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Effect Of Marine Algae Extract And Trichoderma On The Growth And Production Qualities Of Petunia Hybrida

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Abstract

The study was carried out at the University of Anbar, College of Agriculture, Department of Horticulture and Landscape Engineering, to study the effect of seaweed extract and Trichoderma fungus on the growth characteristics and chemical content of Petunia hybrida plant. The experiment was designed according to the randomized completely block design (RCBD), Treatment included three levels of Trichoderma fungus, 0, 5, and 10 grams per kilogram of the weight of soil in the Pot, in a way that touches the roots of the plants. The fungus isolate was obtained from Department of Plant Protection at the College of Agriculture, University of Anbar. The second factor included four concentrations of seaweed extract 0, 100, 200, and 300 mg L^{-1}, Applied two times: the first spray two weeks after planting the seedlings, and the second spray one month after the first spray,. Obtained results of the study summarized the superiority of Trichoderma mushrooms at the level of 10 g per Pot in the total leaf area , Main stem diameter average , relative leaf content of nitrogen, phosphorus, chlorophyll, average number of flowers, average flower diameter, and carotenoids , Infection rate of Trichoderma fungus , and number of spores which amounted to 682.484cm^2 6.926 mm, 1.173%, 0.394%, 26.263, 25.034 flowers per $plant^{-1}$, 29.780 mm, and 2.134 mg per 100, 47.182 % , 85.806 spores 10 g^{-1} soil. the results of marine algae extract showed the main stem diameter average, relative leaf content of nitrogen, phosphorus, chlorophyll, average number of flowers, average flower diameter, and carotenoids, Infection rate of Trichoderma fungus, and number of spores that recorded 7.381mm, 1.702%, 0.418%, 26.734, 28.06 plant $flower^{-1}$, 31.746 mm, and 2.333 mg per 100 g fresh weight, 49.408% , 114.546 spores 10 g^{-1} correspondingly.

Keywords: Foliar spray, Trichoderma fungus, *Petunia hybrid*, Seaweed extract, Namely

INTRODUCTION

Petunia is a genus in the family Solanaceae. It is a genus of 20 species of flowering plants of South American origin. Hybrid petunias possess large, trumpet shaped flowers in an enormous assortment of colors, most of the varieties seen in gardens are hybrids (*Petunia integifolia* and *Petunia atkinsiana*, also known as *Petunia hybrida*), maximum growth occurs in late spring and summer (Gülser Khan et al, 2019 Kamali Aliabad et al, (2024).). The flowers range in hue from white to red, purple and yellow. Petunia varieties are very different from each other not only in terms of growth habit. they also vary in flower shape, colour and size. So, Petunias are a perfect fit for porch, gazebo, garden pergola and balcony decorating with hanging baskets and containers. Petunias are among the most popular flowering annuals for good reason. Petunias are bright and lively, bloom from spring until frost, and scent the air with lovely fragrance. Although it may not be practical to deadhead masses of petunias in the garden, it's a must for flowering annuals in containers (Huda and Muna, 2014; Nouri, et al, 2014) . Trichoderma is a

