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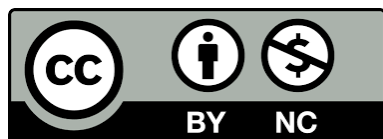
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# The effects of *Trichoderma* fungi symbiosis and nitrogen on essential oil and leaf pigments in green cumin (*Cuminum cyminum* L.) under weed competition

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**Abstract:** Green cumin is one of the most widely utilized medicinal plants both in Iran and globally, which is mainly exploited for the purpose of using its essential oil. It seems necessary to conduct an experiment in this direction to pay attention to sustainable agriculture, which can be achieved by using biofertilizers along with chemical inputs such as nitrogen. This research was carried out in Maraveh-Tappeh county and in two distinct regions (Qarah-Dam and Shalami) during the 2023 crop year, as a split factorial experiment based on randomized complete block design with three replications. The experimental treatments were nitrogen fertilizer levels (control, 50 and 100 % recommended nitrogen from urea) as the main plot, biofertilizer treatments (control, seed inoculation with *Trichoderma longibrachiatum*, *Trichoderma atroviride*, and co-inoculation (*Trichoderma longibrachiatum* and *Trichoderma atroviride*) and weeding management (no weeding and weeding) as sub-plots. The results showed that the highest essential oil content and yield in both regions were achieved under the no weeding condition with 100 % recommended nitrogen and co-inoculation treatments. Also, the use of *Trichoderma* fungi and nitrogen had a positive effect on the content of chlorophyll a, chlorophyll b and total chlorophyll.

**Keywords:** Anthocyanin, biofertilizer, chlorophyll content, essential oil content, nitrogen.

## Introduction

Medicinal plants play a vital role in preserving biodiversity, providing food and medicine, improving soil and water quality, and reducing pollution. Additionally, they strengthen ecosystems and aid in their sustainability (Agbo et al., 2023; Ahmadiania and Heidari, 2023; Hosseinpour Azad, 2023). Green cumin (*Cuminum cyminum* L.) is one of the most important medicinal plants known in Iran and the worldwide, valued for the properties of its seeds (Hadi et al., 2018). These seeds are widely used in the pharmaceutical, culinary and traditional medicine industries due to their unique taste and numerous health benefits (Karik et al., 2021). Cumin seeds are rich in fiber, which naturally stimulates the digestive system and improves digestion. In addition, due to the abundant presence of bioactive compounds, green cumin has anti-cancer, anti-bloating, anti-spasmodic, antioxidant, digestive system strengthening, anti-fungal, anti-microbial and anti-inflammatory properties (Mohammed et al., 2024).

The green cumin plant is cultivated in different regions around the world (Hadi et al., 2018). However, green cumin cultivation is fraught with challenges, especially weed competition, which threatens agricultural sustainability and crop productivity. Weeds compete with crops for light, water, and nutrients. Also, studies have shown that weed competition can reduce crop yield by 20% to more than 80%, depending on the severity of the infestation and the time of weed emergence. To combat this yield reduction, farmers have traditionally relied on chemical herbicides (Scavo and Mauromicale, 2020). However, growing concerns about environmental sustainability and human health have stimulated interest in alternative and environmentally friendly weed management strategies (Kohestani et al., 2023).

In this context, the role of beneficial microorganisms, especially *Trichoderma* fungus, has attracted much attention in agricultural research. These fungi are known for their high ability to increase plant resilience through various mechanisms, including enhancing nutrient availability, improving root growth, and providing biocontrol against soil-borne pathogens (Rostaminia et al., 2021; Kulbat-Warycha et al.,

2024). In addition, *Trichoderma* species can establish symbiotic relationships with plants, which leads to increased stress tolerance and growth even in competitive conditions (Guo et al., 2020). In fact, the use of innovative biological formulations containing microbes, by improving the yield and quality of medicinal plants, provides an opportunity to develop agricultural systems with the least environmental effects in the agricultural production process (Comite et al., 2021).

In addition, nitrogen plays an important role in plant growth, affecting growth rate, biomass accumulation, and yield (Mahmud et al., 2020). Nitrogen plays an important role in the composition of proteins, enzymes, chlorophyll, vitamins, and alkaloids (Mu and Chen, 2021). Nitrogen deficiency in plants causes the growth of vegetative organs to stop and the leaves take on a yellowish-green color, in addition, it causes disruption in the synthesis of chlorophyll and enzymes (Anas et al., 2020; Adl et al., 2023). By providing nitrogen in the soil, the absorption of nutrients and the production of metabolites in the plant increases. Therefore, the use of nitrogen fertilizers improves crop nutrition, which is especially important in nutrient-deficient soils (Hao et al., 2023). However, the interaction between nitrogen application and microbial inoculations such as *Trichoderma* in reducing weed competition, especially in the field of green cumin, has not been widely studied.

Also, some researchers have reported the positive effects of *Trichoderma* fungi on the quantitative and qualitative traits of medicinal plants. For example, the application of *Trichoderma* fungi positively affected the chlorophyll and anthocyanin content in the medicinal plant (*Hibiscus sabdariffa* L.) (Rostaminia et al., 2021), and the increase of the chlorophyll index in the black cumin plant (*Nigella sativa* L.) (Nassif et al., 2023). In another experiment, the use of *Trichoderma* spores added to the soil led to an increase in flavonoid and terpenoid content were found in the leaves of *thyme* species (Kulbat-Warycha et al., 2024). In addition, the results of the effect of *Trichoderma* fungi on yield, photosynthetic activity and production of secondary metabolites of basil showed that photosynthetic efficiency, phenolic acids and rosmarinic acid content increased (Comite et al., 2021).

This study aimed to evaluate the response of essential oil content and yield, representing overall phytochemicals, as well as photosynthetic and non-photosynthetic pigments of green cumin medicinal plant to symbiosis with *Trichoderma* fungus and nitrogen application under weed competition.

## Materials and Methods

### Field experiment locations

This research was carried out in Maraveh-Tappeh county and in two different regions (Qarah-Dam and Shalami) in 2023 crop year as a split factorial experiment based on randomized complete block design with three replications.

### The treatments of the experiment

The experimental treatments included nitrogen fertilizer (control, 50 and 100% recommended nitrogen from urea fertilizer source) as the main factor, biofertilizer (control, seed inoculation with *Trichoderma longibrachiatum*, *Trichoderma atroviride*, and co-inoculation (*Trichoderma longibrachiatum* + *Trichoderma atroviride*)) and weeding management (no weeding and weeding) are sub-factors. The fungi used in this research were obtained from the Environmental Stress Laboratory located at Sari Agricultural Sciences and Natural Resources University.

### Field growth conditions

Before planting, in order to determine the characteristics of the soil, sampling was done from 0-30 cm of the research site (three mixed samples from different parts of the field), and its physical and chemical characteristics were evaluated (Table S1). The characteristics of the meteorological data were shown in Table S2. To experiment, land preparation was completed on February 9, 2023. Planting commenced following the land's leveling on February 14, 2023. The main plots were 21 × 3 m with a distance of one meter from each other and the sub-plots were 3 × 2 m with a distance of 60 cm from each other. In each experimental plot, rows were spaced 20 cm apart, with a plant spacing of 4 cm within each row. As a result, the target density of 125 plants per square meter was effectively achieved. In this experiment, a local variety seeds were used. Before planting, the seeds were pre-treated with fungi. Based on the soil test results, the

chemical fertilizers (e.g., nitrogen (urea source; 150 kg ha<sup>-1</sup>), triple superphosphate (100 kg ha<sup>-1</sup>) and potassium sulfate (100 kg ha<sup>-1</sup>) were applied to the soil. Regarding the nitrogen application, 50 kg ha<sup>-1</sup> was applied before planting and the remainder was applied in two stages: 30 and 60 days after planting. No herbicides, fungicides, or chemical pesticides were used during the growth period. Weed control was carried out manually using mechanical method.

### Measurement of characteristics

After removing a half-meter border around the plot, the plants were harvested from the remaining area, and the seed yield was evaluated per square meter. The seeds were dried in a shaded area to preserve the quality of the seeds and essential oils.

### Measurement of essential oil content and yield

For essential oil extraction, 30 grams of cumin seeds were used. The essential oil was extracted using a Clevenger apparatus. Then the essential oil was dehumidified by sodium sulfate and the amount of essential oil was weighed with a precise balance. The yield of essential oil was calculated by equation (1).

$$\text{Equation (1) } \text{Essential oil yield } \left(\frac{\text{kg}}{\text{ha}}\right) = (\text{Essential oil content} \times \text{seed yield})$$

### Measurement of chlorophyll and anthocyanin

Sampling for physiological characteristics was conducted using young leaves at the flowering stage. The leaf samples were immediately transferred to the freezer at -80 °C. At this stage, characteristics such as chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid Lichtenthaler (1987), and anthocyanin Krizek et al. (1993) were measured by spectrophotometry.

### Data analysis

In order to analyze the data, the homogeneity of the variances of the two research sites was tested using Bartlett's test. The data obtained in the experiments were analyzed by SAS software version 9.1. Least significant difference (LSD) method was used to compare the means. The graphs were drawn using Excel software.

## Results

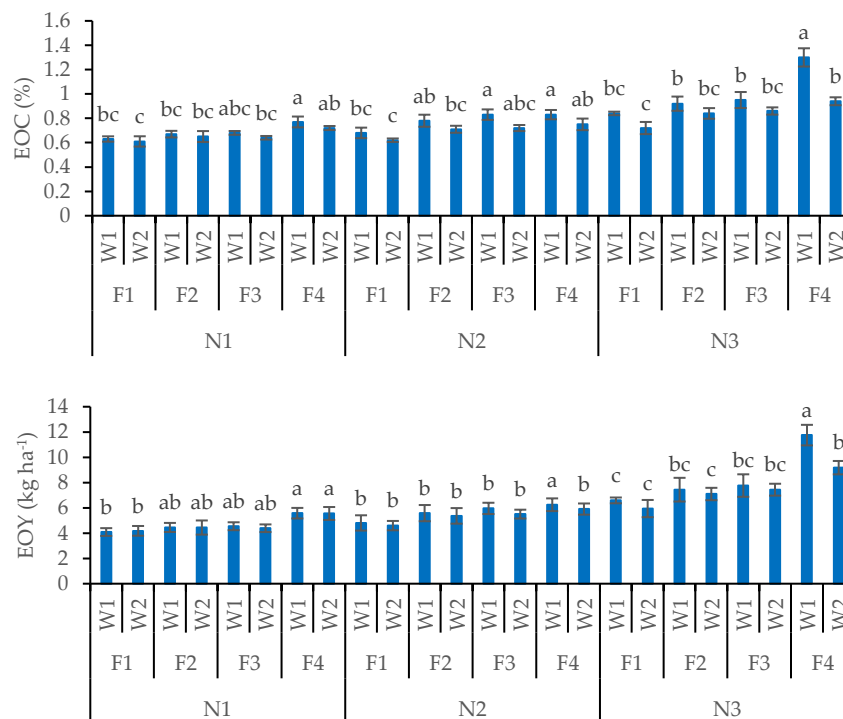
### Phytochemical and physiological characteristics of at Qarah-Dam samples region

### Essential oil content and yield

The results of the analysis of variance showed that the essential oil content was significantly ( $P \leq 0.01$ ) affected by nitrogen, biofertilizer and weed treatments (Table S3). Also, two-way and three-way interaction for essential oil content were significant ( $P \leq 0.01$ ) (Table S3).

