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Effect of biological factors on some growth characteristics of the *Crocus sativus L.* plant

Sheren Ghazi Mahmood[1], Khawlla Mahmood Yahia[1], Huda Hazim Wafy[2], Alaa Younis Dhannoon[1], Noor Fatek Mohammed[1], Zahraa Abdul Rahman Sabri[1], Ali M. Saadi[1]*

[1]Northern Technical University, Mosul, Iraq.
[2]University of Mosul, Mosul, Iraq.
*Corresponding author, e-mail: ali.mohammed@ntu.edu.iq

Abstract

Crocus sativus L. is an autumn-flowering species belonging to the Iridaceae family. This study was conducted under greenhouse conditions at the Plant Production Technology Department, Agricultural Technical College, Northern Technical University, to evaluate the effects of the environmentally friendly biological agents Trichoderma harzianum T-22 and *Bacillus subtilis* on vegetative growth, flowering, stigma production, enzymatic activity, and corm yield of saffron using two application methods. Significant differences were observed between biological treatments and the untreated control. Corm immersion with T. harzianum T-22 promoted the earliest vegetative bud emergence (17.56 days). The same fungal treatment also produced the tallest plants, reaching 8.75 and 7.40 cm depending on the application method, whereas untreated plants reached only 3.42 cm. Both fungal and bacterial treatments significantly reduced the time required for flower bud emergence, with T-22 showing the shortest periods (89.77 and 89.81 days). Corm immersion with fungal and bacterial agents also shortened the time to first flower opening to 125.6 and 126.1 days, respectively. Fresh and dry stigma weights were significantly higher in biologically treated plants than in the control, which recorded only 0.021 and 0.002 g, respectively. Peroxidase activity in saffron leaves was highest in plants treated with T-22, reaching 0.096 unit min^{-1} g^{-1} fresh weight. Biological treatments also significantly increased corm number, corm fresh weight, cormlet number, and cormlet fresh weight compared with the control. Overall, T. harzianum T-22 and B. subtilis improved saffron growth, flowering, physiological activity, and yield under greenhouse conditions.

Keywords: *Bacillus subtilis,* biological fertilizers, *Crocus sativus*, saffron, *Trichoderma harzianum*

Introduction

Saffron (*Crocus sativus L.*) is a perennial herbaceous plant belonging to the Iridaceae family, which also includes other plants such as Freesia and Gladiolus (Mahmood et al., 2024). Saffron is a sterile plant, and the corms are responsible for its propagation. Consisting of fleshy underground corms covered with scales, ranging in diameter from 3 to 5 cm. Flowering occurs from October to the end of January, depending on weather conditions. The saffron flower is bisexual, appearing in purple and white with six petals, and the leaves are dark green, ranging in number from 6 to 9 leaf. The ovary is tubular in shape, and the stigma is pale yellow, thin, and long, with a length of 20-35 mm and a reddish-orange color (Deo, 2003).It is one of the most expensive spices in the world, originating in Asia Minor, where the Mediterranean climate is suitable for saffron growth (Shrestha and Pathak, 2024).Saffron is called red gold because of its high economic value and its flavor, aroma, and color, as it contains safranal, picrocrocin, and crocin, which are responsible for its aroma, taste, and color.(El Hajj et al., 2019; Rashed-Mohassel, 2020). Cultivated saffron species have great commercial value, while many varieties are grown for their beautiful and attractive flowers.(Cardone et al., 2021) It is the most expensive aromatic and medicinal plant in the world. Saffron is mainly used as a spice for coloring and flavoring food. In addition, it is also used as an antioxidant and to treat cancer, depression, coughs, and infections (Alhasan, 2023).

One of the environmentally friendly methods that plays a role in improving plant productivity is the use of nano-biological fertilizers. One of the most effective biological control agents is the fungus *Trichoderma harzianum*, which has been developed into commercial biological control products and is used in fields and

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greenhouses (Harman et al., 2000). *Trichoderma* inhibits the enzymes responsible for disease, namely the enzymes that break down the cell walls of the plant host and cause infection. These include pectolytic, cutinolytic, and cellulolytic enzymes, which play an active role in breaking down the cell walls of the host (Elad, 2000). Biological agents produce many effective enzymes and biomaterials and are considered biological resistance agents due to their ability to interact directly with other species using several methods, including fungal parasitism, competition, biotransformation, and hyphal analysis (Kubicek & Harman, 2002).*Trichoderma* stimulates systemic or local resistance in plants, which is achieved through the production of inducers that activate plant defense responses. *Trichoderma* colonizes plant roots and promotes plant growth by increasing nutrient uptake (Oyesola et al., 2024).

