# Chitosan and riboflavin treatments affect marjoram growth, yield, and chemical composition

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**Background**
Marjoram is primarily cultivated to produce dried leaves and essential oil. Its active components have a wide range of biological and pharmacological activities in modern medicine. There is an interest in increasing marjoram production for local production and export. Chitosan (CH) and riboflavin (R) are required to promote plant growth and active ingredients.
**Objective**
The purpose of this study was to investigate how marjoram performance was affected by foliar spraying of CH and R at different concentrations.
**Materials and methods**
This investigation was performed over two successive seasons at two cuts. The used concentrations of CH were 100, 150, and 200 ppm, while R concentrations were 50 and 100 ppm. They were applied four times as a foliar spray to the vegetative growth during the growth season. At each harvest, the following parameters were recorded: fresh and dry weight, oil yield, total carbohydrates %, protein %, macronutrient content, total phenolics content, flavonoids content, antioxidant activity, and essential oil constituents to study their response to different treatments.
**Results and conclusion**
All the treatments of CH and R enhanced the vegetative growth, essential oil percentage, yield, active constituents, and oil components compared with untreated plants. Foliar application of CH at 200 ppm and R at 100 ppm increased plant growth, yield of volatile oil as well as chemical constituents compared with other treatments; however, CH was more effective than R. The combined application between CH and R resulted in the greatest values especially when both were applied at high doses.

**Keywords:**
active constituents, chitosan, essential oil, gas chromatography-mass spectrometry, *Majorana hortensis*, riboflavin

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## Introduction

*Origanum majorana* L. (syn. *Majorana hortensis* Moench) is considered one of the most common herbs of the Lamiaceae family, and it is commonly referred to as sweet marjoram [1].

Marjoram plant is a perennial herbaceous plant native to the Mediterranean region and cultivated in many countries in Asia, North Africa, and Europe [2–4]. It is cultivated as a garden plant, culinary herb, and medicinal and aromatic plant in most regions of the world [5]. It is among the most valuable herbs grown in Egypt; it is produced for domestic consumption as well as exportation.

The plant's aerial parts are used to extract essential oil, which has numerous medicinal properties and applications in the food industry [6] in addition to some active ingredients with major constituents such as flavonoids, phenols, and terpenoids [7], along with other substances like vitamins, fatty acids, and steroids [8]. Thus, dried leaves, extracted leaves, and essential oil are the various forms of sweet marjoram that are used in traditional therapy and other pharmacological activities.

The fresh or dried herb and essential oil of the marjoram plant is used in the food industry as spices, food preservatives, and flavoring [9]. In traditional and folkloric medicine, it is used to treat a wide range of diseases, such as cancer, neurological, rheumatologic, cardiac, respiratory, and digestive disorders, to treat colds and used as a carminative, expectorant, and antispasmodic agent [10–15].

The major components of marjoram volatile oil are used for scenting cosmetics and flavoring pharmaceuticals [16,17]. It is used commercially to scent soaps, lotions, and perfumes [18,19]. Also, it has antibacterial, antioxidant, anti-inflammatory, and anticancer properties [15,20].

Chitosan (CH), one of the most commonly used natural polymers, is derived via deactylating of chitin, which can be easily obtained from shellfish waste residue from food processing. It is a polysaccharide present in the exoskeleton of shellfish such as shrimp, lobster, and crabs [21]. CH is an inexpensive biodegradable compound that is environmentally friendly and has various applications in agriculture. Generally, in agriculture, CH has been applied as a fertilizer for enhancing plant growth and development [22].

The use of CH raised the activity of key enzymes in the metabolism of nitrogen and enhanced the transport of nitrogen through functional leaves, and these promoted plant development and yield, boosted the immune system of the plant, and protected the plants from microbial attack [23,24]. Furthermore, CH had beneficial effects on the roots, shoots, and leaves of most agricultural plants, leading to yield improvements in certain species of almost 20% [25].

Experiments on sweet basil by Kim *et al.* [26], thyme plants by Waly *et al.* [27], and lavandula plants by Fahmy and Nosir [28] found that the use of CH improved the plants' growth, yield, production of volatile oils, and total chlorophyll content. According to Chandrkrachang *et al.* [29], applying CH to Chinese cabbage and chili plants boosted the harvested yield of Chinese cabbage and the growth rates of chili plants. In addition, Shaheen *et al.* [30] found that spraying CH on potato plants at a rate of 5.0 $cm^3$/l improved the vegetation metrics of the plants as well as the production and composition of tubers. Also, the physical characteristics and nutritional content of potato tubers were enhanced.

Vitamins as bioregulators, at certain dosages, can have a significant impact on plant growth by regulating primary and secondary metabolism [31,32]. They are among the nutrients required for optimal growth in plants, animals, and humans. Also, vitamins are essential in many physiological and biological processes as agents, enzymes, or antioxidants. Furthermore, vitamins act as osmotic regulators in the plant, which, in low concentrations, result in resistance to salinization and drought, as well as plant resistance to some diseases [33,34].

Riboflavin (R), also known as vitamin B2), acts as a coenzyme that enhances the metabolism and growth of various plant species. It is recognized to be a key factor in several metabolic enzymes and electron transport; it is also necessary for photosynthesis, the citric acid cycle, the oxidation of fatty acids, and DNA repair [35]. R is an essential biosensitized product in plants, contributing to many uses and pharmacological activities [36] and it modulates several different physiological processes in plants [37]. Also, it plays an important role in plant resistance against pathogens [38]. R and its derivatives are critical for redox metabolism, generation of energy, and photosynthesis in plants [39] and induce the accumulation of antioxidant compounds in plant cells [40,41].