genus of fungi, which is one of the microorganisms that coexist symbiotically with several plant species, either independently or in a mutually beneficial relationship with beneficial bacteria and fungi that promote plant growth. Trichoderma is considered as a plant growth promoter and biocontrol fungal agents (Vinale and Sivasithamparam, 2020). Trichoderma establishes colonization in the roots, which triggers the release of plant hormones that stimulate plant growth. These hormones bear similarities to auxins, cytokinins, gibberellins, ethylene, and zeatins. Trichoderma plays a role in enhancing the availability of nutrients such as nitrogen, phosphorus, iron, potassium, manganese, zinc, and copper (Saini and Aggarwal, 2019 ; Mahato and Shrestha, 2018). The study of (Ahmed and Kaeel, 2021). shows that the treatment of Tagetes erecta with Trichoderma has led to positive effects on various aspects of vegetative, root, and flower growth. Several characteristics have exhibited an increase as a result of this treatment, such as leaf area, number of leaves, percentage of total chlorophyll in the leaves, percentage of dry matter in the leaves, root length, diameter, and number, number of flowers (the Trichoderma treatment has led to an increase in the number of flowers produced by the Tagetes erecta plants), size of flowers, wet and dry weight of flowers and the flowers on the treated plants have exhibited a longer duration of stay on the plant, implying prolonged flowering and potentially extended ornamental value. Algae are a diverse group of predominantly aquatic photosynthetic organisms, including cyanobacteria, green algae and other eukaryotic algae. They are different from higher plants because they lack roots, stems, and leaves. Algae rely on the nutritive substances available in the seawater where they thrive. Algae exhibit a wide range of sizes, and many species are indeed microscopic, consisting of a single cell or small colonies that can only be observed under a microscope others can be quite large and even reach giant proportions (Ahmed et al, 2021 ; Velasco-Ramírez et al, 2020). Algae, being photosynthetic organisms, do not follow an annual growth cycle like many wild plants. Instead, their growth is influenced by environmental factors such as nutrient availability, light intensity, temperature, and water conditions, their growth is completed in the period extending from February to May of each year (Rashidi and Trindade, 2018). Marine algae extracts have been recognized for their potential benefits in improving root system growth and nutrient absorption in plants, consequently enhancing vegetative growth. These extracts can play a role in promoting plant health and development through various mechanisms (Balaraman et al, 2020 ; saad et al, 2020) . So, this study aims at investigating the effect of different levels of Trichoderma on the growth characteristics of the petunia plant, studying the effect of different levels of marine algae extract, and finding out the bilateral interaction of the study factors on the growth characteristics of the plant.

MATERIALS AND METHODS

The experiment was conducted in the Department of Horticulture and Landscape Architecture in the College of Agriculture/ University of Anbar for the period from September 5, 2023 to June 1, 2024 in order to study the effect of Trichoderma and Marine algae extract on the growth traits of Petunia 'Blue Crown'. All treatments received fertilization according to the recommended dosage using a chemical fertilizer with an NPK formulation grams of the chemical fertilizer. The present experiment used a randomized complete block design (RCD). Each block contained 12 treatments containing three samples grown in an 8 kg Pot . the Pot likely refers to the container or pot used to hold the plants during the experiment. The data collected from the experiment was analyzed using the Genstat program. The probability level chosen for the analysis was 0.05, which indicates that the significance of any observed effects or differences would be evaluated at a 5% level of significance.

FACTORS

The First Factor:

Trichoderma fungus: The first factor being studied is the Trichoderma fungus. Three different concentrations of the fungus were applied to the anvil soil. The concentrations used

were 0 grams per kilogram, 5 grams per kilogram, and 10 grams per kilogram of the weight of the anvil soil. The Trichoderma fungus isolate used in the experiment was obtained from the Prevention Department at the College of Agriculture, University of Anbar.

The Second Factor:

Marine algae extract: The second factor being studied is the marine algae extract. The extract was applied by shoot spraying of plants at four different levels: 0 mg L^{-1} (no extract), 100 mg L^{-1}, 200 mg L^{-1}, and 300 mg L^{-1}. The marine algae extract was applied through two separate sprays. The first spray occurred two weeks after planting the seedlings, while the second spray took place a month after the first spray.

The study indicators mentioned in the experiment are as follows:

Total Leavs area (cm2).
Main stem diameter(mm).
Nitrogen percentage in leaves (%).
Phosphorus percentage in leaves (%).
Chlorophyll concentration in leaves (mg per 100 g fresh weight).
Average number of flowers.
Flower longevity and Carotene pigment concentration (mg per 100 g fresh weight).
Fungal infection rate (%).
Number of spores (Spre 10 gm^{-1} Soil)

RESULTS AND DISCUSSION

1.Total leaf area (cm^2):

The results of Table 1 indicated that there were no significant differences when treated with seaweed extract. Regarding treating Trichoderma fungus, the T2 treatment significantly differed from the other treatments, with a total leaf area of 682.484 cm^2, while the average leaf area in the control treatment was 586.788 cm^2. There were no significant differences in the treatment of the two-way interaction between the study factors.

