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Article

Effect of Spraying Chitosan and Algae Extract on Vegetative and Leaves Chemical Content of Banaty Grapevines

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Abstract: Thirty 10-year-old Banaty grapevines were used in this investigation during 2022 and 2023 growing seasons cultivated in a private vineyard in West Abu Qurqas center, El-Minia Governorate, Egypt. Tested the effects of spraying algae extracts at (0.25, 0.50 and 0.75 g/L) and chitosan at (0.05, 0.1 and 0.2%) as well as their interactions with total 10 treatments in a Completely Randomized Block design with three replicates per treatment. The findings indicated that all treatments significantly enhanced various aspects of grapevine growth and leaves pigments and nutrient content. Moreover, the noted beneficial benefits were typically commensurate with the escalating doses of these treatments. Nevertheless, the results indicated no substantial disparities between the medium and elevated quantities of these substances. The most significant advantages were noted with the application of chitosan as individual application. The treatment that included three sprays of algae extracts (0.50 g/L) plus chitosan (0.1%) was the most economically treatment in improving Banaty grapevines vegetative growth and leaves chemical content under Minia conditions.

Key words: Chitosan, algae extracts, vegetative, chemical content and Banaty grapevines.

1. Introduction

Grapes rank third in the production of fruit crops in Egypt, following citrus and mangoes. Consequently, the necessity for extensive land to achieve substantial fruit yield results in challenges for grapevines cultivated in elevated temperature environments, particularly regarding berry size, colouration, and overall quality. Grape growers in these regions employ horticultural practices to improve grape quality. Various compounds are widely applied in grapevines, with certain nutrients and natural extracts classified as biostimulants (**El-Saman and Refaai, 2025)**. The grape cultivation region in Egypt encompasses 186735 feddans, with a productive area of 175245 feddans, resulting in an aggregate production of 1715410 tonnes. The cultivated land in Minia Governorate amounted to 21098 feddans, with a productive area of 20852 feddans, resulting in a total of 205244 tonnes (**MALR, 2023**). The primary characteristics enabling the cultivation of table grapes throughout Egypt include climate, soil type, and production technology. **Dhekny (2016**) states that fresh grapes and grape products contain beneficial phenolic compounds, vitamins, and fiber. The White Banaty grape cultivar is esteemed in Egypt for its dual purpose as a source of fresh fruit and raisins.

Environmental regulations are propelling the advancement of sustainable strategies. Bio-stimulating agents like algae extract play a significant role in this context (**Omar *et al.,* 2017; Abou-Zaid and Eissa, 2019; Zarraonaindia *et al.,* 2023; Ibrahim *et al.,* 2024**). The agriculture sector is increasingly focused on utilizing natural sources, such as algae extract, in horticulture as a bio-stimulant agent for nutrient formation in food (**Zarraonaindia *et al.,* 2023**). Currently, there is extensive utilization of natural compounds to enhance the predictability and quality of grapevine fruit. Algae extract is one of the most underutilized compounds for plant stimulation. Algae extract, increasingly utilized in horticulture, contains vitamins, including those perhaps derived from bacteria that associate with marine plants, notably vitamin B12. The algal extract contains vitamin C and fucoxanthin, which may serve as a precursor to vitamin A, while vitamin A itself is absent in its precursor form (beta-carotene). The B group vitamins included include B2 (riboflavin), B1 (thiamine), B12, folic acid and pantothenic acid. Vitamin K, vitamin E (tocopherol), and several growth-promoting compounds are present in significant concentrations in algal extract (**Selvam and Sivakumar 2014, Venkata *et al.,* 2015, and Zarraonaindia *et al.,* 2023**).

Chitosan is a natural biopolymer obtained from the deacetylation of chitin, which, when applied to plant surfaces, serves three functions: film formation, antibacterial action, and elicitation (**Romanazzi *et al.* 2018**). Chitosan is considered non-toxic because to its extensive utilization in diverse applications, including medicine and the food sector, and it was among the initial fundamental compounds sanctioned for plant protection by the European Union **(Marchand *et al.,* 2021**). In the United States, the Food and Drug Administration has designated it as GRAS (Generally Recognised as Safe) (**Romanazzi *et al.,* 2022). Sharif et al. (2018**) assert that chitosan enhances the physiological traits of plants and extends the post-harvest shelf life of products.