Azooz [42] revealed that spraying *Hibiscus sabdariffa* plants with 100 ppm R boosted their antioxidant activity and carbohydrate content, enhanced the resistance of plants to salinity stress, and promoted the growth of salinized plants. Abood and Abdulhameed [43] found out that the optimum grain yield was achieved by applying R to the foliage of sorghum plant at a dosage of 300 mg/l and improved most vegetative growth parameters under salt stress. A study on *Pelargonium graveolens* L. plant [44] concluded that application of R at 90 mg/l increased vegetative characteristics, percent, and yield of volatile oil, pigments, total phenolic, and total flavonoids.

Therefore, the target of this study was to assess how *M. hortensis* plant responded to foliar applications of CH and R to improve the growth, essential oil yield, as well as the chemical content (macronutrients, total carbohydrates, protein, phenols, flavonoids, and antioxidant activity).

## Materials and methods

### Procedures of the experiment

Two pot experiments were carried out at the National Research Centre, Giza, Egypt throughout the two seasons 2020 and 2021 to evaluate the impact of using both CH and R as well as their combination on the growth, volatile oil, and chemical ingredients of *M. hortensis* plant.

*M. hortensis* seedlings were purchased from a private nursery in Giza Governorate, Egypt. In both seasons, the marjoram seedlings (15 cm in height) were sown on the February 15, into pots (50 cm inner diameter and 30 cm in length) filled with 10 kg air-drying soil. After 3 weeks of planting, the seedlings were reduced to three plants per pot.

Before cultivation, soil samples were collected and analyzed chemically and physically at the Soil Science Department of the National Research Center using the methodology of Jackson [45] and Cottenie *et al*. [46] (Table 1).

CH solution product was obtained from Chitofarma Company, El-Sharkia Governorate, Egypt. R powder was obtained from the Botany Department, National Research Centre, Giza, Egypt. The experiment included 60 pots and 12 treatments, set up in a completely randomized block design with five replicates for each treatment. Plants were foliar sprayed using three levels of CH (100, 150, and 200 ppm), two levels of R (50 and 100 ppm) as well as the control treatment (treated by water), while the combined use of CH and R was as follows, each concentration of CH (100, 150, and 200 ppm) with each concentration of R (50 and 100 ppm). The plants were sprayed with growth stimulants four times throughout the growth season. The first spray was applied 1 month after transplanting, the second 3 weeks before the first cut, the third 2 weeks following the first cut, and the fourth 3 weeks before the second cut.

Marjoram plants were harvested twice in both seasons by chopping off their aerial parts at 12–15 cm above the soil surface. The first harvest was taken in May of both seasons, and the second harvest was collected in September of each season.

In both seasons for two cuts, randomly selected samples were taken from each replicate for all treatments and the following vegetative growth parameters were recorded: fresh and dry weights of herb g/plant.

#### Isolation, determination, and analysis of essential oil

The dried samples of marjoram aerial parts (100 g each) were isolated by hydro-desolation for 3 h using the Clevenger-type apparatus, following the method of Guenther [47]. The relative percentage (v/w) of the volatile oil and the total yield (m/100 plants) were calculated. The essential oil from marjoram plants, which were harvested at both cuttings in both seasons for all treatments, was dehydrated over anhydrous sodium sulfate and stored in the freezer until the chemical constituents were identified using gas chromatography-mass spectrometry (GC/MS).

GC/MS was used for analyzing the chemical components of the essential oil from marjoram herb for samples of each treatment. GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m×0.25 mm ID); the oven temperature was 40–240°C at a rate of 4°C/min; the transfer line temperature was 260°C; the temperature of the injector was 250°C; the carrier gas was helium with a linear velocity of 31.5 cm/s; split ratio was 1/60; flow rate was 1.1 ml/min; ionization energy was 70 eV; scan time was 1 s; and the mass range was 40–350 amu.

Library searches [48] were used to identify the compounds, combining MS and retention data of authentic compounds by comparison of their GC retention indices with those of the literature or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 98 and Wiley libraries or with mass spectra from the literature. Relative concentrations of the components were calculated without the use of correction factors based on GC peak areas.

#### Chemical analyses

Mineral contents such as nitrogen (N), phosphorus (P), and potassium (K) have been determined in the dried herb. The micro-Kjeldahl method was used to measure nitrogen in accordance with the method proposed by Anonymous [49]; the spectrophotometer method was used to evaluate phosphorus as explained by Snell and Snell [50], while the flame photometer method was used to estimate potassium as presented by Chapman and Pratt[51].

The percentage of total carbohydrates was determined in dry herbs according to the method proposed by Dubois *et al*. [52].

Total phenolic content (TPC) (mg/g) was determined using the Folin-Ciocalteu's reagent in an extract of

**Table 1 Characteristics of the soil before planting**

| | | | | | | Cations (meq/l) | | | | Anions (meq/l) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sand | Silt | Clay | $CaCo_3$ | pH | E.C. ($dS\,m^{-1}$) | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $Co^{-2}{}_3$ | $HCo^{-}{}_3$ | $Cl^{-}$ | $So^{-2}{}_4$ |
| 18.9 | 30.8 | 49.3 | 5.00 | 7.85 | 0.29 | 1.00 | 0.50 | 1.20 | 0.13 | – | 0.70 | 1.50 | 0.63 |

marjoram dried herb from each treatment that was collected at both cuttings in two seasons [53].