Under no nitrogen application, the highest content of essential oil was related to the treatment of simultaneous inoculation of two fungi + no weeding (0.77 %), compared to the treatments of simultaneous inoculation of two fungi + weeding (0.72 %) and *T. atroviride* inoculation + no weeding (0.68%) had no statistically significant difference, and the lowest content of essential oil was observed in the treatment of no inoculation + weeding (0.61 %) (Figure 1). In the conditions of application of 50 % recommended nitrogen fertilizer, treatments of *T. atroviride* inoculation + no weeding (0.83 %) and simultaneous inoculation of two fungi + no

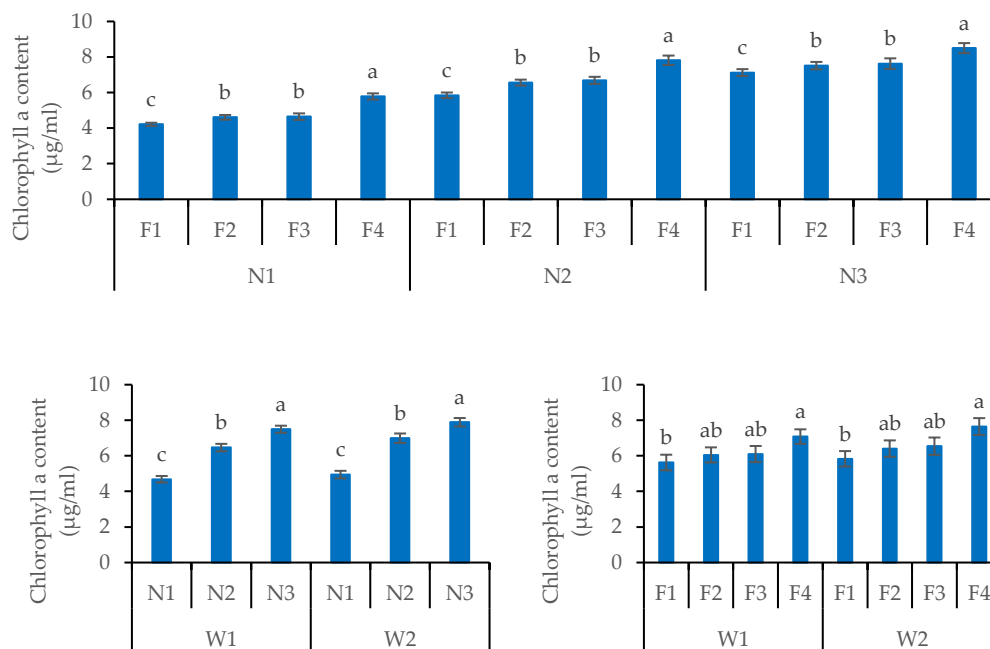
weeding (0.83 %) had the highest content of essential oil and there was no statistically significant difference with simultaneous inoculation of two fungi + weeding (0.75 %), *T. atroviride* inoculation + weeding (0.72 %) and *T. longibrachiatum* inoculation + no weeding (0.78 %) treatments. On the other hand, the lowest content of essential oil in the conditions of application of 50 % recommended nitrogen fertilizer was related to the treatment of no inoculation + weeding (0.62 %). In application of 100 % recommended nitrogen fertilizer, the highest essential oil content was recorded with simultaneous inoculation of two fungi + no weeding (1.30 %). In contrast, the lowest amount of essential oil content was observed under conditions of no inoculation + weeding (0.72 %) without statistically significant difference with no inoculation + no weeding (0.84 %), *T. longibrachiatum* inoculation + weeding (0.84 %) and *T. atroviride* inoculation + weeding (0.86 %) treatments (Figure 1).



**Figure 1.** Influence of nitrogen, biofertilizer and weeding on essential oil content (EOC) and essential oil yield (EOY) characteristics in green cumin plants cultivated in Qarah-Dam region (W1: no weeding, W2: weeding, F1: no inoculation, F2: *T. longibrachiatum* inoculation, F3: *T. atroviride* inoculation, F4: simultaneous inoculation of *T. longibrachiatum* and *T. atroviride*, N1: no nitrogen application, N2: application of 50 % of recommended nitrogen, N3: application of 100 % of recommended nitrogen). Means followed by different letters in the same column for the same factor are significantly different ( $P \leq 0.05$ ) according to the LSD test. Mean comparisons were shown as mean  $\pm$  standard error (SE).

The results of the analysis of variance showed that the essential oil yield was significantly ( $P \leq 0.01$ ) affected by nitrogen, biofertilizer, and weed treatments (Table S3). Also, two-way and three-way interactions for essential oil yield were significant ( $P \leq 0.01$ ). Based on the results, under no nitrogen application, it was observed that the treatments of *T. atroviride* inoculation + no weeding (5.58 kg ha<sup>-1</sup>) and simultaneous inoculation of two fungi + weeding (5.55 kg ha<sup>-1</sup>) had the highest amount of essential oil yield without statistically significant difference with *T. longibrachiatum* inoculation + no weeding (4.45 kg ha<sup>-1</sup>), *T. longibrachiatum* inoculation + weeding (4.4 kg ha<sup>-1</sup>), *T. atroviride* inoculation + no weeding (4.55 kg ha<sup>-1</sup>) and *T. atroviride* inoculation + weeding (4.39 kg ha<sup>-1</sup>) treatments (Figure 1). On the other hand, in the conditions of no nitrogen application, the lowest amount of essential oil yield was related to the treatments of no inoculation + no weeding (4.09 kg

ha<sup>-1</sup>) and no inoculation + weeding (4.18 kg ha<sup>-1</sup>). In the conditions of application of 50 % of recommended nitrogen, the treatment of simultaneous inoculation of two fungi + no weeding (6.24 kg ha<sup>-1</sup>) had the highest amount of essential oil yield, while the other treatments had the lowest essential oil yield (Figure 1). In the conditions of 100% recommended nitrogen, the simultaneous inoculation of two fungi + no weeding (11.75 kg ha<sup>-1</sup>) had the highest amount of essential oil yield; If the treatments of no inoculation + no weeding (6.59 kg ha<sup>-1</sup>), no inoculation + weeding (5.94 kg ha<sup>-1</sup>) and *T. longibrachiatum* inoculation + weeding (7.09 kg ha<sup>-1</sup>) had the lowest essential oil yield and had no statistically significant difference with *T. longibrachiatum* inoculation + no weeding (7.44 kg ha<sup>-1</sup>), *T. atroviride* inoculation + no weeding (7.76 kg ha<sup>-1</sup>) and *T. atroviride* inoculation + weeding (7.44 kg ha<sup>-1</sup>) treatments (Figure 1).



**Figure 2.** Influence of nitrogen, biofertilizer and weeding on chlorophyll a content characteristic in green cumin plants cultivated in Qarah-Dam region (W1: no weeding, W2: weeding, F1: no inoculation, F2: *T. longibrachiatum* inoculation, F3: *T. atroviride* inoculation, F4: simultaneous inoculation of *T. longibrachiatum* and *T. atroviride*, N1: no nitrogen application, N2: application of 50 % of recommended nitrogen, N3: application of 100 % of recommended nitrogen). Means followed by different letters in the same column for the same factor are significantly different ( $P \leq 0.05$ ) according to the LSD test. Mean comparisons were shown as mean  $\pm$  standard error (SE).

### Chlorophyll *a* content

The chlorophyll *a* was significantly ( $P \leq 0.01$ ) affected by the main effects of nitrogen, biofertilizer and weeding. Also, nitrogen  $\times$  biofertilizer interaction ( $P \leq 0.01$ ) and nitrogen  $\times$  weeding and biofertilizer  $\times$  weeding interactions ( $P \leq 0.05$ ) were significant for chlorophyll *a* (Table S3). The results showed that in all nitrogen levels, the use of biofertilizer increased the content of chlorophyll *a* compared to the treatment without the use of biofertilizer. Under conditions of no application of nitrogen, the highest amount of chlorophyll *a* content was observed in the treatment of simultaneous inoculation of two fungi (5.78  $\mu\text{g/ml}$ ), which showed a 37 % increase compared to the treatment of no inoculation of fungi (Figure 2). Under the conditions of application of 50 % recommended nitrogen, treatments of simultaneous inoculation of two fungi and no inoculation of fungi had the highest and lowest amounts of chlorophyll *a* content with 7.82 and 5.85  $\mu\text{g/ml}$ , respectively. The results showed that the application of simultaneous inoculation of two fungi caused a 33 % increase in the content of chlorophyll *a* compared to the treatment of no inoculation of fungi under the conditions of application of 50 % recommended nitrogen (Figure 2).

In addition, the results showed that under the conditions of 100 % recommended nitrogen, the treatment of simultaneous inoculation of two fungi (8.5  $\mu\text{g/ml}$ ) had the highest amount of chlorophyll *a* content, while the treatment of no inoculation of fungi had the lowest amount of chlorophyll *a* content (7.13  $\mu\text{g/ml}$ ) (Figure 2). Under the conditions of 100 % recommended nitrogen, the application of the simultaneous inoculation of two fungi increased the content of chlorophyll *a* by 19 % compared to the treatment of no inoculation of fungi.

The results of interaction between weeding and nitrogen revealed that under no-weeding conditions, the application of nitrogen at 50 and 100 % recommended levels, increases the content of chlorophyll *a* by 38 and 60 % respectively, compared to the treatment of no application of nitrogen. On the other hand, under weeding conditions, the application of nitrogen at the recommended levels of 50 and 100 % increased the content of chlorophyll *a* by 41 and 59 %,

respectively, compared to the no nitrogen application treatment. Under no weeding conditions, the highest amount of chlorophyll *a* content was observed in the treatment of the application of 100 % recommended nitrogen (7.49  $\mu\text{g/ml}$ ). In contrast the lowest amount of chlorophyll *a* was recorded in the treatment of without nitrogen application (4.68  $\mu\text{g/ml}$ ). Also, under weeding conditions, treatments with 100 % recommended and no nitrogen application had the highest and lowest chlorophyll *a* content by 7.89 and 4.94  $\mu\text{g/ml}$ , respectively (Figure 2).

The results of the interaction of biofertilizer  $\times$  weeding showed that in the condition of no weeding, the application of simultaneous inoculation of two fungi, inoculation of *T. atroviride* and inoculation of *T. longibrachiatum* caused an increase in the content of chlorophyll *a* by 26, 9 and 7 %, respectively compared to no inoculating of fungi treatment. On the other hand, in weeding conditions, the application of simultaneous inoculation of two fungi, inoculation of *T. atroviride* and inoculation of *T. longibrachiatum* increased the content of chlorophyll *a* by 31, 12 and 8 %, respectively, compared to the treatment of no inoculation of fungi (Figure 2).