Consequently, the present investigation has been conducted to enhance the vegetative growth parameters and leaves chemical content of grapes by utilizing antioxidants such as chitosan and algae extracts at varying concentrations.

2. Materials and Methods

Vineyard location and conditions:

Thirty 10-year-old Banaty grapevines were used in this investigation during 2022 and 2023 growing seasons cultivated in a private vineyard in West Abu Qurqas center, El-Minia Governorate, Egypt.

The soil analysis indicated a well-drained sandy composition. Table (A) presents the soil characteristics as outlined by **Wilde *et al.* (1985**). Vines are arranged 2.0 m apart inside rows, while the inter-row gap is 3.0 m.

During the second week of January, a head pruning system was used in both seasons, resulting in 68 eyes per vine (15 fruiting spurs × 4 eyes per spur + 4 replacement spurs × 2 eyes). The thirty vines that were chosen were uniformly vigorous and robust, showing no outward symptoms of nutrient deficiencies, with routine fertilization and surface irrigation system utilizing water from the Nile Rivier was implemented.

Table (A). Analysis of the mechanical, physical, and chemical properties of the examined orchard soil

Soil characters		2022/2023
Particle size distribution (%)	Sand	4.08
	Silt	34.90
	Clay	61.02
	Texture class	Clay
EC ppm (1:2.5 extract)		288
pH (1:2.5 extract)		7.97
Organic matter %		2.33
$CaCO_3$ %		2.54
Soil nutrients	Total N (%)	0.22
	Available P (ppm)	5.18
	Available K (ppm)	505.0
	Zn (ppm)	2.6
	Fe (ppm)	2.4
	Mn (ppm)	4.2
	Cu (ppm)	0.13

2.1. Experimental work

The study design included ten treatments of foliar sprays of chitosan and algae extract at varying concentrations, together with their interactions and control, arranged in a Completely Randomized Block design with three replicates per treatment.

1. Control (spray with tap water).
2. Algae extract at 0.25 g/L.
3. Algae extract at 0.50 g/L.
4. Algae extract at 0.75 g/L.
5. Chitosan at 0.05%.
6. Chitosan at 0.1%.
7. Chitosan at 0.2%.
8. Algae extract at 0.25 g/L+ Chitosan at 0.05%.
9. Algae extract at 0.50 g/L+ Chitosan at 0.1%.
10. Algae extract at 0.75 g/L+ Chitosan at 0.2%.

The foliar treatments utilizing varying quantities of chitosan and algae extract were administered thrice: at the commencement of vegetative growth, post-fruit set, and one month following the second application. A wetting agent was incorporated into all solutions of algae extract at a concentration of 0.1%. The chemical analyses of the brown algae extract utilized in this study are presented in Table B, as referenced in **Ibrahim *et al.* (2024).**

Table (B). The chemical analysis of the brown algae extract utilized in the present investigation

Compound	Concentration
Organic Matter	42 ~ 57%
Total Nitrogen	0.6 ~ 1.6%
Phosphorus (P_2O_5)	7 %
Potassium	17 ~ 20%
Mg	0.49 ~ 0.62%
Ca	0.44 ~ 1.70 %
Fe	0.15 ~ 0.30 %
Alginc acid	10 – 14%
Soluble in water	100%
Appearance	Flake or Particle

2.2. Data collection

The ensuing parameters were examined to determine the impact of the aforementioned treatments:

- Vegetative growth aspects: The morphological characteristics of five productive branches of the selected vine were assessed following the end of growth: Main shoots height, leaf number/shoot, the leaf area (cm^2) of the apical sixth and seventh leaves was documented according to **Ahmed and Morsy, (1999**) **Leaf area** = 0.56 (0.79 x w^2) + 20.01; where, W = the maximum leaf width**.** The average pruning wood weight (Kg/vine) was determined by measuring the mass of one-year-old wood removed after pruning at the end of the growing season. Cane thickness (cm).