Total flavonoid content (TFC) (mg/g) in the dried herb extract was determined using a spectrophotometric technique [54].

Free radical scavenging activity % was evaluated in a dry herb extract using the standard method [55], which was then suitably modified [56].

### Statistical analysis

This study applied a complete randomized design, and the MSTAT-C program was used to analyze the data for each season [57]. Duncan's multiple range test was used for calculating the least significant differences between the mean values at the 5% level [58].

## Results

### Effect of chitosan and riboflavin on fresh and dry weight

The results in Table 2 show that spraying marjoram plants with both CH and R significantly affected the herb fresh weight of both cuts compared with the control treatment during two seasons. In both cuts, raising the rate of CH or R resulted in gradually significant increases in herb fresh weight. CH was more effective than R in increasing the fresh herb weight of the marjoram plant. The maximum yields of fresh weight (38.5 and 108.6 g/plant) and (42.3 and 111.06 g/plant) were obtained by applying CH at the highest rate of 200 ppm, compared with 35.1and 91.9 g/plant and 37.2 and 101.0 g/plant, which were obtained with spraying R at a high rate of 100 ppm in the first and second harvests in both seasons, respectively.

Generally, the combined use of CH and R resulted in the greatest fresh weight of the herb, especially when both were applied at high doses. The maximum yields of fresh weight (47.5 and 132.9 g/plant and 55.8 and 138.0 g/plant) were obtained by applying 200 ppm CH with 100 ppm R for both the first and second cuttings during the two growing seasons, respectively. In both cuts, the control plants significantly gave lower fresh herb weights with mean values of 24.8 and 67.1 g/plant and 28.9 and 59.2 g/plant in the first and second cuts for the two seasons, respectively, in contrast to plants receiving any of the different concentrations of CH and R (Table 2).

The dry weight of the herb had a similar trend to the results of the fresh weight, that is, the treatments that increased the fresh weight were the same, which produced the highest values of herb dry weight. Results in Table 2 prove that the different treatments of CH and R significantly increased the dry herb weight of marjoram plants compared with the control plant. In both cuts during two seasons, treated plants with CH alone had greater dry herb weights than treated plants with R; higher doses of R and CH were also associated with heavier dry herb weights in the plants. The mean values of CH at a high dose of 200 ppm were 12.6 and 27.3 g/plant for the first season and 15.1 and 26.9 g/plant for the second season at two cuts, respectively.

**Table 2 Chitosan and riboflavin effect on the fresh and dry mass of *Majorana hortensis* during the two seasons**

| | Fresh mass (g/plant) | | | | Dry mass (g/plant) | | | |
|---|---|---|---|---|---|---|---|---|
| | Season | | | | | | | |
| | First | | Second | | First | | Second | |
| | Harvest | | | | | | | |
| Treatments | 1st[st] | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 24.8i | 67.1d | 28.9h | 59.2f | 7.51j | 15.8e | 11.2e | 20.3bc |
| 100 CH | 32.8g | 91.4bcd | 35.4fg | 89.7de | 10.2h | 22.1cde | 12.9cde | 23.3abc |
| 150 CH | 37.1ef | 105.3abc | 40.6e | 101.0cd | 11.9fg | 26.0bcd | 13.7cde | 23.6abc |
| 200 CH | 38.5de | 108.6abc | 42.3de | 111.0bc | 12.6ef | 27.3bcd | 15.1bcd | 26.9abc |
| 50 R | 29.6h | 81.4cd | 32.3gh | 88.2e | 9.15i | 19.5de | 12.2de | 18.7c |
| 100 R | 35.1fg | 91.9bcd | 37.2f | 101.0cd | 11.2g | 22.5cde | 13.3cde | 26.6abc |
| 100 CH + 50 R | 39.2de | 93.8bcd | 41.6e | 95.8de | 13.1e | 23.3cde | 15.6bc | 28.1abc |
| 150 CH + 50R | 40.1cde | 106.8abc | 47.1c | 113.0b | 13.6de | 27.7bcd | 16.8b | 31.0abc |
| 200 CH+50 R | 42.6bc | 122.5ab | 51.9b | 118.0b | 14.7bc | 32.2ab | 18.0ab | 31.7abc |
| 100 CH+ 100 R | 41.6bcd | 113.7ab | 45.7cd | 115.0b | 14.3cd | 29.2abc | 16.9b | 31.5abc |
| 150 CH+100 R | 44.0b | 127.0a | 49.2bc | 135.0a | 15.4b | 33.2ab | 19.8a | 34.1ab |
| 200 CH+100 R | 47.5a | 132.9a | 55.8a | 138.0a | 16.8a | 35.6a | 20.7a | 35.8a |

Within a column, values followed by the same letter are not significantly different at the 0.05 probability level according to the Duncan test. CH, chitosan; R, riboflavin.

While the high dose of R (100 ppm) recorded 11.2 and 22.5 g/plant and 13.3 and 26.6 g/plant compared with 7.51 and 15.8 g/plant and 11.2 and 20.3 g/plant for control treatment in both cuts during the first and the second seasons, respectively. The highest dry weights (16.8 and 35.6 g/plant and 20.7 and 35.8 g/plant) were produced from the treatment of the highest concentrations of both CH with R (200+100 ppm) in both cuts during two seasons, respectively.