Table 1. The Effect of Trichoderma and Marine algae extract on the Total leaf area (cm^2)

معدل S	T_2	T_1	T_0	
581.388	594.197	581.807	568.160	S_0
623.454	657.553	637.230	575.580	S_1
648.128	692.783	664.760	586.840	S_2
696.796	785.403	688.413	616.570	S_3
	682.484	643.053	586.788	معدل T
0.05	N.S		LSD S	
	118.441		LSD T	
	N.S		LSD S*T	

2. Main Stem Diameter Average (mm):

The results of Table 2 confirmed significant differences in the average diameter of the main stem when treated with seaweed extract (S3), which measured 7.381 mm, while the average diameter of the main stem decreased in the control treatment, measuring 5.293 mm. In the treatment with the biofertilizer represented by Trichoderma fungi, the treatment T2 was significantly distinguished from the other treatments in the average diameter of the main stem, measuring 6.926 mm, whereas the control treatment recorded the lowest average for the main stem diameter at 5.652 mm. Regarding the interaction between the study treatments, the

treatment (F3T2) significantly outperformed the other treatments in the average diameter of the main stem, measuring 8.733 mm. In contrast, the average diameter of the main stem decreased in the control treatment (F0T0), measuring 5.163 mm.

Table 2. The Effect of Trichoderma and Marine algae extract on the Main Stem Diameter Average (mm)

معدل S	T_2	T_1	T_0	
5.293	5.467	5.250	5.163	S_0
6.052	6.273	6.267	5.617	S_1
6.524	7.230	6.697	5.647	S_2
7.381	8.733	7.230	6.180	S_3
	6.926	6.361	5.652	معدل T
0.05	0.103		LSD S	
	0.089		LSD T	
	0.178		LSD S*T	

3. The nitrogen content in the leaves%:

The results in (table1) show that there are significant differences in the nitrogen content of the leaves based on the different treatments. The treatment with a concentration of 300 mg L^{-1} of the marine algae extract showed significantly higher nitrogen content in the leaves compared to the other treatments. The nitrogen content was found to be 1.702% higher in this treatment compared to the rest. Among the different concentrations of Trichoderma, the treatment with a concentration of 10 grams per plant demonstrated superior results in terms of the nitrogen content in the leaves. The interaction between the marine algae extract treatment S3 (concentration of 300 mg L^{-1}) and the Trichoderma treatment T2 (concentration of 10 grams per plant) resulted in the highest nitrogen content in the leaves. The nitrogen content in this combined treatment (S3T2) was significantly superior, amounting to an increase of 2.187% compared to the other treatments.

Table 3. The Effect of Trichoderma and Marine algae extract on the nitrogen content in the leaves

Seaweed	Trichoderma			Mean
	T0	T1	T2	
S0	1.047	1.137	1.173	1.119
S_1	1.147	1.207	1.280	1.211
S_2	1.183	1.287	1.400	1.290
S_3	1.227	1.693	2.187	1.702
Mean	1.151	1.331	1.510	mean
LSD S		0.038		0.05
LSD T		0.033		
LSD S*T		0.066		

4. Phosphorus Percentage in Leaves (%):

the results in (table 2) illustrate that that there are significant differences in an unspecified parameter between the treatments. The treatment using seaweed extract at a concentration of 300 mg L^{-1} demonstrated superiority, reaching a value of 0.418% for the unspecified parameter. This indicates that this treatment resulted in a higher value compared to the other treatments. Trichoderma treatment, specifically at a concentration of 0.394%, was found to be superior for

the unspecified parameter. This suggests that this particular concentration resulted in a higher value compared to the other concentrations of Trichoderma used in the experiment. As for Bilateral interaction effect, the interaction between the seaweed extract treatment S3 (concentration of 300 mg L^{-1}) and the Trichoderma treatment T2 (concentration not specified) yielded the highest value for the unspecified parameter. The value obtained for this combined treatment (S3T2) was superior, with an increase of 0.493% compared to the other treatments.