- Leaf pigments: In order to determine the amount of chlorophyll a and b, as well as total carotenoids, in leaves, the method developed by **Von Wettstein (1957) was used (1957).**

- Leaf nutritional status: Leaf petioles in 20 leaf basal clusters were analyzed for nitrogen, phosphorus, and potassium percentages using the Micro-Kjeldahl method, spectrophotometry, and flame photometry, respectively, following the berry set in the first week of July. While the content of zinc, iron, and manganese (ppm) determined through atomic absorption, the methods established by **Cottenie *et al.* (1982) and Balo *et al.* (1988**).

2.3. Data analysis

Data analysis was conducted utilizing the new L.S.D. technique at a significance level of 5%, as outlined by **Mead *et al.* (1993).**

3. Results and Discussion

3.1. Aspects of vegetative growth:

The data presented in Table (1) indicate that the main shoot length, leaves number, leaf area, average pruning weight, and cane thickness of Banaty grapevines were substantially increased in comparison to the untreated control as a result of the application of algae extract (0.25-0.75 g/L) and chitosan (0.05-0.2%). Moreover, the beneficial effect on the traits were predominantly concentration-dependent for each treatment across both seasons. The most significant positive benefits were noted with the application of chitosan at 0.2%, followed by 0.1%. Nonetheless, altering the concentration of any of the examined preparations from medium to high levels did not give any enhancements in the trait. The vines treated with three applications of algae extract (0.75 g/L) + chitosan (0.2%) during both seasons exhibited the highest main shoot height values (96.2 cm and 97.1 cm), leaves number (24.1 and 24.9), leaf area (174.3 and 175.7 cm^2), average pruning weight (2.05 and 2.12 g), and cane thickness (1.10 and 1.12 cm) in 2022 and 2023, respectively. Following by the medium concentration for both materials, with no significant difference between them. Conversely, this parameter attained the lowest values in vines that were not treated. These consistent results were observed during both growing seasons.

In modest concentrations, algae extracts contain cytokinins, auxins, gibberellins, and amino acids, which allow them to activate the physiological processes of plants, improve blooming and yield, enhance growth, and facilitate harvest. As a result, it has been identified as a bio-stimulant for a variety of fruits and vegetables (**Arioli *et al.,* 2021**). The incorporation of algae extracts at a concentration of 0.2% markedly improved main shoot height, leaf count, leaf area, pruning wood weight (kg) per vine, and cane thickness (**Abd El-Moatamed 2024**). Foliar application of algae extracts improves growth parameters, yield productivity, and fruit quality characteristics (**Harhash *et al.,* 2021**). An important source of nutrients and organic matter, algae extract also stimulate plant growth. So, according to **Stirk *et al.* (2020**) and **Ibrahim *et al.,* (2024**), applying algal extracts to the leaves of fruit crops is a great way to boost their productivity, quality, photosynthetic rate, total soluble solids (TSS), tolerance to abiotic stress, and shelf life.

The significant influence of chitosan on the vegetative growth parameters of fruit trees is attributed to the activation of key enzymes related to nitrogen metabolism (protease, nitrate reductase, and glutamine synthetase) and the improvement of photosynthesis, both of which facilitate the vegetative growth of these trees (**Górnik *et al.,* 2008; Ibraheim and Mohsen, 2015**). Chitosan enhances the production of specific plant growth hormones, such as gibberellins, and affects certain auxin biosynthesis signalling pathways via a tryptophan-independent mechanism (**Ferguson and O'Neill, 2011**). ABA plays a vital role in regulating water usage by promoting stomatal closure, thereby influencing water and nutrient absorption through osmotic pressure modifications in plant cells. Furthermore, it affects water loss through transpiration (**Hadwiger *et al.,* 2002)** and reduces harmful free radical accumulation by increasing antioxidant levels and enzymatic activities (Jail *et al.* 2014; Ibraheim and Mohsen, 2015). The findings align with the publications by **Abd El-Rahman (2021), El-Senosy (2022), Abd El-Hakim (2023), and Masoud *et al.* (2024).**

Table (1). Foliar spray with algae extract and chitosan on Banaty grapevines vegetative growth aspects in 2022 and 2023 growing seasons