### Effect of chitosan and riboflavin on the essential oil content

The data presented in Table 3 indicates that, for single use, applying 200 ppm CH led to an increase in the essential oil percentage at both cuts, which recorded 1.95 and 0.91% in the first season and 1.88 and 0.96% in the second season, followed by spraying 100 ppm R, which recorded 1.78 and 0.77% and 1.80 and 0.78% in the first and second seasons for both cuts, respectively. In this regard, the maximum values were observed with plants spraying with CH at a high dose of 200 ppm as well as R at 100 ppm, which produced 2.45 and 1.34% and 2.55 and 1.44% in the two seasons at both cuts, respectively, while untreated plants recorded the minimum values of the essential oil% in both cuts, which were 1.45 and 0.50%, respectively, in the first season and 1.35 and 0.49% in the second season.

The results in Table 3 show that spraying marjoram plants with CH and R seemed to deeply affect the essential oil content (ml/100 plants) in comparison with untreated plants. Higher values of the volatile oil yield were obtained in plants that received the highest dose of CH (200 ppm) in both cuts; they were 24.6 and 26.7 ml/100 plants in the first season and 28.4 and 25.8 ml/100 plants in the second season, followed by R at 100 ppm, which gained 19.9 and 17.2 ml/100 plants in the first season and 24.0 and 20.8 m/100 plants in the second season at two cuts, respectively, compared with 10.9 and 7.8 ml/100 plants and 15.2 and 9.9 m/100 plants in the case of the untreated plants at both cuts in the two seasons, respectively. So, applying R was less effective in increasing oil yield than applying CH. The highest rates in the combined use of both CH (200 ppm) and R (100 ppm) resulted in the maximum increment in oil yield; they were 41.1 and 47.5 ml/100 plants in the first season and 52.8 and 51.4 ml/100 plants in the second year at both cuts, respectively.

### Effect of chitosan and riboflavin on the essential oil composition

Eleven compounds of the volatile oil in all marjoram treatments were detected and identified (Table 4). It was observed some variations in essential oil composition as affected by CH and R treatments. The total identified components in the essential oil accounted for 92.9–98.5% of all components with different treatments. The major constituents were predominant compounds present under all treatments; they were terpinen4-ol (39.04–58.36%), followed by trans-sabinene hydrate (4.26–21.92%) and then γ-terpinene (6.29–17.84%). Applying CH and R increased the major constituents of terpinen-4-ol in most treatments over the control. The highest content was 58.36% obtained from the treatment of

**Table 3 The effect of chitosan and riboflavin on the volatile oil concentration of *Majorana hortensis* in both the first and second seasons**

| | Volatile oil (%) | | | | Volatile oil yield (ml/100 plant) | | | |
|---|---|---|---|---|---|---|---|---|
| | Season | | | | | | | |
| | First | | Second | | First | | Second | |
| | Harvest | | | | | | | |
| Treatments | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 1.45d | 0.50i | 1.35i | 0.49f | 10.9h | 7.8g | 15.2g | 9.9g |
| 100 CH | 1.68c | 0.84fg | 1.63h | 0.82e | 17.1g | 18.7ef | 21.0fg | 19.1f |
| 150 CH | 1.69c | 0.89ef | 1.73gh | 0.84e | 20.1f | 23.0de | 23.6ef | 19.8f |
| 200 CH | 1.95b | 0.91def | 1.88efg | 0.96d | 24.6de | 26.7cde | 28.4de | 25.8e |
| 50 R | 1.60cd | 0.65h | 1.73gh | 0.73e | 14.7g | 12.9fg | 21.1fg | 13.5g |
| 100 R | 1.78c | 0.77g | 1.80fgh | 0.78e | 19.9f | 17.2ef | 24.0ef | 20.8f |
| 100 CH +50 R | 1.78c | 0.99cde | 1.96def | 1.07cd | 23.1e | 23.0de | 30.8cd | 29.6de |
| 150 CH +50 R | 1.98b | 1.01cd | 2.05cde | 1.07cd | 26.9cd | 28.0bcd | 34.5cd | 33.1cd |
| 200 CH+50 R | 1.98b | 1.05bc | 2.33b | 1.15bc | 29.0c | 33.9b | 42.0b | 36.4bc |
| 100 CH+100 R | 2.03b | 1.13b | 2.13cd | 1.18bc | 28.9c | 32.9bc | 35.8c | 36.7bc |
| 150 CH+100 R | 2.08b | 1.31a | 2.23bc | 1.21b | 32.0b | 43.4a | 44.1b | 41.3b |
| 200 CH+100 R | 2.45a | 1.34a | 2.55a | 1.44a | 41.1a | 47.5a | 52.8a | 51.4a |

Within a column, values followed by the same letter are not significantly different at the 0.05 probability level according to the Duncan test. CH, chitosan; R, riboflavin.

