium have been reported in recent studies on plant growth (Sustr *et al.,* 2019), photosynthetic capacity (Tränker *et al.,* 2018), and complex plant functional mechanisms in response to various stresses (Jia *et al.,* 2018). Potassium fertilization has been demonstrated to boost yield because of its important roles in numerous plant processes, such as nitrogen fixation, protein biosynthesis like ferredoxin and cytochrome, the electron transport chain complex, and the nitrate-absorbing enzyme structure (Mohamed *et al.,* 2023). Potassium is crucial for the proliferation of leaves because it helps the plant produce more dry matter and increases leaf area. Potassium plays a significant role in photosynthesis, and its availability increases the net photosynthetic rate and plant production. This may explain why plants develop more quickly when potassium is applied topically to them (Marschner, 1995). Furthermore, K is required for the activation of enzymes, processes involved in photosynthesis, the organization of osmotic pressure, the movement of stomata, energy transport, and the maintenance of the cation-anion balance in soil (Marschner, 2012).

Potassium promotes qualitative aspects of production, such as color, taste, consistency, and fruit preservation, by activating a variety of enzymes involved in plant development and vigor (Dhillon *et al.,* 1999). The improvement in sugar transport into berries is due to the rise in total TSS brought on by potassium foliar spray (Kumaran *et al.,* 2019). These outcomes are consistent with those that were found by Abd El-Razek *et al.* (2011), who made the claim that Crimson Seedless grape T.S.S. was raised by high K fertilization. Mohsen (2011) reported that the TSS of Crimson Seedless grapes was dramatically raised by potassium treatments at fruit set and the start of harvest. Martin *et al.* (2004) found that a greater potassium supply led to higher total soluble solids content and lower overall acidity in berries. The current outcomes align with those attained by Upadhyay *et al.* (2019), Baghdady *et al.* (2020), and Salem *et al.* (2021).

Abiotic stress disrupts the balance of physiological, biochemical, and metabolic processes in plant cells. This disruption results in decreased protein turnover, altered post-translational modification of proteins, diminished photosynthetic activity, and increased formation of reactive oxygen species (Singh *et al.,* 2019). Strong antioxidant defense systems, such as antioxidant enzymes and non-enzymatic antioxidants, are found in the plant body itself. To reduce oxidative damage and scavenge reactive oxygen species (ROS), plants develop more antioxidants when under stress (Rahman *et al.,* 2021). The findings of the current study revealed that the activities of POD, PPO, and CAT were significantly increased in control groups with a significant decrease in PAL activity compared to those in the treated grapevines (Figure 4). The positive effects observed may be attributed to the protective function of the MLE, dry yeast, and potassium thiosulfate in preserving the stability of the cell membrane, promoting plant growth, and regulating osmoregulation, as well as reducing oxidative stress by regulating enzyme activity (Arif *et al.,* 2023; Nimsi *et al.,* 2023). In addition, the amount of phenolic compounds in plants has a positive correlation with PAL activity (Christopoulos and Tsantili, 2015). Phenylalanine ammonia-lyase is the primary enzyme involved in phenolic biosynthesis, which is responsible for various phenolic-related quality properties (Tomás-Barberán and Espín, 2001). A significant reduction in PAL activity may be explained by the oxidative damage of enzyme proteins caused by ROS buildup in plant cells (Piechowiak and Balawejder, 2019). The application of bio-stimulants resulted in a decrease in the production rate of oxygen radicals under abiotic stress, as well as a reduction in the activity of POD and SOD (Hasanuzzaman *et al.,* 2021). The protective impact of MLE against salinity stress has been well documented in many plant species (Hassanein *et al.,* 2019), where they reported that the decline in catalase enzyme levels may be attributed to the improved efficiency of the Halliwell-Asada cycle.

Conclusion

In conclusion, Thompson seedless yield and berry quality were seriously enhanced by various treatments in the following order: MLE + dry yeast + potassium thiosulfate > MLE > dry yeast > potassium thiosulfate. All tested treatments significantly increased chlorophyll pigment and suppressed the oxidative stress enzymes that ultimately increased the tolerance of grapevines to abiotic stress. Moreover, MLE + dry yeast + potassium thiosulfate notably circumvented the deleterious effects of abiotic stress on grapevines, evident from improved vegetative growth, yield components, and berry quality. Thus, it can be recommended to use MLE and dry yeast as an alternative to chemical fertilizers, which are eco-friendly and safe for humans.

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تأثير مستخلص أوراق المورينجا الطبيعية والخميرة الجافة وثيوكبريتات البوتاسيوم على صنف عنب طومسون سيدلس تحت الظروف المناخية لأسيوط

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الملخص

أجريت هذه الدراسة في موسمي 2022 ،2023 بالمزرعة البحثية بكلية الزراعة – جامعة أسيوط – مصر لدراسة تأثيرات الرش بمستخلص أوراق المورينجا بتركيز 3٪ والخميرة الجافة 1٪ وثيوكبريتات البوتاسيوم 1٪ ومستخلص أوراق المورينجا 1.5٪ + الخميرة الجافة 0.5٪ + ثيوكبريتات البوتاسيوم 0.5٪ كمواد تزيد من قدرة النبات على تحمل الضغوط البيئية الغير حيوية حيث تم رشها أثناء بداية النمو، وبعد عقد الثمار، وبعد شهر واحد من الرشة الثانية على النمو الخضري والمحصول وجودة الحبات لصنف العنب طومسون سيدلس في ظل الظروف المناخية لأسيوط وكان تصميم التجربة قطاعات كاملة العشوائية وثلاث مكررات لكل معاملة.

أظهرت النتائج أن الرش الورقي لجميع المعاملات المدروسة أدى إلى تحسين مساحة الأوراق ومحتوى الكلوروفيل ووزن خشب التقليم والمحصول وجودة الحبات ومقاومة الإجهادات غير الحيوية . وسجلت أعلى نسبة لعقد الحبات (17.21%) والمحصول لكل كرمة (14.69 كجم) في المعاملة المركبة مقارنة بالكنترول والتي أنتجت أقل قيمة لنسبة العقد (15.6%) والمحصول (9.94 كجم/كرمة) وفي نفس الاتجاه حققت المعاملة المركبة أعلى نسبة للمواد الصلبة الذائبة الكلية (21.45%) والسكريات المختزلة (16.26%) وأقل حموضة (0.301%)، بينما كانت النسب في الكنترول 19.05%، 14.84%، 0.481% على التوالي.

من نتائج الدراسة يمكن التوصية أن معاملة الخليط هي الأكثر فعالية في تحسين خصائص العنقود ومقاومة الاجهاد البيئي الغير حيوي بالإضافة الى انها مواد صديقة للبيئة.

***الكلمات المفتاحية**: الاجهاد البيئي الغير حيوي، البوتاسيوم، المحصول، المورينجا، جودة الحبات.*