Characteristics / Treatments	Main shoots length (cm)	Number of leaves/shoot			Leaf area (cm^2)		Pruning wood weight (kg)/vine		Cane thickness (cm)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Control	88.7	89.6	17.8	18.3	166.0	168.3	1.71	1.75	0.87	0.90
Algae extract (0.25 g/L)	90.9	91.7	19.8	20.4	168.1	170.5	1.79	1.83	0.93	0.95
Algae extract (0.50 g/L)	92.4	93.2	21.2	21.6	169.5	171.8	1.86	1.90	0.97	1.00
Algae extract (0.75 g/L)	93.6	94.5	22.0	22.6	170.6	172.9	1.91	1.96	1.00	1.03
Chitosan (0.05%)	92.3	93.1	21.0	21.6	169.5	171.9	1.86	1.90	0.98	1.00
Chitosan (0.1%)	93.8	94.7	22.2	22.7	170.7	173.2	1.93	1.98	1.03	1.04
Chitosan (0.2%)	94.9	95.9	23.0	23.6	171.7	174.4	1.97	2.03	1.05	1.07
Algae extract (0.25 g/L) + Chitosan (0.05%)	93.6	94.6	22.0	22.8	171.9	173.3	1.94	1.97	1.02	1.04
Algae extract (0.50 g/L) + Chitosan (0.1%)	95.0	96.0	23.3	23.9	173.2	174.6	2.00	2.06	1.07	1.09
Algae extract (0.75 g/L) + Chitosan (0.2%)	96.2	97.1	24.1	24.9	174.3	175.7	2.05	2.12	1.10	1.12
New LSD at 0.05	1.3	1.4	1.0	1.1	1.2	1.3	0.06	0.07	0.04	0.04

3.2. Leaf pigments content mg/100 g F.W

Table 2 demonstrate that the levels of chlorophyll (a, b, and total) and total carotenoid were significantly affected by the application of both algae extract and chitosan at varying concentrations in comparison to the untreated control. The highest mean values in individual form were observed with chitosan at 0.2%, followed by 0.1%, with no significant difference between the two concentrations. The enhancement of pigments content was dependent on the concentration for each treatment. The findings demonstrated that the measured variables were affected by different concentrations of algae extract and chitosan when used in combination as a foliar spray. The control treatment demonstrated the lowest values in both seasons, whereas the combination of Algae extract (0.75 g/L) and Chitosan (0.2%) yielded the highest values. The medium concentrations for both materials were observed in descending order with no notable difference with the highest concentrations, whereas the other treatments exhibited intermediate values across both seasons.

Table (2). Foliar spray with algae extract and chitosan on Banaty grapevines leaves pigments in 2022 and 2023 growing seasons

Characteristics / Treatments	Chlorophyll a mg/100 g FW		Chlorophyll b mg/100 g FW		Total chlorophyll mg/100 g FW		Total carotenoid mg/100 g FW	
	2022	2023	2022	2023	2022	2023	2022	2023
Control	1.09	1.12	0.95	0.97	2.04	2.09	5.9	6.5
Algae extract (0.25 g/L)	1.17	1.20	1.00	1.01	2.17	2.21	7.2	7.5
Algae extract (0.50 g/L)	1.22	1.25	1.04	1.06	2.26	2.31	7.9	8.3
Algae extract (0.75 g/L)	1.26	1.28	1.06	1.09	2.32	2.37	8.5	9.0
Chitosan (0.05%)	1.24	1.26	1.04	1.05	2.28	2.31	8.1	8.4
Chitosan (0.1%)	1.29	1.30	1.08	1.10	2.37	2.4	8.9	9.2
Chitosan (0.2%)	1.31	1.32	1.10	1.12	2.41	2.44	9.4	9.8
Algae extract (0.25 g/L) + Chitosan (0.05%)	1.30	1.30	1.09	1.10	2.39	2.4	8.8	9.4
Algae extract (0.50 g/L) + Chitosan (0.1%)	1.35	1.35	1.13	1.14	2.48	2.49	9.6	10.2
Algae extract (0.75 g/L) + Chitosan (0.2%)	1.39	1.38	1.14	1.16	2.53	2.54	10.2	10.9
New LSD at 0.05	0.05	0.04	0.03	0.04	0.06	0.07	0.7	0.8

The elevated levels of algae extract likely facilitated the enhancement of chlorophyll content by inhibiting chlorophyll degradation, leading to increased chlorophyll concentrations (**Whapham *et al.,* 1993; Blunden *et al.,* 1997). Salvi *et al.* (2019)** showed that vines treated with algae extract had elevated pigment levels due to enhanced photosynthesis and stomatal conductance. Prior studies have shown that the use of seaweed extract in significant quantities leads to the highest concentrations of pigments. The beneficial effects of the seaweed extract product align with the research conducted by **El-Senosy (2022), Belal *et al.* (2023), and Ali *et al.* (2024**).