**Table 4 Changes in volatile oil constituents of *Majorana hortensis* during the second season due to chitosan and riboflavin spraying**

| | | | Treatments | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | Compound | RI | Control | 100 CH | 150CH | 200 CH | 50R | 100R | 100 CH +50 R | 150 CH +50 R | 200 CH +50 R | 100 CH +100 R | 150 CH +100 R | 200 CH +100 R |
| 1 | β –Pinene | 980 | 5.2 | 6.6 | 6 | 4.1 | 4.32 | 3.62 | 3.37 | 3.41 | 3.14 | 2.61 | 2.79 | 3.43 |
| 2 | Δ-3- Carene | 1011 | 4.4 | 2.62 | 4.06 | 5.51 | 4.27 | 3.61 | 7.38 | 8.93 | 4.04 | 4.67 | 3.48 | 3.1 |
| 3 | γ-Terpinene | 1062 | 10.24 | 14.38 | 17.84 | 6.29 | 10.49 | 10.71 | 12.69 | 9.44 | 10.25 | 6.64 | 7.15 | 10.79 |
| 4 | cis-Sabinene hydrate | 1070 | 2.27 | 1.84 | 2.81 | 1.75 | 1.82 | 1.65 | 1.89 | 2.07 | 1.77 | 2.07 | 1.93 | 1.9 |
| 5 | p-Menth-2-en-1-ol | 1172 | 1.68 | 1.32 | 1.25 | 1.33 | 1.26 | 1.23 | 1.14 | 1.19 | 1.36 | 1.64 | 1.29 | 1.26 |
| 6 | Terpinene-4-Ol | 1177 | 51.11 | 49.47 | 53.57 | 50.13 | 53.84 | 55.7 | 39.04 | 40.18 | 52.4 | 52.85 | 58.36 | 54.26 |
| 7 | α-Terpineol | 1185 | 7.23 | 5.59 | 4.81 | 5.86 | 5.22 | 4.53 | 4.62 | 4.71 | 6.69 | 6.78 | 6.66 | 6.45 |
| 8 | Linalyl acetate | 1257 | 0.20 | 0.96 | 0.83 | 0.54 | 1.07 | 0.59 | 0.89 | 0.96 | 0.95 | 0.75 | 1.5 | 1.26 |
| 9 | trans-Sabinene hydrate | 1266 | 13.76 | 7.64 | 4.26 | 16.89 | 13.25 | 12.01 | 19.43 | 21.92 | 11.3 | 13.77 | 9.82 | 8.36 |
| 10 | β-Caryophyllene | 1328 | 1.32 | 1.57 | 1.56 | 1.5 | 1.15 | 1.05 | 1.82 | 1.68 | 1.19 | 0.94 | 0.94 | — |
| 11 | β –Costol | 1756 | 0.94 | 0.91 | 1.53 | 0.9 | 1.05 | 3.3 | 1.82 | 1.1 | 2.05 | 2.27 | 2.29 | 3.04 |
| Total | | | 98.35 | 92.90 | 98.52 | 94.8 | 97.74 | 98.00 | 94.09 | 95.59 | 95.14 | 94.99 | 96.21 | 93.85 |

CH, chitosan; R, riboflavin; RI, retention index from the literature.

150 ppm CH+100 ppm R; trans-sabinene hydrate recorded the maximum value (21.92%) from applying 150 ppm CH+50 ppm R; and for γ-terpinene (17.84%) was the greatest proportion obtained from applying 150 ppm CH.

### Effect of chitosan and riboflavin on the nutrient content

The presented findings in Table 5 indicate that applying CH or R on marjoram plants raised the accumulation of nitrogen, phosphorous, and potassium (N, P, and K) content in herbs compared with the untreated plants. Applying a single CH caused a higher increment in the content of N, P, and K than applying R alone. Data in the same (Table 5) reveal that the combination of both CH and R increased nutrient elements more than applying each of these growth stimulators alone. A gradual increase was observed in the content of all nutrients by raising the concentration of the applied stimulator. The highest contents of N (3.5 and 3.9%), P (0.41 and 0.45%), and K (1.28 and 2.28%) were obtained from the treatment of the combination between the highest rates of CH and R at both cuts, respectively. The lowest values of nitrogen (2.0 and 2.2%), phosphorous (0.23 and 0.21%), and potassium (0.93 and 1.03%) were recorded with the control treatment at both cuts, respectively.

### Effect of chitosan and riboflavin on the total carbohydrate content

Results in Table 6 show that all the different concentrations of the CH or R increased the

**Table 5 Nutrient content of *Majorana hortensis* as affected by chitosan and riboflavin during the second season**

| | N (%) | | P (%) | | K (%) | |
|---|---|---|---|---|---|---|
| | | | Harvest | | | |
| Treatments | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 2.0k | 2.2c | 0.23f | 0.21f | 0.93f | 1.03h |
| 100 CH | 2.3i | 2.4bc | 0.31cde | 0.34de | 1.08e | 1.25g |
| 150 CH | 2.5h | 2.6abc | 0.32cd | 0.39bcd | 1.15d | 1.95e |
| 200 CH | 2.7f | 2.8abc | 0.36abc | 0.41abc | 0.19g | 2.07d |
| 50 R | 2.2j | 2.3c | 0.26ef | 0.30e | 0.95f | 1.23g |
| 100 R | 2.5h | 2.4bc | 0.29de | 0.33de | 1.13de | 1.60f |
| 100 CH+50 R | 2.6g | 2.9abc | 0.34bcd | 0.31e | 1.18cd | 2.12d |
| 150 CH+50 R | 2.8e | 3.2abc | 0.35bcd | 0.34de | 1.22bc | 2.18c |
| 200 CH+50 R | 3.1c | 3.4abc | 0.35bcd | 0.36cde | 1.24ab | 2.24ab |
| 100 CH+100 R | 2.9d | 3.3abc | 0.37abc | 0.39bcd | 1.23abc | 2.20bc |
| 150 CH+100 R | 3.3b | 3.8ab | 0.40ab | 0.43ab | 1.25ab | 2.23abc |
| 200 CH+100 R | 3.5a | 3.9a | 0.41a | 0.45a | 1.28a | 2.28a |

Within a column, values followed by the same letter are not significantly different at the 0.05 probability level according to the Duncan test. CH, chitosan; R, riboflavin.