Under no weeding conditions, the highest amount of chlorophyll *a* content was observed in the treatment of simultaneous inoculation of two fungi (7.08  $\mu\text{g/ml}$ ), which had no statistically significant difference with *T. atroviride* inoculation (6.10  $\mu\text{g/ml}$ ) and *T. longibrachiatum* inoculation (6.05  $\mu\text{g/ml}$ ) treatments. In contrast, the lowest amount of chlorophyll *a* content was observed in no inoculation treatment (5.62  $\mu\text{g/ml}$ ). Under weeding conditions, the highest content of chlorophyll *a* was related to the treatment of simultaneous inoculation of two fungi (7.64  $\mu\text{g/ml}$ ), which had no statistically significant difference with *T. atroviride* inoculation (6.54  $\mu\text{g/ml}$ ) and *T. longibrachiatum* inoculation (6.40  $\mu\text{g/ml}$ ). On the other hand, the lowest amount of chlorophyll *a* content was related to the treatment of no inoculation of fungi (5.83  $\mu\text{g/ml}$ ) (Figure 2).

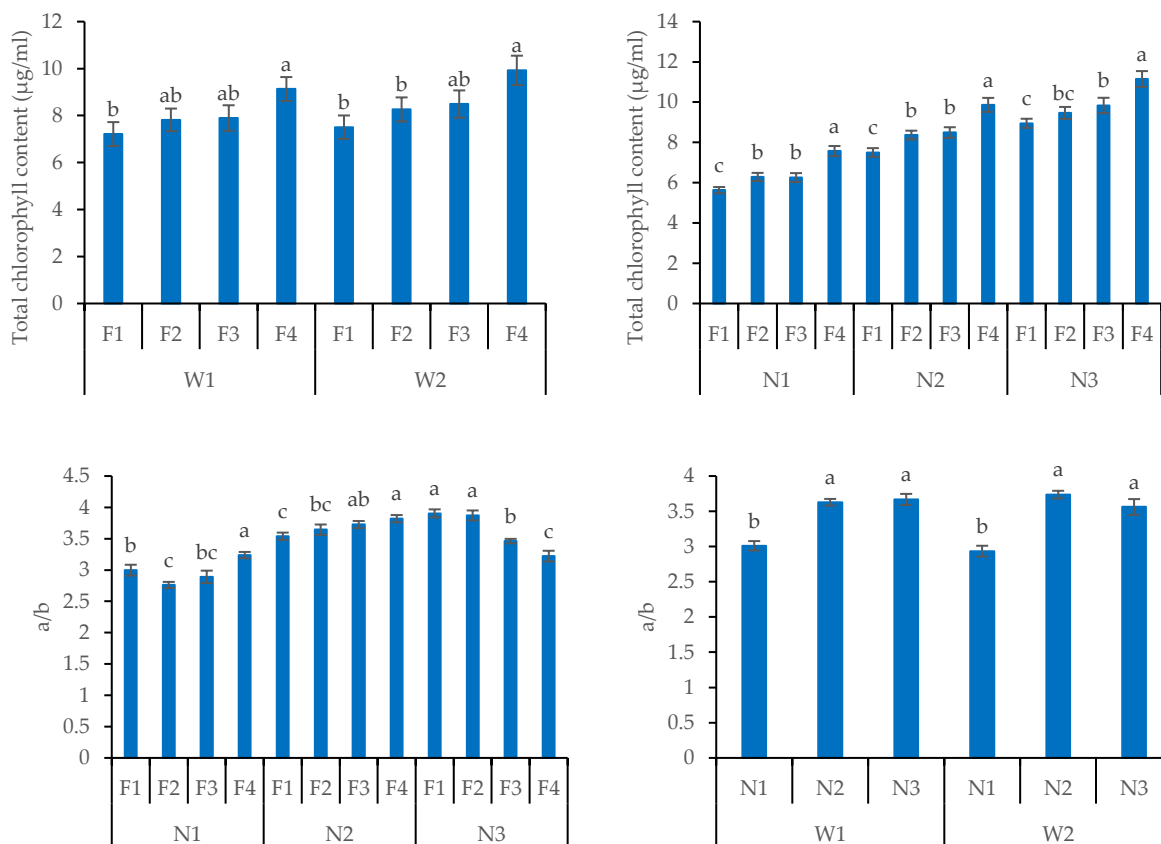
### Chlorophyll *b* content

The results showed that the chlorophyll *b* was significantly ( $P \leq 0.01$ ) affected by the main effects of nitrogen, biofertilizer and weeding (Table S3). Also, the interactions of nitrogen  $\times$  biofertilizer ( $P \leq 0.01$ ),

nitrogen  $\times$  weeding ( $P \leq 0.05$ ) and biofertilizer  $\times$  weeding interactions ( $P \leq 0.01$ ) were significant for chlorophyll *b*. Chlorophyll *b* was significantly affected by the three-way interaction of nitrogen  $\times$  biofertilizer  $\times$  weeding ( $P \leq 0.01$ ).

The interaction of nitrogen  $\times$  biofertilizer  $\times$  weeding showed that under no nitrogen application, the simultaneous inoculation of two fungi combined with weeding (1.83  $\mu\text{g/ml}$ ) had the highest amount of chlorophyll *b* content. This was not statistically different from treatments involving simultaneous inoculation of two fungi + no weeding (1.75  $\mu\text{g/ml}$ ), inoculation of *T. atroviride* + weeding (1.69  $\mu\text{g/ml}$ ), inoculation of *T. longibrachiatum* + weeding (1.74  $\mu\text{g/ml}$ ) and *T. longibrachiatum* inoculation + no weeding (1.61  $\mu\text{g/ml}$ ). On the other hand, the lowest

amount of chlorophyll *b* content was recorded in the treatment without inoculation of fungi combined with no weeding (1.35  $\mu\text{g/ml}$ ) (Figure 3). In the conditions of application of 50 % of the recommended nitrogen, the simultaneous inoculation of two fungi + weeding (2.12  $\mu\text{g/ml}$ ) had the highest amount of chlorophyll *b* content, which had no statistically significant difference with simultaneous inoculation of two fungi + no weeding (1.98  $\mu\text{g/ml}$ ), inoculation of *T. atroviride* + weeding (1.86  $\mu\text{g/ml}$ ) and inoculation of *T. longibrachiatum* + weeding (1.83  $\mu\text{g/ml}$ ) treatments; whereas the lowest amount of chlorophyll *b* content was related to the no inoculation + no weeding (1.63  $\mu\text{g/ml}$ ) treatment.



**Figure 3.** Influence of nitrogen, biofertilizer and weeding on chlorophyll *b* content, total chlorophyll content and ratio of chlorophyll *a* to *b* characteristics in green cumin plants cultivated in Qarah-Dam region (W1: no weeding, W2: weeding, F1: no inoculation, F2: *T. longibrachiatum* inoculation, F3: *T. atroviride* inoculation, F4: simultaneous inoculation of *T. longibrachiatum* and *T. atroviride*, N1: no nitrogen application, N2: application of 50 % of recommended nitrogen, N3: application of 100 % of recommended nitrogen). Means followed by different letters in the same column for the same factor are significantly different ( $P \leq 0.05$ ) according to the LSD test. Mean comparisons were shown as mean  $\pm$  standard error (SE).

With applying 100 % of the recommended nitrogen, the treatment of simultaneous inoculation of two fungi + weeding (2.89  $\mu\text{g/ml}$ ) had the highest amount of chlorophyll *b* content. While the lowest amount of chlorophyll *b* content was related to the treatments of no inoculation + no weeding (1.79  $\mu\text{g/ml}$ ), no inoculation + weeding (1.86  $\mu\text{g/ml}$ ) and *T. longibrachiatum* inoculation + no weeding (1.91  $\mu\text{g/ml}$ ) which had no statistically significant difference with *T. longibrachiatum* inoculation + weeding (1.99  $\mu\text{g/ml}$ ) and *T. atroviride* inoculation + no weeding (2.12  $\mu\text{g/ml}$ ) (Figure 3).

#### **Total chlorophyll content**

Based on the results, the total chlorophyll content was significantly affected by the main effects of nitrogen, biofertilizer, and weeding ( $P \leq 0.01$ ). The interactions of nitrogen  $\times$  biofertilizer ( $P \leq 0.05$ ) and biofertilizer  $\times$  weeding ( $P \leq 0.01$ ) were significant for the total chlorophyll trait (Table S3).

Based on the interaction of biofertilizer and weeding, under no weeding conditions, the simultaneous inoculation of two fungi (9.13  $\mu\text{g/ml}$ ) resulted in the highest amount of total chlorophyll content, with no statistically significant difference observed between *T. atroviride* inoculation (7.89  $\mu\text{g/ml}$ ) and *T. longibrachiatum* inoculation (7.81  $\mu\text{g/ml}$ ). On the other hand, the lowest amount of total chlorophyll content was observed in the treatment of no inoculation of fungi (7.21  $\mu\text{g/ml}$ ) (Figure 3). In the condition of no weeding, the simultaneous inoculation of two fungi, inoculation of *T. atroviride* and inoculation of *T. longibrachiatum* increased the total chlorophyll content by 26, 9 and 8 %, respectively, compared to the no inoculation of fungi (Figure 3).

In addition, the results showed that in weeding conditions, the simultaneous inoculation of two fungi (9.92  $\mu\text{g/ml}$ ) had the highest total chlorophyll content, which had no statistically significant difference with *T. atroviride* inoculation (8.49  $\mu\text{g/ml}$ ); However, the no inoculation (7.51  $\mu\text{g/ml}$ ) and *T. longibrachiatum* inoculation (8.26  $\mu\text{g/ml}$ ) treatments had the lowest total chlorophyll content (Figure 3). The results in weeding conditions showed that the application of simultaneous inoculation of two fungi and inoculation of *T. atroviride* inoculation increased the total chlorophyll content by 32 and

13 %, respectively, compared to the no inoculation of fungi treatment (Figure 3).

The results of the interaction of nitrogen and biofertilizer showed that in the condition of no nitrogen application, the simultaneous inoculation of two fungi (7.57  $\mu\text{g/ml}$ ) had the highest amount of total chlorophyll content and the lowest amount of this trait was observed in the no inoculation treatment (5.63  $\mu\text{g/ml}$ ) (Figure 3). In the conditions of no nitrogen application, the treatment of simultaneous inoculation of two fungi increased the total chlorophyll content by 34 % compared to the treatment of no inoculation of fungi. In the conditions of application of 50 % of the recommended nitrogen, the simultaneous inoculation of two fungi (9.87  $\mu\text{g/ml}$ ) had the highest amount of total chlorophyll content and was able to increase the total chlorophyll content by 32 % compared to the no inoculation of fungi (Figure 3). In the condition of applying 100 % of the recommended nitrogen, the highest amount of total chlorophyll content was observed in the treatment of simultaneous inoculation of two fungi (11.15  $\mu\text{g/ml}$ ); while the lowest amount of total chlorophyll content was observed in the treatment of no inoculation of fungi (8.95  $\mu\text{g/ml}$ ) and had no statistically significant difference with *T. longibrachiatum* inoculation (9.47  $\mu\text{g/ml}$ ) (Figure 3). In the condition of applying 100 % of the recommended nitrogen, the treatment of simultaneous inoculation of two fungi increased the total chlorophyll content by 24 % compared to the treatment of no inoculation of fungi (Figure 3).