Table 4. The Effect of Trichoderma and Marine algae extract on the Phosphorus Percentage in Leaves

Seaweed	Trichoderma			Mean
	T0	T1	T2	
S_0	0.247	0.300	0.330	0.292
S_1	0.303	0.347	0.370	0.340
S_2	0.320	0.367	0.383	0.357
S_3	0.333	0.427	0.493	0.418
Mean	0.301	0.360	0.394	
LSD S		0.018		
LSD T		0.015		0.05
LSD S*T		0.031		

5. Total Chlorophyll (mg per 100 g):

The results in ((table 3) display that there are significant differences in the chlorophyll content of the leaves among the different treatments. The treatment using the extract at a concentration of 300 mg L^{-1} resulted in a significantly higher chlorophyll content compared to the other treatments. The chlorophyll content reached 26.743 mg per 100 grams, indicating a substantial increase compared to the other treatments. The Trichoderma treatments showed superiority over the Trichoderma with a concentration of 10 grams per plant in terms of chlorophyll content. The specific concentration of the mushroom treatments is not mentioned, but they recorded a chlorophyll content of 26.263 mg per 100 grams, and Bilateral interaction effect, the interaction between the extract treatment S3 (concentration of 300 mg L^{-1}) and Trichoderma treatment T2 (concentration of 10 grams per plant) resulted in the highest chlorophyll content among the treatments. The chlorophyll content for this combined treatment (S3T2) was significantly superior, amounting to 30.290 mg per 100 grams.

Table 5. The Effect of Trichoderma and Marine algae extract on the Total Chlorophyll Percentage in Leaves

Seaweed	Trichoderma			Mean
	T0	T1	T2	
S_0	20.150	22.147	23.587	21.961
S_1	21.223	23.170	24.223	22.872
S_2	22.203	24.177	26.953	24.444
S_3	22.733	27.207	30.290	26.743
Mean	21.578	24.175	26.263	
LSD S		0.513		0.05

LSD T	0.445	
LSD S*T	0.889	

6. Average number of flowers:

The results of (table 4) show that there are significant differences in the number of flowers per plant (plant^{-1}) among the different treatments. The marine algae extracts at a concentration of 300 mg L^{-1} resulted in a significantly higher average number of flowers per plant compared to the other treatments. The number of flowers recorded was 28,063 flowers per plant^{-1}, indicating a substantial increase compared to the other treatments. The Trichoderma with a concentration of 10 grams per plant showed superiority in terms of the average number of flowers per plant compared to the other treatments. The number of flowers recorded was 25,024 flowers per plant^{-1}, which was significantly higher compared to the other treatments. Bilateral interaction effect: The interaction between the seaweed extract treatment S3 (concentration of 300 mg L^{-1}) and the Trichoderma treatment T2 (concentration of 10 grams per plant) resulted in the highest average number of flowers per plant. The number of flowers for this combined treatment (S3T2) was significantly superior, amounting to 30,510 flowers per plant^{-1}.

Table 6. The Effect of Trichoderma and Marine algae extract on Average number of flowers Percentage in Leaves

Seaweed	Trichoderma			Mean
	T0	T1	T2	
S_0	16.817	18.560	19.643	18.340
S_1	18.903	20.897	23.057	20.952
S_2	21.920	24.887	26.887	24.564
S_3	24.820	28.860	30.510	28.063
Mean	20.615	23.301	25.024	
LSD S		0.698		
LSD T		0.604		0.05
LSD S*T		1.209		

7. Flower longevity:

The results in (Table 5) indicate that there are significant differences in flower longevity as treating plants using the marine algae extracts at a concentration of 300 mg L^{-1} resulted in significant differences in flower longevity. The treatment with Trichoderma at a concentration of 10 grams per plant was found to be superior in terms of flower longevity on the plant. The exact duration is not mentioned, but it amounted to 29.780 units. This indicates that Trichoderma treatment had a positive effect on prolonging flower longevity compared to the other treatments. As concerning Bilateral interaction effect, the interaction between the seaweed extract treatment S3 (concentration of 300 mg L^{-1}) and the Trichoderma treatment T2 (concentration of 10 grams per plant) resulted in the highest flower longevity. The specific duration for this combined treatment (S3T2) is not mentioned, but it reached 35.523 units.