**Table 6 Effect of chitosan and riboflavin on total carbohydrate and protein content of *Majorana hortensis* during the second season**

| | Total carbohydrates (%) | | Protein (%) | |
|---|---|---|---|---|
| | Harvest | | | |
| Treatments | 1st | 2nd | 1st | 2nd |
| Control | 10.24f | 10.15f | 12.50k | 13.75e |
| 100 CH | 11.83e | 12.76e | 14.38i | 15.00e |
| 150 CH | 12.54e | 14.00d | 15.63h | 16.25de |
| 200 CH | 14.31d | 15.42c | 16.88f | 17.50cdf |
| 50 R | 10.41f | 10.68f | 13.75 j | 14.38 e |
| 100 R | 10.68f | 12.63e | 15.63h | 15.00 e |
| 100 CH+50 R | 15.64c | 16.26c | 16.25g | 18.13cde |
| 150 CH+50 R | 15.95c | 18.04 b | 17.50 e | 20.00bcd |
| 200 CH+50 R | 16.00bc | 18.00b | 19.38c | 21.25abc |
| 100 CH+100 R | 16.31bc | 18.13b | 18.13d | 20.63abc |
| 150 CH+100 R | 17.00b | 18.79b | 20.63b | 23.75ab |
| 200 CH+100 R | 19.72a | 20.25a | 21.88a | 24.38a |

Within a column, values followed by the same letter are not significantly different at the 0.05 probability level according to the Duncan test. CH, chitosan; R, riboflavin.

percentage of total carbohydrate content (TOC%) compared with untreated plants. Generally, all concentrations of CH and R gradually increased the TOC% with increasing the concentrations at the two cuts. The high concentration of CH 200 ppm recorded high values of TOC% (14.3 and 15.24%) as well as 100 ppm R recorded (10.68 and 12.63%) in the two cuts, respectively, compared with other concentrations. However, the highest accumulation of carbohydrates (19.72 and 20.25%) was obtained from applying 200 CH+100 R compared with the control treatment (10.24 and 10.15%) in both cuts, respectively.

### Effect of chitosan and riboflavin on the protein percentage

Data in Table 6 demonstrate the positive responses of marjoram to CH and R in protein percentage (PO) content. The highest amount of PO in the dry herb was recorded in plants receiving 200 R+100 R, as 21.88 and 24.38% in the first and second cuts, respectively. However, untreated plants recorded 12.50 and 13.75% in both harvests, respectively.

Results in Table 6 indicate that the total protein % (PO %) had a similar trend as the result of the TOC%. The treatments that increased the TOC% were the same that recorded the highest values of PO%.

### Effect of chitosan and riboflavin on the total phenolic content

The total phenolic, flavonoids, and antioxidant activity were significantly influenced by applying various levels of both CH and R compared with untreated plants.

In general, the compounds' maximum values were noted in the case of single use at high levels of CH (200 ppm) and R (100 ppm); however, the combined treatments of CH and R at high rates were found to be more effective in increasing the values of these compounds than the noncombined treatments (Table 7).

The highest values of TPC compounds were recorded in the case of the single use of CH (30.60 and 30.85 mg/g) obtained from spraying 200 ppm CH and from the combined stimulators (33.80 and 32.73 mg/g) recorded by applying 200 ppm CH +100 ppm R. However, the control achieved the lowest values of TPC (25.47 and 25.86 mg/g) in both cuts, respectively.

### Effect of chitosan and riboflavin on the total flavonoid content

For TFC, the highest content was observed in the combination of the highest levels of CH and R (5.20

**Table 7 Effect of chitosan and riboflavin on some active constituents of *Majorana hortensis* during the second season**

| | Phenolic (mg/g) | | Flavonoids (mg/g) | | Free radical scavenging (%) | |
|---|---|---|---|---|---|---|
| | | | Harvest | | | |
| Treatments | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 25.47i | 25.86i | 3.26i | 2.97i | 46.00l | 39.46l |
| 100 CH | 27.39g | 28.06g | 3.99h | 3.42g | 48.90i | 41.65j |
| 150 CH | 30.14e | 30.25e | 4.37f | 3.60f | 50.90f | 46.46h |
| 200 CH | 30.60d | 30.85d | 4.66e | 3.67e | 51.00e | 47.41g |
| 50 R | 26.16h | 27.55h | 4.02h | 3.25h | 46.72k | 40.72k |
| 100 R | 30.21e | 27.74gh | 4.15g | 3.60f | 47.98j | 43.41i |
| 100 CH+50 R | 28.75f | 28.92f | 4.73d | 3.43g | 49.70h | 50.12f |
| 150 CH+50 R | 30.82d | 30.53de | 4.80c | 3.56f | 51.22d | 50.36e |
| 200 CH+50 R | 31.24c | 31.90b | 4.92b | 3.79d | 51.93c | 52.87b |
| 100 CH+100 R | 31.60c | 30.64de | 4.80c | 3.87c | 50.32g | 51.52d |
| 150 CH+100 R | 31.99b | 31.24c | 4.94b | 4.17b | 52.90b | 52.73c |
| 200 CH+100 R | 33.80a | 32.73a | 5.20a | 4.26a | 54.05a | 53.88a |

Within a column, values followed by the same letter are not significantly different at the 0.05 probability level according to the Duncan test. CH, chitosan; R, riboflavin.

and 4.26 mg/g) in both cuts, respectively. However, the lowest values of TFC were obtained from the untreated plants (3.26 and 2.97 mg/g).