#### **Ratio of chlorophyll *a* to *b***

The results showed that among the main effects, only the main effect of nitrogen was significant ( $P \leq 0.01$ ) for the ratio of chlorophyll *a* to *b*. Also, the interactions of nitrogen  $\times$  biofertilizer ( $P \leq 0.01$ ) and nitrogen  $\times$  weeding ( $P \leq 0.05$ ) were significant for the ratio of chlorophyll *a* to *b* (Table S3).

The results of the interaction of nitrogen and biofertilizer showed that in the condition of no application of nitrogen, the simultaneous inoculation of two fungi (3.24) had the highest value of the ratio of chlorophyll *a* to *b*; but on the other hand, the lowest amount of the ratio of chlorophyll *a* to *b* was observed in the treatment of *T. longibrachiatum* inoculation (2.76), which had no

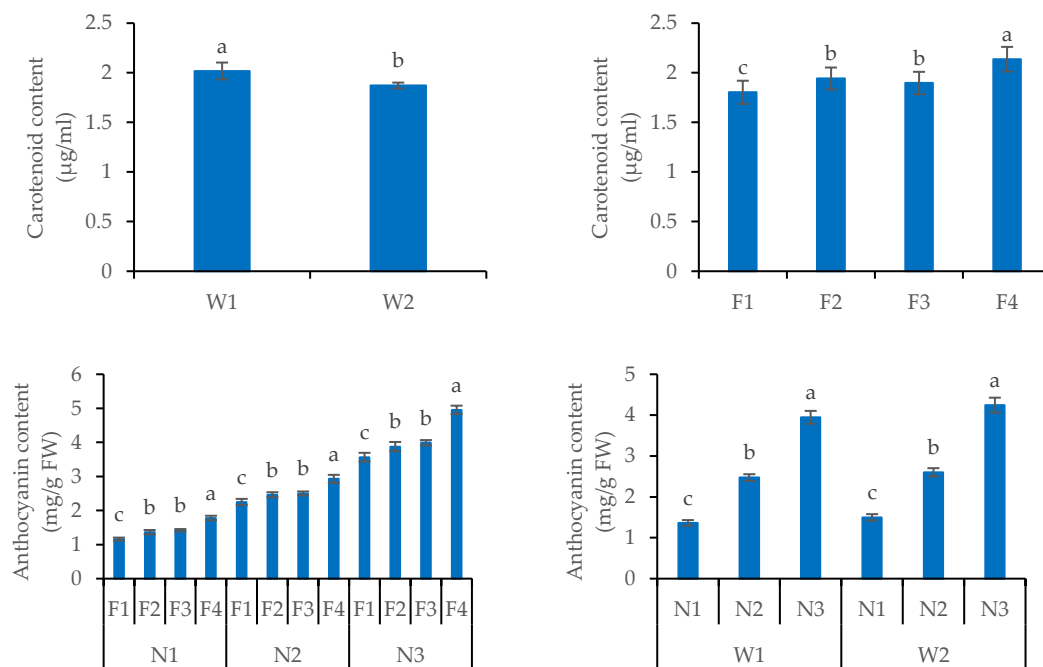
statistically significant difference with *T. atroviride* inoculation (2.89) (Figure 3). In addition, in the condition of applying 50 % of the recommended nitrogen, the treatment of simultaneous inoculation of two fungi (3.82) had the highest ratio of chlorophyll *a* to *b*, which had no statistically significant difference with *T. atroviride* treatment (3.73); on the other hand, the lowest amount of the ratio of chlorophyll *a* to *b* was observed in the treatment of no inoculation of fungi (3.54). In the conditions of application of 100 % of recommended nitrogen, the treatments of no inoculation of fungi (3.9) and inoculation of *T. longibrachiatum* (3.87) had the highest amount of the ratio of chlorophyll *a* to *b*, and the treatment of simultaneous inoculation of two fungi (3.22) had the lowest amount of chlorophyll *a* to *b* ratio (Figure 3).

The interaction of nitrogen and weeding showed that in the condition of no weeding, the use of recommended 50 and 100 % nitrogen treatments could increase the ratio of chlorophyll *a* to *b* by 20 and 22 %, respectively, compared to the treatment

of no nitrogen application. In addition, in weeding conditions, the use of recommended 50 and 100 % nitrogen treatments could increase the ratio of chlorophyll *a* to *b* by 27 and 21%, respectively, compared to the no nitrogen application treatment (Figure 3).

#### Carotenoid content

The results of analysis of variance showed that the carotenoid content was significantly affected by the main effects of nitrogen, biofertilizer and weeding ( $P \leq 0.01$ ) (Table S3). The two-way and three-way interactions for carotenoid content were not significant. The highest amount of carotenoid content was observed in the treatment of simultaneous inoculation of two fungi (2.13  $\mu\text{g/ml}$ ), which showed an increase of 18 % compared to the no inoculation of fungi treatment. There was no significant difference between the *T. longibrachiatum* inoculation and *T. atroviride* inoculation in terms of carotenoid content (Figure 4).



**Figure 4.** Influence of nitrogen, biofertilizer and weeding on carotenoid and anthocyanin content characteristics in green cumin plants cultivated in Qarah-Dam region (W1: no weeding, W2: weeding, F1: no inoculation, F2: *T. longibrachiatum* inoculation, F3: *T. atroviride* inoculation, F4: simultaneous inoculation of *T. longibrachiatum* and *T. atroviride*, N1: no nitrogen application, N2: application of 50 % of recommended nitrogen, N3: application of 100 % of recommended nitrogen). Means followed by different letters in the same column for the same factor are significantly different ( $P \leq 0.05$ ) according to the LSD test. Mean comparisons were shown as mean  $\pm$  standard error (SE).

### Anthocyanin content

Based on the results, the main effects of nitrogen, biofertilizer, and weeding were significant for the anthocyanin content ( $P \leq 0.01$ ). Also, the content of anthocyanin was significantly affected by the interactions of nitrogen  $\times$  biofertilizer and nitrogen  $\times$  weeding ( $P \leq 0.01$ ) (Table S3). The results of the weeding treatment showed that the amount of carotenoid content was reduced by 7 % compared to the no-weeding treatment (Figure 4). The interaction of nitrogen and biofertilizer showed that in the condition of no application of nitrogen, the simultaneous inoculation of two fungi (1.78 mg/g FW) had the highest amount of anthocyanin content and the lowest amount of anthocyanin content was observed in the no inoculation of fungi (1.16 mg/g FW) treatment (Figure 4).

Also, at the no application of nitrogen level, the treatments of *T. longibrachiatum* inoculation, *T. atroviride* inoculation, and simultaneous inoculation of two fungi increased anthocyanin content by 18, 23 and 53 %, respectively, compared to the no inoculation of fungi treatment (Figure 4). Under conditions of application of 50 % recommended nitrogen, treatments of simultaneous inoculation of two fungi (2.93 mg/g FW) and no inoculation of fungi (2.25 mg/g FW) had the highest and lowest amount of anthocyanin content respectively (Figure 4). In the conditions of application of 50 % of recommended nitrogen, application of *T. longibrachiatum* inoculation, *T. atroviride* inoculation, and simultaneous inoculation of two fungi increased anthocyanin content by 10, 11 and 30 %, respectively, compared to the no inoculation of fungi (Figure 4).

In addition, under the condition of applying 100 % of the recommended nitrogen, the highest amount of anthocyanin content was observed in the simultaneous inoculation of two fungi (4.96 mg/g FW). The lowest amount of anthocyanin content was related to the no inoculation of fungi (3.57 mg/g FW) (Figure 4). At the condition of applying 100 % of the recommended nitrogen, *T. longibrachiatum* inoculation, *T. atroviride* inoculation, and simultaneous inoculation of two fungi increased the amount of anthocyanin content by 9, 12 and 39 %, respectively, compared to no inoculation of fungi (Figure 4). The interaction of nitrogen and weeding

showed that in the condition of no weeding, the use of recommended 50 and 100 % nitrogen treatments could increase anthocyanin content compared to the treatment of no nitrogen application; the highest and lowest amounts of anthocyanin content were related to the 100 % recommended nitrogen treatments (3.94 mg/g FW) and no nitrogen application (1.36 mg/g FW), respectively (Figure 4). In addition, in weeding conditions, the application of nitrogen had a positive effect on anthocyanin synthesis; So that the highest amount of anthocyanin content was observed in the treatment of application of 100 % of recommended nitrogen (4.24 mg/g FW) and the lowest amount of anthocyanin content was related to the treatment of no nitrogen application (1.5 mg/g FW) (Figure 4).

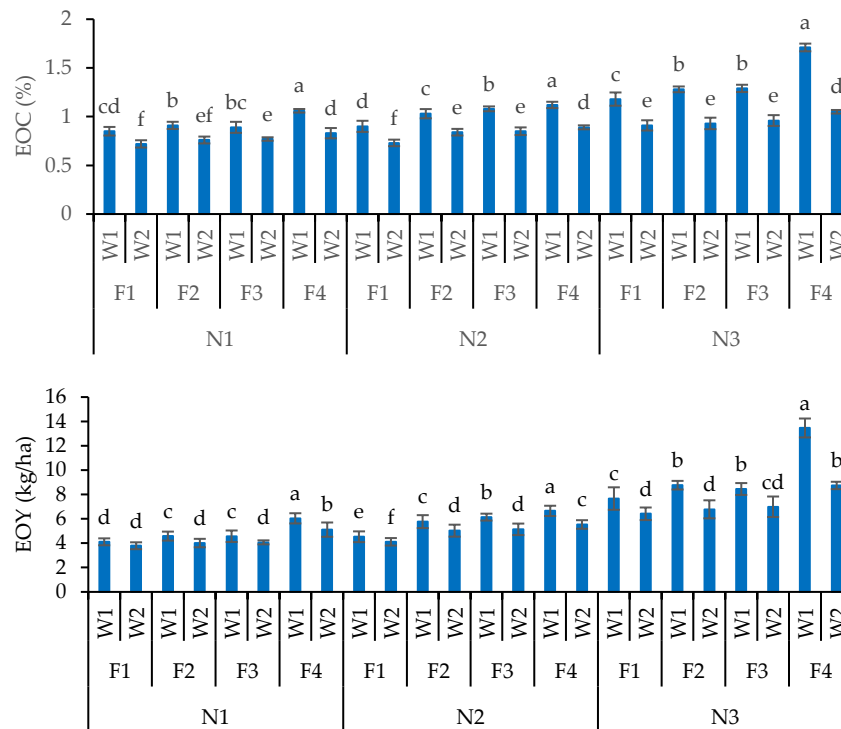
### Phytochemical and physiological characteristics at Shalami region

#### Essential oil content and yield

The results showed that the essential oil content and yield were significantly affected by the main effects of nitrogen, biofertilizer, and weeding ( $P \leq 0.01$ ). Also, two-way and three-way interactions for these traits were significant ( $P \leq 0.01$ ) (Table S4). Based on the results obtained from the interaction of weeding, nitrogen fertilizer and biofertilizer, it was observed that in the conditions of no nitrogen application, the simultaneous inoculation of two fungi + no weeding (1.06 %) had the highest content of essential oil; while the lowest amount of essential oil content was observed in the treatment of no inoculation of fungi + weeding (0.72 %), which had no statistically significant difference with *T. longibrachiatum* inoculation + weeding (0.76 %) (Figure 5). In the conditions of application of 50 % of the recommended nitrogen, the treatment of simultaneous inoculation of two fungi + no weeding (1.12 %) had the highest content of essential oil. In comparison, the lowest content of essential oil was observed in the treatment of no inoculation of fungi + weeding (0.73 %) (Figure 5). In addition, the results showed that the treatment of simultaneous inoculation of two fungi + no weeding (1.71 %) had the highest content of essential oil under the conditions of 100 % recommended nitrogen; while the treatments of no inoculation of fungi + weeding (0.91 %), *T. longibrachiatum* inoculation + weeding (0.93 %) and *T. atroviride* inoculation + weeding

(0.96 %) had the lowest content of essential oil (Figure 5). The results of weeding, nitrogen fertilizer and biofertilizer interactions showed that in the conditions of no nitrogen application, the treatment of simultaneous inoculation of two fungi + no weeding (6.04 kg/ha) had the highest amount of essential oil yield. The lowest amount of essential oil

yield was observed in the treatments of no inoculation of fungi + no weeding (4.11 kg/ha), no inoculation of fungi + weeding (3.79 kg/ha), *T. longibrachiatum* inoculation + weeding (4.01 kg/ha) and *T. atroviride* inoculation + weeding (4.07 kg/ha) (Figure 5).