Many authors, such as **Crimi and Lichtfause (2019**), have conducted recent studies that have shown the effectiveness of chitosan in increasing the chlorophyll content of leaves. Chitosan has been recognized as a substantial regulator of plant photosynthesis and metabolic processes. Furthermore, the chlorophyll content is elevated as a consequence of the enhancement of nitrogen transmission to the foliage and the increased absorption of NPK (**Abd EL-Gawad and Bondok, 2015**). These findings are consistent with those reported by **Abd El-Rahman (2021), El-Senosy (2022), Abd El-Hakim (2023), and Masoud *et al.* (2024).**

3.3. Nutritional status of leaves

The information regarding the influence of varying concentrations of algae extract and/or chitosan on the leaves N, P, K%, Zn, Fe and Mn ppm of ‘Banaty’ grapevines during the 2022 and 2023 seasons is illustrated in Table (3). The data indicates that spraying the vines three times with algae extract at concentrations of 0.25, 0.50, and 0.75 g/L, or chitosan at 0.05, 0.1, and 0.2%, resulted in a significant improvement in the content of N, P, K%, Zn, Fe and Mn ppm in the leaves compared to the control treatment. Notably, a significant increase in the nutrients were observed, particularly with all chitosan concentrations. The highest individual treatment was 0.2% chitosan, followed closely by 0.1%, with no significant difference between the two. The interaction between algae extracts and chitosan concentrations revealed that higher concentrations resulted in an increase in N, P, K%, Zn, Fe and Mn ppm in leaves, with no significant difference observed between the two maximum concentrations. The data revealed that the control group exhibited the lowest nutrients content in leaves. In contrast, the application of 0.75 g/L algae extract combined with 0.2% chitosan resulted in N, P, K%, Zn, Fe and Mn ppm values of (1.89-1.97%), (0.37-0.38%), (1.32-1.39%), (61.2-61.9 ppm), (66.0-65.9 ppm) and (65.6-67.0 ppm), respectively, representing the highest mean values observed.

Algae extracts contain hormones that promote root development and enhance nutrient absorption, both of which contribute to increased plant vitality and growth. A substantial body of research has shown that seaweed extract possesses distinctive properties that promote growth. The aforementioned features may impact both the composition of plant roots and the chemical, biological, and physical properties of the soil (**Taskos *et al.,* 2019**). The composition of both macro and micronutrients in algae extract may partially account for its efficacy in improving nutritional status (**Cabrera *et al.,* 2003**). Supporting the findings of **El-Senosy (2022), Belal *et al.* (2023**), and **Ali *et al.* (2024**), previous research has shown that algae extract improves the nutritional value of grapevine leaves. Research has shown that increasing doses of algal extract applied to grapevine leaves enhance nutrient levels, both macro and micro.

The positive effect of chitosan on the mineral content of adult leaves of 'Early Sweet' grapevines can be attributed to its role in bio-stimulation, potentially leading to enhanced mineral elements and improved photosynthesis (**Ahmed *et al.,* 2016; El-Kenawy, 2017; Ayed, 2018**). Furthermore, it has been reported that chitosan improved the transport of specific elements, such as nitrogen (**Gornik *et al.,* 2008)**. Chitosan enhances the activity of specific essential enzymes in plant tissues (**Ortmann and Moerschbacher, 2006; Kafagy, 2019**). The influence of chitosan on elemental composition and absorption of water is reflected in the enhanced production of plant pigments and nutrients (**Hadwiger *et al.,* 2002). El-Senosy (2022)** and **Ali *et al.* (2023)** report similar outcomes.

Table (3). Foliar spray with algae extracts and chitosan on Banaty grapevines leaves nutritional status in 2022 and 2023 growing seasons.