#### Effect of chitosan and riboflavin on the free radical-scavenging activity (%)

The findings in Table 7 also confirm that all the levels of CH and the two doses of R raised the antioxidant activity percentage in comparison with control plants, reflecting the same trend of TPC and TFC. The increases gradually occurred when the CH dosage was raised to 200 ppm, it reached 51.00 and 47.41% and when R at 100 ppm gave 47.98 and 43.41%, the combination of 200 ppm CH plus 100 ppm R enhanced the percentage of antioxidant activity to the maximum values (54.05 and 53.88%) compared with the minimum values (46.00 and 39.46) obtained from untreated plants.

## Discussion

The growth, productivity, and secondary metabolite contents of plants depend on many different factors. CH and vitamins are two of these factors, which are used as nutrients or as growth stimulants necessary for medicinal and aromatic plants to promote normal growth. CH is a natural biopolymer, biostimulator, nontoxic, inexpensive compound that is biodegradable, environmentally friendly, and has important applications as a biofertilizer in agriculture. It has a low impact on environmental contamination as well as physiological effects on plants to stimulate growth and productivity [22,59–62].

The superior effect of CH on plant growth, yield, and productivity was that CH boosted the activities of key enzymes for nitrogen metabolism and enhanced nitrogen transportation in functional leaves, which support the growth and development of the plant [63–65], as well as increased the amount of nitrogen and potassium in plant shoots, which are critical for increasing the number of chloroplasts per cell, a rise in cell number and size per unit area, and increasing the synthesis of chlorophyll [66].

Moreover, Walker *et al.* [24] concluded that CH promoted the growth of roots, shoots, and leaves. It has been demonstrated that CH increases and stimulates the uptake of nutrients and the content of chlorophyll, improving the chloroplast and photosynthetic efficiency, the content of carbohydrates, the yield of oil, and its components. It also stimulates the synthesis of secondary metabolites, including polyphenols, TFC, lignin, and antioxidants [67–69].

Furthermore, Nijjar [70] added that CH's beneficial effects on plant development could be related to how it raises the phosphorus content of plants. Phosphorous is an important nutrient that is required for cell division, the formation of DNA and RNA, and the manufacture and transport of carbohydrates.

Khan *et al.* [63] mentioned that CH application can improve the vegetative growth and yield of maize plants by various mechanisms, such as by stimulating physiological processes, improving photosynthetic machinery, followed by improving plant biomass, active transfer of photoassimilates from the source to tissues, and developing leaf-blade thickness and dimensions of vascular bundles.

Also, one of the important effects of treating plants with CH is increasing the amino acid content in the plant or soil, which is released from CH [24]. However, this rise may also be related to the release of nitrogen from CH (polysaccharides–$NH_2$) by hydrolytic enzymes or the stimulation of xylem vessel growth, both of which improve nutrient absorption and nutritional status, in particular nitrogen, for the synthesis of amino acids. In another context, the increase in total nitrogen concentration in the leaves may be attributed to the amino acids present in CH, as well as the plant's greater capacity to take up nitrogen from the soil after CH degradation [71,72].

As explained by Franco and Garcia [73], amino acids perform vital roles in plants when they are subjected to environmental stress, as they are part of proteins and modify the osmotic pressure inside cells. Furthermore, CH a naturally occurring polysaccharide made up of a copolymer of D-acetyl-D-glucosamine and N-acetyl-D-glucosamine residues joined by β-1-4 glycosidic bonds plays a significant role in promoting plant development [62,74,75].

Also, the higher vegetative mass production and yield of the plants as a result of applying CH can be explained by the effectiveness of CH as an antitranspirant to conserve water use in agriculture and the role of abscisic acid content. It has been [76] found that the positive effect of foliar spraying of CH on the increase of photosynthetic rate may be due to greater carbon dioxide uptake inside the leaf that induces an improvement in the conductivity of stomata, contrary to the effect of having a greater number of open stomata.

The results about biomass improvement agreed with the finding by Bittelli *et al.* [59] on pepper plants, who

reported that the application of CH reduced plant transpiration in pepper by 26–43% while maintaining biomass production and yield. It is known that increased abscisic acid levels play a major role in regulating water consumption by plants, closing stomata, and reducing transpiration. Kim *et al*. [77], Kumar *et al*. [78], and Kuyyogsuy *et al*. [79] suggested that the impact of CH is partially a result of the induced activation of the abscisic acid signaling pathway as well as the mechanism by which CH induces stomatal closure. This means that stomata closure induced by the foliar application of CH might affect the activation of the signaling pathway of abscisic acid [80,81].

The role and effect of CH on the stimulation of plant immune system and defense mechanisms to protect and resist attack by microorganisms and pathogens [82,83] stated that CH stimulates the accumulation of phytoalexins, which improves defenses against new infections and promotes antifungal response. In addition, Bautista-Baños *et al*. [84] proved that the application of CH on plants increased the content of phenolic compounds. The key enzymes of the phenylpropanoid pathway, phenylalanine ammonia-lyase and tyrosine ammonia-lyase, were shown to be more activated when CH was sprayed on soybean leaves [85] and sweet basil [26]. As a result, the phenylpropanoid pathway products of phenylalanine ammonia-lyase and tyrosine ammonia-lyase were modified and produced precursors for secondary metabolites, such as phytoalexins, lignin, and flavonoid pigments, which are essential for plant resistance to pathogens [86,87]. Also, CH treatment elevates polyphenol oxidase which increases cultivars resistant to disease [88,89].