**Figure 5.** Influence of nitrogen, biofertilizer and weeding on essential oil content (EOC) and essential oil yield (EOY) characteristics in green cumin plants cultivated in Shalami region (W1: no weeding, W2: weeding, F1: no inoculation, F2: *T. longibrachiatum* inoculation, F3: *T. atroviride* inoculation, F4: simultaneous inoculation of *T. longibrachiatum* and *T. atroviride*, N1: no nitrogen application, N2: application of 50 % of recommended nitrogen, N3: application of 100 % of recommended nitrogen). Means followed by different letters in the same column for the same factor are significantly different ( $P \leq 0.05$ ) according to LSD test. Mean comparison were shown as mean  $\pm$  standard error (SE).

In addition, under the conditions of application of 50 % of recommended nitrogen, the treatment of simultaneous inoculation of two fungi + no weeding (6.65 kg/ha) had the highest amount of essential oil yield. On the other hand, the lowest amount of essential oil yield was observed in the treatment of no inoculation of fungi + weeding (4.11 kg/ha) (Figure 5).

Also, under the conditions of 100 % recommended nitrogen, the results showed that the treatment of simultaneous inoculation of two fungi + no weeding (13.46 kg/ha) had the highest amount of essential oil

yield; In the event that the lowest amount of essential oil yield was observed in the treatments of no inoculation of fungi + weeding (6.41 kg/ha) and *T. longibrachiatum* inoculation + weeding (6.78 kg/ha) which had no statistically significant difference with *T. atroviride* inoculation + weeding (6.99 kg/ha) treatment (Figure 5).

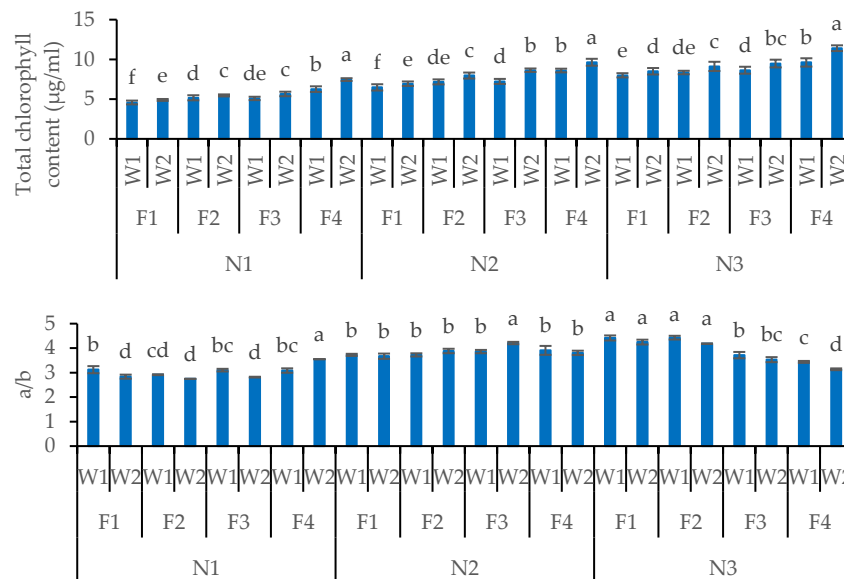
#### Chlorophyll a content

The results of the analysis of variance showed that the main effects of nitrogen, biofertilizer, and weeding were significant for chlorophyll *a*, chlorophyll *b* and total chlorophyll content ( $P \leq 0.01$ )

(Table S4). Also, two-way and three-way interactions for chlorophyll *a*, chlorophyll *b*, and total chlorophyll content were significant ( $P \leq 0.01$ ). The main effects of nitrogen ( $P \leq 0.01$ ), biofertilizer ( $P \leq 0.01$ ) and weeding ( $P \leq 0.05$ ) were significant for the ratio of chlorophyll *a* to *b*. Also, two-way and three-way interactions were significant ( $P \leq 0.01$ ) for the ratio of chlorophyll *a* to *b* (Table S4).

Based on the results of weeding, nitrogen fertilizer and biofertilizer interactions, it was observed that in the conditions of no nitrogen application, the simultaneous inoculation of two fungi + weeding (5.82  $\mu\text{g/ml}$ ) had the highest amount of chlorophyll *a* content, and the lowest amount of chlorophyll *a* content was observed in the treatment of no inoculation of fungi + no weeding (3.47  $\mu\text{g/ml}$ ), which had no statistically significant difference with no inoculation of fungi + weeding (3.62  $\mu\text{g/ml}$ )

(Figure 6). The results in conditions of no nitrogen application showed that the treatment of simultaneous inoculation of two fungi + weeding caused a 68 % increase in chlorophyll *a* content, compared to the treatment of no inoculation of fungi + no weeding (Figure 6). In addition, in the condition of applying 50 % of the recommended nitrogen, the treatment of simultaneous inoculation of two fungi + weeding (7.63  $\mu\text{g/ml}$ ) had the highest amount of chlorophyll *a* content; In the case, the lowest amount of chlorophyll *a* content was observed in the treatment of no inoculation of fungi + no weeding (5.11  $\mu\text{g/ml}$ ) (Figure 6). The results under the conditions of 50 % recommended nitrogen showed that the treatment of simultaneous inoculation of two fungi + weeding caused a 49 % increase in chlorophyll *a* content compared with no inoculation of fungi + no weeding (Figure 6).



**Figure 6.** Influence of nitrogen, biofertilizer and weeding on chlorophyll *a* content, chlorophyll *b* content, total chlorophyll content and ratio of chlorophyll *a* to *b* characteristics in green cumin plants cultivated in Shalami region (W1: no weeding, W2: weeding, F1: no inoculation, F2: *T. longibrachiatum* inoculation, F3: *T. atroviride* inoculation, F4: simultaneous inoculation of *T. longibrachiatum* and *T. atroviride*, N1: no nitrogen application, N2: application of 50 % of recommended nitrogen, N3: application of 100 % of recommended nitrogen). Means followed by different letters in the same column for the same factor are significantly different ( $P \leq 0.05$ ) according to the LSD test. Mean comparisons were shown as mean  $\pm$  standard error (SE).

Under the conditions of 100 % recommended nitrogen, the simultaneous inoculation of two fungi + weeding (8.65  $\mu\text{g/ml}$ ) had the highest amount of

chlorophyll *a* content, and the lowest amount of chlorophyll *a* was observed in the treatment of no inoculation of fungi + no weeding (6.52  $\mu\text{g/ml}$ )

which had no statistically significant difference with *T. longibrachiatum* inoculation + no weeding (6.81 µg/ml) and *T. atroviride* inoculation + no weeding (6.81 µg/ml) (Figure 6). The results of applying 100 % recommended nitrogen showed that the treatment of simultaneous inoculation of two fungi + weeding treatment caused a 33 % increase in chlorophyll *a* content compared to the treatment of no inoculation of fungi + no weeding (Figure 6).

#### **Chlorophyll *b* content**

The results of the interaction of weeding, nitrogen fertilizer and biofertilizer showed that in the condition of no nitrogen application, the simultaneous inoculation of two fungi + weeding (1.64 µg/ml) had the highest amount of chlorophyll *b* content; However, the lowest amount of chlorophyll *b* content was observed in the treatment of no inoculation of fungi + no weeding (1.11 µg/ml) (Figure 6). In conditions of no nitrogen application, the results showed that simultaneous inoculation of two fungi + weeding caused a 48 % increase in chlorophyll *b* content compared to the treatment of no inoculation of fungi + no weeding (Figure 6). In the conditions of application of 50 % of the recommended nitrogen, the results showed that the treatment of simultaneous inoculation of two fungi + weeding (2.01 µg/ml) had the highest content of chlorophyll *b* and the lowest amount of chlorophyll *b* content was observed in the no inoculation of fungi + no weeding (1.37 µg/ml) (Figure 6). Under the conditions of 50 % nitrogen, the results showed that the application of the simultaneous inoculation of two fungi + weeding caused a 47 % increase in chlorophyll *b* content compared to the treatment of no inoculation of fungi + no weeding (Figure 6).

On the other hand, under the conditions of 100 % recommended nitrogen, the results showed that the treatment of simultaneous inoculation of two fungi + weeding (2.76 µg/ml) had the highest amount of chlorophyll *b* content; If the treatment of no inoculation of fungi + no weeding (1.48 µg/ml) had the lowest amount of chlorophyll *b* content, which had no statistically significant difference with *T. longibrachiatum* inoculation + no weeding (1.54 µg/ml) (Figure 6). Under the conditions of 100 % recommended nitrogen, the results showed that the application of the simultaneous inoculation of two fungi + weeding resulted in an 86 % increase in

chlorophyll *b* content compared to the treatment of no inoculation of fungi + no weeding (Figure 6).

#### **Total chlorophyll content**

The results of the interaction of weed, nitrogen fertilizer and biofertilizer showed that in the condition of no nitrogen application, the simultaneous inoculation of two fungi + weeding (7.46 µg/ml) had the highest amount of total chlorophyll content and the lowest amount of total chlorophyll content was observed in the treatment of no inoculation of fungi + no weeding (4.58 µg/ml) (Figure 6). Based on the results obtained in the conditions of no nitrogen application, the use of simultaneous inoculation of two fungi + weeding, increased the total chlorophyll content by 63 % compared to the treatment of no inoculation of fungi + no weeding (Figure 6).