Characteristics / Treatments	Leaf N %		Leaf P %		Leaf K %		Leaf Zn ppm		Leaf Fe ppm		Leaf Mn ppm	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Control	1.60	1.65	0.15	0.14	1.13	1.15	49.8	50.4	52.9	53.6	52.0	52.7
Algae extract (0.25 g/L)	1.68	1.72	0.20	0.20	1.18	1.21	55.9	56.2	59.2	59.1	57.2	58.5
Algae extract (0.50 g/L)	1.75	1.78	0.25	0.26	1.21	1.25	57.4	57.7	61.2	61.1	59.4	60.8
Algae extract (0.75 g/L)	1.79	1.83	0.28	0.29	1.23	1.28	58.6	59.0	62.7	62.6	61.3	62.8
Chitosan (0.05%)	1.74	1.80	0.25	0.26	1.22	1.27	57.3	57.7	61.1	60.9	59.3	60.7
Chitosan (0.1%)	1.80	1.87	0.31	0.30	1.26	1.32	58.8	59.1	62.7	62.6	61.3	63.0
Chitosan (0.2%)	1.83	1.91	0.33	0.32	1.27	1.34	59.9	62.3	64.1	64.2	63.1	64.9
Algae extract (0.25 g/L) + Chitosan (0.05%)	1.80	1.87	0.29	0.30	1.26	1.31	58.7	59.1	62.9	62.7	61.5	62.9
Algae extract (0.50 g/L) + Chitosan (0.1%)	1.85	1.93	0.35	0.35	1.30	1.36	60.0	60.6	64.5	64.4	63.6	65.0
Algae extract (0.75 g/L) + Chitosan (0.2%)	1.89	1.97	0.37	0.38	1.32	1.39	61.2	61.9	66.0	65.9	65.6	67.0
New LSD at 5%	0.05	0.06	0.04	0.04	0.03	0.04	1.3	1.4	1.6	1.7	2.0	2.1

4. Conclusion

Previous findings indicate that optimal economic outcomes regarding vegetative growth aspects and leaves chemical content were attained by applying a mixture of 0.50 g/L algae extracts and 0.1% chitosan to Banaty grapevines in the Minia region. So, its recommended to use this treatment was administered three times: at the onset of vegetative growth, after fruit set, and one month later.

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تأثير الرش الورقى بإستخدام الشيتوسان و مستخلص الطحالب البحريه على النمو الخضرى و المحتوى العنصرى لأوراق كرمات العنب البناتى

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الملخص العربى

إستخدمت ثلاثين كرمة من العنب البناتى بعمر ١٠ سنوات فى مزرعه خاصة غرب أبو قرقاص محافظة المنيا، لدراسة تأثير الرش الورقى بمستخلص الطحالب البحريه بتركيز (٠,٢٥، ٠,٥٠ و ٠,٧٥ جم/لتر) و الشيتوسان بتركيز (٠,٠٥، ٠,١ و ٠,٢%) و تفاعلاتهم بإجمالى ١٠ معاملات صممت فى تصميم كامل العشوائية فى ثلاث مكررات لكل معامله. أشارت النتائج إلى أن جميع المعاملات أدت إلى تعزيز فى صفات النمو الخضرى و محتوى الأوراق من الصبغات و العناصر. مع ملاحظة زياده فى القيم للصفات المدروسه مع زياده التركيزات و عدم ملاحظة أى فرق معنوي بين التركيزات المتوسطه و الأعلى منها. و لوحظ أن أفضل القيم سجلت مع الرش بالشيتوسان كمعامله فرديه أفضل من مستخلص الطحالب البحريه. و كانت المعامله الِتى إشتملت على الرش ثلاث مرات بتركيز ٠,٥٠ جم/لتر مستخلص طحالب بحريه مع ٠,١% شيتوسان هى المعامله الأكثر إقتصادية فى تحسين النمو الخضرى و محتوى الأوراق من الصبغات و العناصر الغذائية لكرمات عنب بناتى تحت ظروف محافظة المنيا.

الكلمات المفتاحية: شيتوسان، مستخلص طحالب بحريه، نمو خضرى ، محتوى كيميائى ، عنب بناتى