Table 7. The Effect of Trichoderma and Marine algae extract on Flower longevity Percentage in Leaves

Seaweed	Trichoderma			Mean
	T0	T1	T2	
S_0	22.567	24.270	25.280	24.039

S_1	24.750	26.780	28.130	26.553
S_2	26.663	28.660	30.187	28.503
S_3	28.767	30.947	35.523	31.746
Mean	25.687	27.664	29.780	
LSD S		0.703		
LSD T		0.609		0.05
LSD S*T		1.218		

8. Carotene pigment concentration (mg per 100 g fresh weight):

The results of this study show that the S3 plant extract treatment, which presumably refers to the seaweed extract treatment at a concentration of 300 mg L^{-1}, was found to be superior in terms of the concentration of carotene pigment. The specific concentration is mentioned as 2.333 mg 100 gm, indicating a higher concentration compared to the other treatments. Trichoderma recorded the highest concentration of carotene pigment among the treatments, amounting to 2.134 mg 100 gm. As for bilateral interaction effect, the interaction between the seaweed extract treatment S3 (concentration of 300 mg L^{-1}) and Trichoderma T2 (concentration not specified) resulted in the highest concentration of carotene pigment. The specific concentration for this combined treatment (S3T2) is mentioned as 2.980 mg 100 gm, indicating a substantial increase compared to the other treatments.

Table 8. The Effect of Trichoderma and Marine algae extract on Carotene pigment concentration Percentage in Leaves

Seaweed	Trichoderma			Mean
	T0	T1	T2	
S_0	1.173	1.553	1.583	1.437
S_1	1.607	1.870	1.917	1.798
S_2	1.817	1.947	2.057	1.940
S_3	1.880	2.140	2.980	2.333
Mean	1.619	1.878	2.134	
LSD S		0.120		0.05
LSD T		0.104		
LSD S*T		0.208		

9. Infection rate of Trichoderma fungus (%):

The results presented in Table 9 indicate significant differences in the infection rate of Trichoderma fungus when treated with seaweed extract (F3), which reached 49.408%, while the infection rate of the fungus decreased to 13.660%. In the case of treatment with Trichoderma fungus, the infection rate increased in treatment T2, reaching 47.182%, while the infection rate in the control treatment decreased to 15.928%. Regarding the interaction between the study treatments, the treatment (F3T2) significantly outperformed the other treatments and reached 80.367%, while the infection rate in the control treatment decreased to 10.200%.

Table 9. The Effect of Trichoderma and Marine algae extract on the Infection rate of Trichoderma fungus (%)

معدل S	T_2	T_1	T_0	
13.660	17.480	13.300	10.200	S_0
29.441	40.347	35.523	12.453	S_1
32.763	50.533	30.250	17.507	S_2
49.408	80.367	44.303	23.553	S_3
	47.182	30.844	15.928	معدل T
0.05	9.268		LSD S	
	8.026		LSD T	
	16.053		LSD S*T	

10. Number of spores (spores 10 g^{-1} soil):

The results of Table 10 confirmed a significant difference in the average number of spores when treated with seaweed extract at treatment S3, which recorded 114.546 spores 10 g-1 soil, while the number of spores decreased in the control treatment, reaching 40.230 spores 10 g-1. In the treatment with Trichoderma fungus, treatment T2 significantly outperformed the other treatments, reaching 85.806 spores 10 g-1, while the control treatment recorded 44.038 spores 10 g-1. The interaction treatment (F3T2) also showed a significant superiority, reaching 188.117 spores 10 g-1, whereas the average number of spores in the control treatment decreased to 34.607 spores 10 .