The results of application of 50 % of the recommended nitrogen showed that the treatments of simultaneous inoculation of two fungi + weeding (9.64 µg/ml) and no inoculation of fungi + no weeding (6.48 µg/ml) had the highest and lowest content of total chlorophyll, respectively (Figure 6). Under the conditions of 50 % recommended nitrogen application, the results showed that the use of simultaneous inoculation of two fungi + weeding, caused a 46 % increase in total chlorophyll content compared to the treatment of no inoculation of fungi + no weeding (Figure 6). In addition, under the conditions of 100 % recommended nitrogen, the results showed that the treatment of simultaneous inoculation of two fungi + weeding (11.41 µg/ml) had the highest amount of total chlorophyll content; If the treatment of no inoculation of fungi + no weeding (8 µg/ml) had the lowest amount of total chlorophyll content, which had no statistically significant difference with *T. longibrachiatum* inoculation + no weeding (8.35 µg/ml) (Figure 6). Under the conditions of 100 % recommended nitrogen, the results showed that the use of simultaneous inoculation of two fungi + weeding, resulted in a 43 % increase in total chlorophyll content compared to the treatment of no inoculation of fungi + no weeding (Figure 6).

#### **Ratio of chlorophyll *a* to *b***

The results of the interactions of the weeding, nitrogen fertilizer and biofertilizer showed that in the condition of no nitrogen application, the

simultaneous inoculation of two fungi + weeding (3.55) had the highest ratio of chlorophyll *a* to *b*; In contrast, the lowest amount of chlorophyll *a* to *b* was observed in no inoculation of fungi + weeding (2.84), *T. longibrachiatum* inoculation + weeding (2.75) and *T. atroviride* inoculation + weeding (2.82) treatments, which had no statistically significant difference with *T. longibrachiatum* inoculation + no weeding (2.91) (Figure 6). In addition, under the conditions of application of 50 % of the recommended nitrogen, the inoculation of *T. atroviride* + weeding (4.21) had the highest amount of the ratio of chlorophyll *a* to *b*, and other treatments at this level of nitrogen had the lowest amount of the chlorophyll *a* to *b* (Figure 6). Under the conditions of 100 % recommended nitrogen, the results showed that the treatments of no inoculation of fungi + no weeding (4.42), no inoculation of fungi + weeding (4.25), *T. longibrachiatum* inoculation + no weeding (4.43) and *T. longibrachiatum* inoculation + weeding (4.18) had the highest amount of chlorophyll *a* to *b* ratio, and on the other hand, the lowest amount of chlorophyll *a* to *b* ratio was observed in the treatment of simultaneous inoculation of two fungi + weeding (3.14) (Figure 6).

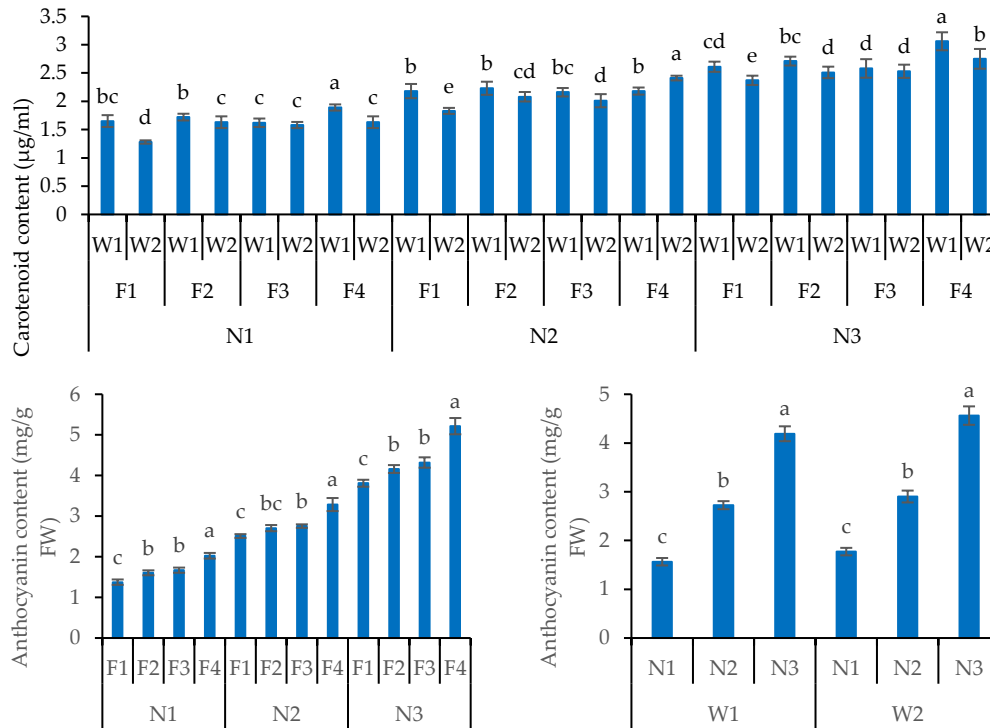
#### Carotenoid content

The results of the analysis of variance showed that the main effects of nitrogen, biofertilizer and weeding as well as the two-way and three-way interactions of these factors were significant for the carotenoid content ( $P \leq 0.01$ ) (Table S4). The results of weed, nitrogen fertilizer and biological fertilizer interactions showed that in the condition of no nitrogen application, the highest amount of carotenoid content was observed in the simultaneous inoculation of two fungi + no weeding (1.89  $\mu\text{g/ml}$ ); in contrast, the lowest amount of carotenoid content was observed in no inoculation of fungi + weeding (1.28  $\mu\text{g/ml}$ ) (Figure 7). In the conditions of application of 50 % of the recommended nitrogen, the highest and lowest amount of carotenoid content were observed in simultaneous inoculation of two fungi + weeding (2.41  $\mu\text{g/ml}$ ) and no inoculation of fungi + weeding (1.83  $\mu\text{g/ml}$ ) treatments, respectively. On the other hand, under the conditions of 100 % recommended nitrogen, the highest and lowest amount of carotenoid content were observed in simultaneous

inoculation of two fungi + no weeding (3.06  $\mu\text{g/ml}$ ) and no inoculation of fungi + weeding (2.37  $\mu\text{g/ml}$ ), respectively (Figure 7).

#### Anthocyanin content

Based on the results, the main effects of nitrogen, biofertilizer and weeding were significant for the anthocyanin content ( $P \leq 0.01$ ) (Table S4). Also, the interactions of nitrogen  $\times$  biofertilizer and nitrogen  $\times$  weeding were significant for anthocyanin content ( $P \leq 0.01$ ). The results of the nitrogen and biofertilizer interaction showed that in the condition of no nitrogen application, the highest amount of anthocyanin content was observed in the treatment of simultaneous inoculation of two fungi (2.02 mg/g FW) and the lowest amount of anthocyanin content was observed in the treatment of no inoculation of fungi (1.37 mg/g FW) (Figure 7). In the conditions of no nitrogen application, the use of *T. longibrachiatum* inoculation, *T. atroviride* inoculation and simultaneous inoculation of two fungi increased the anthocyanin content by 17, 22 and 47 %, respectively, compared to the treatment of no inoculation of fungi (Figure 7). Under the conditions of application of 50 % of the recommended nitrogen, the highest amount of anthocyanin content was observed in the treatment of simultaneous inoculation of two fungi (3.28 mg/g FW) and the lowest amount of anthocyanin content was observed in the treatment of no inoculation of fungi (2.51 mg/g FW) which had no statistically significant difference with *T. longibrachiatum* inoculation (2.71 mg/g FW) (Figure 7). In the conditions of 50 % recommended nitrogen, the use of *T. longibrachiatum* inoculation, *T. atroviride* inoculation and simultaneous inoculation of two fungi increased the anthocyanin content by 8, 9 and 31 %, respectively, compared to the no inoculation of fungi (Figure 7). Under the conditions of 100 % recommended nitrogen, the highest amount of anthocyanin content was observed in the treatment of simultaneous inoculation of two fungi (5.21 mg/g FW) and the lowest amount of anthocyanin content was observed in the treatment of no inoculation of fungi (3.81 mg/g FW) (Figure 7). In the conditions of 100 % recommended nitrogen, the use of *T. longibrachiatum* inoculation, *T. atroviride* inoculation and simultaneous inoculation of two fungi increased the anthocyanin content by 9, 13 and 37 %, respectively.



**Figure 7.** Influence of nitrogen, biofertilizer and weeding on carotenoid and anthocyanin content characteristics in green cumin plants cultivated in Shalami region (W1: no weeding, W2: weeding, F1: no inoculation, F2: *T. longibrachiatum* inoculation, F3: *T. atroviride* inoculation, F4: simultaneous inoculation of *T. longibrachiatum* and *T. atroviride*, N1: no nitrogen application, N2: application of 50 % of recommended nitrogen, N3: application of 100 % of recommended nitrogen). Means followed by different letters in the same column for the same factor are significantly different ( $P \leq 0.05$ ) according to the LSD test. Mean comparisons were shown as mean  $\pm$  standard error (SE).

respectively, compared to the no inoculation treatment (Figure 7). The results of the nitrogen and weeding interaction showed that in the condition of no weeding, the treatment with 100 % recommended nitrogen (4.19 mg/g FW) had the highest amount of anthocyanin content and the lowest amount of anthocyanin content was observed in no nitrogen application (1.56 mg/g FW). In the condition of weeding, the treatment with 100 % recommended nitrogen (4.56 mg/g FW) had the highest amount of anthocyanin content, while the lowest amount of anthocyanin content was observed in the treatment of no nitrogen application (1.77 mg/g FW)

## Discussion

Photosynthetic pigments are considered as an important source of production of photosynthetic products in plants (Silva et al., 2020). On the other

hand, the efficiency and preservation of the function of the photosynthetic apparatus in the face of plants with different environmental and soil conditions is one of the important topics of plant physiology and can play a role as an important criterion in determining the proper growth of plants (Manzoor et al., 2021). The results of the present study showed that the simultaneous application of nitrogen and inoculation with *Trichoderma longibrachiatum* and *Trichoderma atroviride* could significantly increase the amount of chlorophyll *a*, chlorophyll *b* and total chlorophyll content compared to the treatment of not using them. Several researchers stated that nitrogen application and inoculation with *Trichoderma* fungi increase the chlorophyll content in plant leaves, which is consistent with our results ((Ji et al., 2020; Liang et al., 2020; Vukelić et al., 2021). Chlorophyll synthesis is influenced by several factors, such as the physicochemical properties of

the soil, nutrients, temperature, water balance in plants, etc. (Li et al., 2024). It seems that the application of *Trichoderma* fungi has improved the physicochemical properties of the soil and thereby increased the synthesis of chlorophyll, which is consistent with the results of Guo et al. (2020) in the peppermint plant.

Also, inoculation with *Trichoderma* fungi increases the absorption of nutrients in the plant, and for this reason, the synthesis of chlorophyll content is affected (Andrzejak and Janowska, 2022). On the other hand, *Trichoderma* fungi by preserving water in the environment around the root causes the plant to obtain more water and protects the plant from the damage of temporary moisture stress, therefore, increasing the amount of chlorophyll content (Khoshmanzar et al., 2020).