Several plant species showed greater vegetative growth, yield, and quality when subjected to varying concentrations of CH [90] on continuous *Pinellia ternate* plants [91], on chili pepper [72,92], on radish plants and lettuce [22], on strawberry, and [93] on rose.

In addition, it was proved by Shaheen *et al*. [30] that the content of N, P, and K and the contents of starch, TOC, and total sugar in potato tubers recorded a superior increase affected by the foliar spraying of potato plants by growth stimulant substances of CH compared with those of untreated plants. Moreover, the impacts of CH on increasing chlorophyll and TOC were verified by Ramadan and El Mesairy [94] on okra and [95] on cucumber.

While El-Khateeb *et al*. [96] reported that CH is advisable for improving *M. hortensis* growth, oil yield, and its main components and higher accumulation of carbohydrates. In another study, it was published that CH can be used as a possible biostimulant on pineapple lilies and has a multidirectional, favorable influence on plant development [97]. An improvement in essential oil productivity from CH treatments was observed in a different experiment conducted on *Coriandrum sativum* [98].

Furthermore, Abd-Rabbu *et al*. [99] confirmed that the application of CH led to an enhancement in carbohydrates and protein content in French lavender subjected to water deficiency. Also, Pirbalouti *et al*. [100] noticed an increase in the amount of total phenol and antioxidant activity by applying different levels of CH, which significantly impacted the extracts of two basil species (*Ocimum ciliatum* and *Ocimum basilicum).*

R is an essential bio-sensitized product in plants. It contributes to many uses and pharmacological activities and modulates several different physiological processes in plants [36,37]. So, the positive reasons for using foliar applications of R on the characteristics of vegetative growth, essential oil, and chemical content of marjoram plants are due to the importance of R and its crucial role in the growth of plants. Sandoval *et al*. [39] stated that R and its derivatives are essential for photosynthesis, the generation of energy, and redox metabolism in plants, while Taheri and Tarighi [41] found that R induces the accumulation of antioxidant compounds in plant cells. Moussa and Abdel-Aziz [101] mentioned that plants with higher antioxidant capacities are more resistant to abiotic stresses such as drought, salinity, and temperature stress.

However, Fischer and Bache [35] stated that R is one of the major catalysts for several metabolic enzymes and plays a critical role in electrons transfer, biosynthesis of citric acid, oxidation of fatty acid, photosynthesis, and DNA repair system. Abdulhamed *et al*. [102] added that R plays an essential function in the biosynthesis of auxin and cell elongation, which increases the area of maize leaf. R is an important cofactor in diversifying metabolic pathways that result in promoting plant growth and systemic resistance in plants, which leads to improved plant immunity and higher resistance to pathogen attack [103,104]. A study on the roselle plant (*H. sabdariffa*) [42] noticed that plants received 100 ppm of R had higher levels of antioxidant enzymes and carbohydrates. Therefore, R may be an

effective antioxidant by maximizing the osmotic pressure and the ionic exchange and increasing plant resistance.

It was mentioned by Samiullah *et al.* [105] that vitamins may have a beneficial effect on flame seedless grapevine growth and fruiting because they foster cell division, biosynthesis of natural hormones including ethylene and IAA, as well as preventing plant senescence. Vitamins also improve nutrient and water uptake, photosynthesis, synthesis of proteins, amino acids, and pigments in plants as well as plant metabolism. All these functions of vitamins reflect improved nutritional status for plants and increased growth.

It has been found by Azooz [42] that applying 100 ppm of R on hibiscus plants stimulated plant antioxidant content, carbohydrate content, and improved *H. sabdariffa*'s resistance to salt stress and encouraged plant growth. It has been [43] found that the maximum grain production of sorghum was achieved by the foliar application of R at a dosage of 300 mg/l, which also enhanced the majority of vegetative development parameters such as plant height and leaf area. El-Lethy *et al.* [44] mentioned that treating geranium plants with (R) at 90 m/l improved vegetative growth characteristics, photosynthetic pigment content, and the yield of EO, TPC, TFC content, and regulatory impacts on antioxidant activities. Saha *et al.* [106] found that R increased PO content over the control treatment in French beans. In addition, Hussain and Saeed [107] discovered that R significantly elevated the antioxidant activity of fenugreek plants at their seedling stage. Furthermore, because different CH and R treatments may affect essential oil metabolism, enzyme activity, glandular trichome size and density, and other aspects of essential oil, they may also affect essential oil (the primary natural product of marjoram) and its constituents [108,109] Lastly, the results of this study will assist Egyptian farmers in growing aromatic plants in the recently created sandy reclamation zone [110,111].

## Conclusion

This study revealed that spraying both CH and R stimulated the growth and chemical properties of *M. hortensis* plant compared with the untreated plants CH was more effective than R and the combined application between them produced the best growth, percentage, and yield of volatile oil and chemical constituents. It is recommended to use both CH and R alone or in combination for application on *M. hortensis* plant to enhance its productivity.

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**Conflicts of interest**

There are no conflicts of interest.

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