Table 10. The Effect of Trichoderma and Marine algae extract on the Number of spores (spores 10 g^{-1} soil)

معدل S	T_2	T_1	T_0	
40.230	43.483	42.600	34.607	S_0
47.747	50.410	47.567	45.263	S_1
54.680	61.213	55.810	47.017	S_2
114.546	188.117	106.257	49.263	S_3
	85.806	63.058	44.038	معدل T
0.05	2.303		LSD S	
	1.994		LSD T	
	3.988		LSD S*T	

The results showed significant differences in Trichoderma fungi regarding leaf area rate, main stem diameter, and nitrogen and phosphorus ratios. Chlorophyll content in leaves, number of flowers, flower diameter, carotenoid pigmentation, infection rate by Trichoderma fungi, and spore count. This may be attributed to the numerous functions performed by the fungus, including the transformation of the growth medium from a basic to a neutral medium due to the organic acids it produces, its interaction with beneficial microorganisms in the soil for plants, and the increase in root surface area due to fungal hyphae, as mentioned by Ahmed and Kaeel (2021). Additionally, the capability of Trichoderma fungi to produce or secrete a range of compounds plays a significant role in enhancing physiological processes and increasing

plants' capacity to absorb water, macronutrients, and micronutrients. That, in turn, leads to improved plant growth, which is consistent with Ghorbanpour et al. (2018). This may be attributed to the secretion of a type of enzyme by Trichoderma known as Acc-Deminase. This is involved in the biosynthetic pathway of ethylene and inhibits its action. Thereby delaying plant aging by preserving chlorophyll from degradation and sustaining the process of photosynthesis (Vukelić et al., 2021).

The Trichoderma fungus inoculum has a clear effect on the process of biological nitrogen fixation, which in turn contributes to the production of various plant hormones. This includes auxins and gibberellins. These hormones, in turn, activate cell division and elongation, thereby increasing plant height, leaf area, and stem diameter. Additionally, it plays a role in enhancing the absorption of other nutrients, such as potassium, which contributes to the formation of carbohydrates and proteins. Potassium also aids in activating the division and expansion of cambium cells. All these factors have led to an increase in the average stem diameter (Yasmeen & Shaheed, 2017).

The study results indicated a significant superiority when treated with seaweed extract at a concentration of 300 mg L^{-1} in terms of the diameter of the main stem, the percentage of nitrogen and phosphorus, and chlorophyll content in the leaves. Also, the number of flowers, flower diameter, carotenoid pigmentation, the percentage of infection by the Trichoderma fungus, and the average number of spores. This may be attributed to the numerous functions performed by the seaweed extract due to its content of essential nutrients such as nitrogen, phosphorus, potassium, and magnesium. As well as micronutrients such as zinc, iron, boron, and copper. In addition, it contains natural hormones such as auxins, cytokinins, gibberellins, salicylic acid, steroids, jasmonic acid, vitamins, organic acids, amino acids, and phenolic compounds. It also includes hormone-like substances and contains good quantities of proteins and carbohydrates that provide the necessary energy for growth and development (Taiz & Zeiger, 2010). In addition to containing some essential nutrients necessary for vital plant activities such as photosynthesis, respiration, carbohydrate metabolism, the activation of the enzymatic system, and various metabolic processes that play a significant role in the division and elongation of cells (Marschner, 2012). It is positively correlated with vegetative growth processes. Furthermore, the extract contains amino acids that provide the plant with a ready-made formulation of the building blocks of proteins. In addition to their role in constructing nucleic acids and increasing the ability of cells to elongate and divide (Hildbrandt et al., 2015). It also contributes to enhancing the efficiency of the photosynthesis process and stimulates the formation of chlorophyll, which contributes to increased vegetative growth (D'Mello, 2015). Moreover, certain amino acids, particularly tryptophan, phenylalanine, and arginine, play a role in the biosynthesis of several plant hormones, such as auxins, cytokinins, and polyamines (Davies, 2010).

CONCLUSIONS

From previous results, it is concluded that spraying the plants with a concentration of 300 mg L^{-1} led to an increase in vegetative and flowering characteristics in the study. Furthermore, treating plants with Trichoderma at a concentration of 10 mg L^{-1} led to a significant increase in all vegetative and flowering traits. The interaction treatment between seaweed extract with concentration300 mg L^{-1} and Trichoderma 10 fungus led to a significant increase in vegetative and floral traits.

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Conflicts of Interest:

The authors declare no conflict of interest.

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