Unfavorable conditions such as insufficient absorption of nutrients by the plant, lack of sufficient water in the surrounding environment of the roots increase the activity of the chlorophyllase enzyme and as a result reduce the synthesis of chlorophyll *a*, chlorophyll *b*, and carotenoid as well as decrease protein bands which decrease (Siddiqui et al., 2022). A number of researchers showed that the chlorophyllase enzyme causes the degradation of chlorophyll under the influence of increasing the amount of ethylene and abscisic acid hormones (Bai et al., 2021). Other reasons for the reduction of plant chlorophyll include the activation of the chlorophyll catabolism pathway or lack of chlorophyll synthesis (Hu et al., 2021). The simultaneous use of fungus and nitrogen prevents unfavorable conditions for plant growth, and as a result, the amount of chlorophyllase enzyme decreases, as a result, the plant will be in a favorable condition in terms of photosynthetic pigments, which is consistent with the results of our experiment. Inoculation with fungi has many benefits for plant growth, one of which is the increase of photosynthetic pigments (Liang et al., 2020). Inoculation with fungi increases the root growth in the plant and in this way improves the absorption of nutrients and water by the plants, as a result of the absorption of sufficient nutrients and water by the plants, adverse conditions will be lighter and as a result induces the plant growth (Khoshmanzar et al., 2020).

Alipour et al. (2021) showed that the inoculation of the seeds of the fennel (*Foeniculum vulgare*) with

*mycorrhizal* fungi increased plant pigments and thus improved plant growth. Since the fungus depends on the cells of the host plant to grow and complete its life cycle, it seems that under the conditions of nitrogen application, the amount of chlorophyll synthesis in the plant increases. As a result, more photosynthetic pigments are produced by the plant and greater amount of assimilate have been provided to the fungus. With the increase in the activity of the fungus, the absorption of nutrients and water by the plant has increased, and for this reason, it has had a positive effect on increasing the amount of chlorophyll content and the synthesis of secondary metabolites (Han et al., 2020).

The researchers stated that by applying different treatments due to the decrease in the function of chlorophyll *a*, the role of chlorophyll *b* and carotenoid as auxiliary pigments to transfer the received energy to chlorophyll *a* and also to compensate for the deficiencies related to chlorophyll *a* had great importance. (Hosseini et al., 2021). Several researchers pointed to the role of chlorophyll *b* and carotenoids as auxiliary photosynthetic pigments and showed that auxiliary pigments can play an effective role in the performance of the photosynthetic apparatus (Simkin et al., 2022; Zhao et al., 2022; Cheaib et al., 2023).

Based on the results of the present experiment, the application of nitrogen and inoculation with fungi, due to providing favorable conditions for plant growth, were able to increase the seed yield compared to control (without nitrogen and inoculation with fungi). During an experiment on wheat plants, the researchers showed that the simultaneous application of nitrogen and inoculation with fungi increased seed yield Meena et al. (2017), which is consistent with the results of the present experiment. It appears that in our experiment, the use of nitrogen and inoculation with fungi has increased the synthesis of secondary metabolites compared to the control treatment. This can be attributed to the positive effect on plant growth, increased absorption of nutrients, increased production of sugar and chlorophyll, and photosynthetic activity. Since the synthesis of phytochemicals requires energy and the factors that boost plant energy can lead to an increase in these bioactive compounds (Ghasemy-Piranloo et al.,

2022). The researchers showed that with the simultaneous application of nitrogen and inoculation with fungi, the growth rate of the product and the leaf area index had a significant superiority over the control treatment in wheat plants (Mohammed et al., 2024). Greenness index with simultaneous application of nitrogen and inoculation with fungus showed a significant increase compared to control treatment in soybean plant (Spagnoletti et al., 2020). The inoculation of mycorrhizal fungi and the application of nitrogen improve the absorption of nutrients, increase the amount of chlorophyll, strengthen the photosynthetic activity, increase the amount of carbohydrates and amino acids in the leaves of the plant, and in this way provide the necessary energy to increase the synthesis of essential oil content (Xie et al., 2022).

The effect of environmental factors such as light, water, nutrients, soil micro-organisms, and agronomic practices on the synthesis of plant secondary metabolites varies (Khalvandi et al., 2019; Hosseini et al., 2021; Soleimani et al., 2022). It can be stated that the treatments used in this experiment positively contributed to the increase in the content of secondary metabolites. The pathways of the synthesis of secondary metabolites are extremely complex and are affected by environmental factors, and the amount of synthesis of secondary metabolites may decrease or increase with the availability of favorable conditions (Mansinhos et al., 2024). In an experiment, researchers showed that the amount of essential oil production in peppermint plant increased by inoculation with *Trichoderma* fungus, which is consistent with the results of the present experiment (Guo et al., 2020). Also, Alhasan and Hussein (2022) showed during an experiment that the application of nitrogen fertilizer increased the synthesis of essential oil in *Mentha spicata* L., which alligns with the results of our experiment. On the other hand, in the present experiment, the competition of weeds with cumin plants led to the reduction of photosynthetic pigments. It seems that the competition with weeds reduced the absorption of nutrients, water, and light by the cumin plant, and as a result, the synthesis of photosynthetic pigments decreased. Our finding is consistent with the results of Fawad and Khan (2022) in the tomato plant. On the other

hand, the amount of carotenoid increased in the conditions of competition with weeds; In this regard, it can be stated that plants increase the amount of carotenoid pigment when faced with stress to increase energy production by absorbing more light and making it available to chlorophyll *a* in the conditions of competition with weeds (Abdelaal et al., 2022). In the present experiment, competition with weeds increased the content of essential oil in some treatments. This weed competition, considered as stress, appears to stimulate an increase in synthesis rate of essential oil. In this regard, the researchers stated that the increase in the synthesis of bioactive compounds in the face of plants with stress has a defensive aspect and increases the tolerance of plants against adverse conditions (Amini et al., 2020; Soleimani et al., 2022).

### Conclusion

The results of this study showed that the application of nitrogen and inoculation with *T. longibrachiatum* and *T. atroviride* fungi improved the essential oil content and yield of green cumin plants. The simultaneous use of nitrogen and inoculation with fungi led to an increase in chlorophyll *a*, chlorophyll *b*, and total chlorophyll pigments in the cumin plant, which increased energy production, essential oil content, and essential oil yield with the production of more photosynthetic assimilates. The co-inoculation of *T. longibrachiatum* and *T. atroviride* fungi had a better effect compared to their separate use. Also, the highest amount of synthesis of photosynthetic pigments was obtained when 100% recommended nitrogen was applied. Competition with weeds decreased the content of photosynthetic pigments and in some cases, carotenoid synthesis increased. Competition with weeds increased the essential oil content in some treatments. Finally, it can be stated that the use of nitrogen treatments, along with inoculation of *T. longibrachiatum* and *T. atroviride* fungi can be recommended as an effective strategy for promoting sustainable and organic agriculture.

### Supplementary Materials

The supplementary material for this article can be found online at: [https://www.jpmb-gabit.ir/article\\_719402.html](https://www.jpmb-gabit.ir/article_719402.html).

Supplementary Table 1. Physiochemical properties of soil related to Qarah-Dam and Shalami regions.

Supplementary Table 2. Meteorological characteristics of the Qarah-Dam region.

Supplementary Table 3. Analysis of variance of different characteristics related to Qarah-Dam region.

Supplementary Table 4. Analysis of variance of different characteristics related to Shalami region.

### Author Contributions

Conceptualization, A.A. and F.Z.; methodology, A.J.A.; software, R.A.; validation, A.A., R.A. and A.J.A.; formal analysis, A.J.A.; investigation, A.J.A.; resources, A.A.; data curation, R.A.; writing—original draft preparation, A.J.A.; writing—review

and editing, A.A.; visualization, F.Z.; supervision, A.A.; project administration, A.J.A.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

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### Conflict of Interest Statement

The authors declare no conflict of interest.

### References

- Abdelaal, K., Alsubeie, M.S., Hafez, Y., Emeran, A., Moghanm, F., Okasha, S., Omara, R., Basahi, M.A., Darwish, D.B.E., and Ibrahim, M.F. (2022). Physiological and biochemical changes in vegetable and field crops under drought, salinity and weeds stresses: control strategies and management. *Agriculture* 12(12): 2084.
- Adl, S., Masoudian, N., Roudi, B., and Ebadi, M. (2023). Response of wheat to drought stress: focus on root and shoot nutrients, as well as leaf chlorophyll and glycine betaine. *J. Plant Mol. Breed.* 11(2): 39-54.
- Agbo, R.I., Missihoun, A.A., Montcho, D., Kpanou, L., Sedah, P., Avohou, G., Djedatin, G.L., and Agbangla, C. (2023). Assessment of the genetic diversity of onion cultivars (*Allium cepa*, *Amaryllidaceae*) collected in southern Benin. *J. Plant Mol. Breed.* 11(2): 107-118.
- Ahmadinia, H., and Heidari, P. (2023). Effects of polyploidy induction on the performance of anise (*Pimpinella anisum* L.). *J. Plant Mol. Breed.* 11(2): 17-30.
- Alhasan, A.S., and Hussein, H.A. (2022). Effect of applying different levels of nitrogen fertilizer on growth and essential oil of spearmint (*Mentha spicata* L.). *Int. J. Agricu. Stat. Sci* 18(1): 137-140.
- Alipour, A., Rahimi, M.M., Hosseini, S.M.A., and Bahrani, A. (2021). Mycorrhizal fungi and growth-promoting bacteria improves fennel essential oil yield under water stress. *Ind Crops Prod* 170: 113792.
- Amini, R., Ebrahimi, A., and Nasab, A.D.M. (2020). Moldavian balm (*Dracocephalum moldavica* L.) essential oil content and composition as affected by sustainable weed management treatments. *Ind Crops Prod.* 150: 112416.
- Anas, M., Liao, F., Verma, K.K., Sarwar, M.A., Mahmood, A., Chen, Z.-L., Li, Q., Zeng, X.-P., Liu, Y., and Li, Y.-R. (2020). Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. *Biol. Res.* 53: 1-20.
- Andrzejak, R., and Janowska, B. (2022). *Trichoderma* spp. improves flowering, quality, and nutritional status of ornamental plants. *Int. J. Mol. Sci.* 23(24): 15662.
- Bai, Q., Huang, Y., and Shen, Y. (2021). The physiological and molecular mechanism of abscisic acid in regulation of fleshy fruit ripening. *Front. Plant Sci.* 11: 619953.
- Cheuib, A., Mahmoud, L.M., Vincent, C., Killiny, N., and Dutt, M. (2023). Influence of anthocyanin expression on the performance of photosynthesis in sweet orange, *Citrus sinensis* (L.) Osbeck. *Plants* 12(23): 3965.
- Comite, E., El-Nakhel, C., Roupheal, Y., Venterino, V., Pepe, O., Borzacchiello, A., Vinale, F., Rigano, D., Staropoli, A., and Lorito, M. (2021). Bioformulations with beneficial microbial consortia, a bioactive compound and plant biopolymers modulate sweet basil productivity, photosynthetic activity and metabolites. *Pathogens* 10(7): 870.

- Fawad, M., and Khan, M.A. (2022). Impact of irrigation timing and weed management practices on chlorophyll content and morphological traits of tomato (*Solanum lycopersicum* Mill.). *Gesunde Pflanzen* 74(2): 317-332.
- Ghasemy-Piranloo, F., Kavousi, F., and Kazemi-Abharian, M. (2022). Comparison for the production of essential oil by conventional, novel and biotechnology methods. *J. Essent. Oil Res.* 34(5): 455-478.
- Guo, K., Sui, Y., Li, Z., Huang, Y., Zhang, H., and Wang, W. (2020). Colonization of *Trichoderma viride* Tv-1511 in peppermint (*Mentha piperita* L.) roots promotes essential oil production by triggering ROS-mediated MAPK activation. *Plant Physiol. Biochem.* 151: 705-718.
- Hadi, A., Mohammadi, H., Hadi, Z., Roshanravan, N., and Kafeshani, M. (2018). Cumin (*Cuminum cyminum* L.) is a safe approach for management of lipid parameters: A systematic review and meta - analysis of randomized controlled trials. *Phytother Res* 32(11): 2146-2154.
- Han, Y., Feng, J., Han, M., and Zhu, B. (2020). Responses of arbuscular mycorrhizal fungi to nitrogen addition: a meta - analysis. *Glob. Change Biol.* 26(12): 7229-7241.
- Hao, D., Li, X., Kong, W., Chen, R., Liu, J., Guo, H., and Zhou, J. (2023). Phosphorylation regulation of nitrogen, phosphorus, and potassium uptake systems in plants. *Crop J.* 11(4): 1034-1047.
- Hosseini, S.J., Tahmasebi - Sarvestani, Z., Pirdashti, H., Modarres - Sanavy, S.A.M., Mokhtassi - Bidgoli, A., Hazrati, S., and Nicola, S. (2021). Investigation of yield, phytochemical composition, and photosynthetic pigments in different mint ecotypes under salinity stress. *Food Sci. Nutr.* 9(5): 2620-2643.
- Hosseinpour Azad, N. (2023). Genetic diversity of *Satureja bachtiarica* Bunge species collected from north-west Iran. *J. Plant Mol. Breed.* 11(2): 31-38.
- Hu, X., Gu, T., Khan, I., Zada, A., and Jia, T. (2021). Research progress in the interconversion, turnover and degradation of chlorophyll. *Cells* 10(11): 3134.
- Ji, S., Liu, Z., Liu, B., Wang, Y., and Wang, J. (2020). The effect of *Trichoderma* biofertilizer on the quality of flowering Chinese cabbage and the soil environment. *Sci. Horti.* 262: 109069.
- Karik, U., Demirbolat, I., Toluk, Ö., and Kartal, M. (2021). Comparative study on yields, chemical compositions, antioxidant and antimicrobial activities of cumin (*Cuminum cyminum* L.) seed essential oils from different geographic origins. *J. Essent. Oil-Bear. Plants* 24(4): 724-735.
- Khalvandi, M., Amerian, M., Pirdashti, H., Keramati, S., and Hosseini, J. (2019). Essential oil of peppermint in symbiotic relationship with *Piriformospora indica* and methyl jasmonate application under saline condition. *Ind. Crops. Prod.* 127: 195-202.
- Khoshmanzar, E., Aliasgharzad, N., Neyshabouri, M., Khoshru, B., Arzanlou, M., and Asgari Lajayer, B. (2020). Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. *Int. J. Environ. Sci. Technol.* 17: 869-878.
- Kohestani, R., Ahangar, L., Zarei, M., Gholamalipour Alamdari, E., and Avarseji, Z. (2023). Allelopathic effect of *Rapistrum rugosum* L. weed on growth, physiological and biochemical parameters of *Hibiscus sabdariffa* L. *J. Plant Mol. Breed.* 11(2): 55-65.
- Krizek, D.T., Kramer, G.F., Upadhyaya, A., and Mirecki, R.M. (1993). UV - B response of cucumber seedlings grown under metal halide and high pressure sodium/deluxe lamps. *Physiol. Plant.* 88(2): 350-358.
- Kulbat-Warycha, K., Nawrocka, J., Kozłowska, L., and Żyżelewicz, D. (2024). Effect of light conditions, trichoderma fungi and food polymers on growth and profile of biologically active compounds in *Thymus vulgaris* and *Thymus serpyllum*. *Int. J. Mol. Sci.* 25(9): 4846.
- Li, X., Zhang, W., Niu, D., and Liu, X. (2024). Effects of abiotic stress on chlorophyll metabolism. *Plant Sci:* 112030.
- Liang, Z., Soranno, P.A., and Wagner, T. (2020). The role of phosphorus and nitrogen on chlorophyll a: Evidence from hundreds of lakes. *Water Res.* 185: 116236.
- Lichtenthaler, H.K. (1987). "Chlorophylls and carotenoids: pigments of photosynthetic biomembranes," in *Methods in enzymology*. Elsevier, 350-382.

- Mahmud, K., Makaju, S., Ibrahim, R., and Missaoui, A. (2020). Current progress in nitrogen fixing plants and microbiome research. *Plants* 9(1): 97.
- Mansinhos, I., Gonçalves, S., and Romano, A. (2024). How climate change-related abiotic factors affect the production of industrial valuable compounds in Lamiaceae plant species: a review. *Front. Plant Sci.* 15: 1370810.
- Manzoor, N., Ahmed, T., Noman, M., Shahid, M., Nazir, M.M., Ali, L., Alnusaire, T.S., Li, B., Schulin, R., and Wang, G. (2021). Iron oxide nanoparticles ameliorated the cadmium and salinity stresses in wheat plants, facilitating photosynthetic pigments and restricting cadmium uptake. *Sci. Total Environ.* 769: 145221.
- Meena, S.K., Rakshit, A., Singh, H.B., and Meena, V.S. (2017). Effect of nitrogen levels and seed bio-priming on root infection, growth and yield attributes of wheat in varied soil type. *Biocatal Agric Biotechnol* 12: 172-178.
- Mohammed, F.S., Sevindik, M., Uysal, İ., Česko, C., and Koraqi, H. (2024). Chemical composition, biological activities, uses, nutritional and mineral contents of cumin (*Cuminum cyminum*). *Plant Physiol. Biochem.* 158: 76-82.
- Mu, X., and Chen, Y. (2021). The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiol. Biochem.* 158: 76-82.
- Nassif, A.H., Abd, R.M., and Al-Zubaidi, N.A.J. (2023). Improving the growth of black cumin (*Nigella sativa* L.) by humic acid and *Trichoderma harzianum* as biofertilizer. *Mod. Phyt.* 17: 37-40.
- Rostaminia, M., Habibi, D., Shahbazi, S., Sani, B., and Pazoki, A. (2021). Effect of different species of *Pseudomonas* and *Trichoderma* on several morpho-physiological traits of roselle (*Hibiscus sabdariffa* L.). *Acta Physiol. Plant* 43: 1-8.
- Scavo, A., and Mauromicale, G. (2020). Integrated weed management in herbaceous field crops. *Agronomy* 10(4): 466.
- Siddiqui, S.A., Khatri, K., Patel, D., and Rathore, M.S. (2022). Photosynthetic gas exchange and chlorophyll a fluorescence in *Salicornia brachiata* (Roxb.) under osmotic stress. *J. Plant Growth Regul.* 41: 429-444.
- Silva, V.M., Tavanti, R.F.R., Gratão, P.L., Alcock, T.D., and Dos Reis, A.R. (2020). Selenate and selenite affect photosynthetic pigments and ROS scavenging through distinct mechanisms in cowpea (*Vigna unguiculata* (L.) walp) plants. *Ecotoxicol. Environ. Saf.* 201: 110777.
- Simkin, A.J., Kapoor, L., Doss, C.G.P., Hofmann, T.A., Lawson, T., and Ramamoorthy, S. (2022). The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. *Photosynth. Res.* 152(1): 23-42.
- Soleimani, M., Arzani, A., Arzani, V., and Roberts, T.H. (2022). Phenolic compounds and antimicrobial properties of mint and thyme. *J. Herb. Med.* 36: 100604.
- Spagnoletti, F.N., Cornero, M., Chiocchio, V., Lavado, R.S., and Roberts, I.N. (2020). Arbuscular mycorrhiza protects soybean plants against *Macrophomina phaseolina* even under nitrogen fertilization. *Eur. J. Plant Pathol.* 156: 839-849.
- Vukelić, I.D., Prokić, L.T., Racić, G.M., Pešić, M.B., Bojović, M.M., Sierka, E.M., Kalaji, H.M., and Panković, D.M. (2021). Effects of *Trichoderma harzianum* on photosynthetic characteristics and fruit quality of tomato plants. *Int. J. Mol. Sci.* 22(13): 6961.
- Xie, K., Ren, Y., Chen, A., Yang, C., Zheng, Q., Chen, J., Wang, D., Li, Y., Hu, S., and Xu, G. (2022). Plant nitrogen nutrition: The roles of arbuscular mycorrhizal fungi. *J. Plant Physiol.* 269: 153591.
- Zhao, X., Zhang, Y., Long, T., Wang, S., and Yang, J. (2022). Regulation mechanism of plant pigments biosynthesis: anthocyanins, carotenoids, and betalains. *Metabolites* 12(9): 871.

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# اثر همزیستی قارچ تریکودرما و کاربرد نیتروژن بر خصوصیات اسانس و رنگدانه‌های برگ در زیره سبز (*Cuminum cyminum* L.) تحت رقابت با علف‌های هرز

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**چکیده:** زیره سبز یکی از پر مصرف‌ترین گیاهان دارویی در ایران و جهان است که عمدتاً برای استفاده از اسانس آن مورد بهره‌برداری قرار می‌گیرد. در راستای کشاورزی پایدار، طراحی آزمایشی بمنظور بررسی کارکرد کودهای زیستی در کنار نهاده‌های شیمیایی نظیر نیتروژن ضروری به نظر می‌رسد. این تحقیق در سال زراعی ۱۴۰۳-۱۴۰۲ در شهرستان مراوه تپه و در دو منطقه مجزا (قره دام و شلمی) به صورت آزمایش اسپلیت فاکتوریل در قالب طرح بلوک‌های کامل تصادفی با سه تکرار انجام شد. تیمارهای آزمایشی شامل سطوح کود نیتروژن (شاهد، ۵۰ و ۱۰۰ درصد نیتروژن توصیه شده از منبع اوره) به عنوان عامل اصلی، تیمارهای کود زیستی (شاهد، تلقیح بذریه با *Trichoderma longibrachiatum*، *Trichoderma atroviride*) و تلقیح همزمان دو قارچ) و مدیریت علف‌های هرز (وجین و عدم وجین) از عوامل فرعی هستند. نتایج نشان داد که بیشترین مقدار و عملکرد اسانس در هر دو منطقه مربوط به تیمار بدون وجین + ۱۰۰ درصد نیتروژن توصیه شده + استفاده همزمان از دو قارچ بود. همچنین استفاده از قارچ تریکودرما و نیتروژن تأثیر مثبتی بر محتوای کلروفیل a، کلروفیل b و کلروفیل کل داشت.

**کلمات کلیدی:** آنتوسیانین، کود زیستی، محتوای اسانس، محتوای کلروفیل، نیتروژن.