The bacterial biological *Bacillus subtilis* is used as a cell factory to produce microbes for chemicals, enzymes, and antimicrobial substances for industry, agriculture, and medicine due to its highly efficient protein secretion system and adaptable metabolism (Su Yuan et al., 2020). In addition, it exhibits high antifungal activity against plant pathogenic fungi (Narmukhamedova et al., 2024). and has high resistance to environmental stress, effectively tolerating a variety of harsh conditions, such as acidic environments and high temperatures during the pelleting process (Lin et al.,2020). *B. subtilis* does not have an outer membrane or periplasm, which may significantly increase protein secretion efficiency (Souza et al., 2020). *B. subtilis* has a strong ability to express its own proteins and secrete them outside the cell (Kaspar et al., 2019).

Given the importance of saffron for its aesthetic and economic value, we decided to conduct this study, which aims to evaluate the effectiveness of biological agents (fungal and bacterial biological fertilizers) as environmentally and human-friendly agents and their effect on some vegetative, floral, and root growth parameters of saffron plants.

Materials and Methods

The study was conducted in the greenhouse of the Plant Production Technology Department at the Agricultural Technical College/Northern Technical University in 2024 to study The effect of biological factors represented by the fungus *Trichoderma harzianum* (T-22) and the bacterium *Bacillus subtilis*, two methods were used: immersing the corms and watering the cultivated soil, and their effect on some growth parameters of the saffron plant *Crocus sativus* L.

The treatments studied in the experiment were as follows:

1- Standard treatment (comparison) without any additions.

2- The fungal biological factor *Trichoderma harzianum* at a rate of 5 g/L.

3- The bacterial biological factor *Bacillus subtilis*.

Using two methods immersing the saffron corms for 20 minutes before planting in 12 cm pots containing sterilized soil sealed at a temperature of 121°C and pressure of 1 atm; and watering the sterilized soil in which the corms were planted at a rate of 10 ml per pot.

The results were tested using Duncan's multiple range test at a 5% probability level.

The following growth indicators were measured:

Growth indicators:

Vegetative growth characteristics

1- Number of days for vegetative bud emergence (days).

2 - Plant height (cm).

Floral growth characteristics

1- Number of days required for flower bud emergence (days).

2 - Number of days required for the first flower to open (days).

3- Fresh weight of stigma (g).

4- Dry weight of stigma (g)

Estimation of Peroxide Enzyme in Saffron Leaves (unit/ minute/gram fresh weight).

The samples were prepared by taking leaf samples weighing 0.5 g from each plant and crushing them after cleaning off any adhering dust, adding 10 ml of cold potassium phosphate buffer with a pH of 7 in a sterilized ceramic mortar, then filtering the solution through filter paper and subjecting it to centrifugation using a centrifuge at a speed of 4000 rpm for 10 minutes. The peroxidase enzyme was estimated by preparing a reaction mixture consisting of 1 ml of quercetin solution, 1 ml of hydrogen peroxide, and 1 ml of the prepared sample. The change in light absorption was monitored every 30 seconds for 3 minutes at a wavelength of 420 nm (Pittotti et al., 1994).

The results were recorded for three replicates of each treatment, and enzyme activity was calculated using the following equation:

$$\text{Number of enzyme units/ml} = \frac{\Delta A \text{ Device reading} \times 3}{0{,}001 \times \Delta t}$$

ΔA: Change in light absorption.Δ

Δt: Time taken for the change in absorption to occur.

One unit of enzyme is the amount of enzyme that causes an increase in light absorption of 0.01 units per minute at a wavelength of 420 nanometers.

Root growth characteristics

1. Number of corms.
2. Wet weight of corms (g).
3. Number of creams.
4. Wet weight of creams (g).

Results and discussion

Vegetative growth characteristics

The results of the statistical analysis in **Table 1** for vegetative growth characteristics showed significant differences between the comparison treatment and the other treatments in the number of days required for emergence, which was 26.56 days. The statistically significant superiority was observed in the biological factor treatment (T-22) using the immersion method for saffron corms, which reached 17.65 days. The second place was for the bacterial agent treatment using the immersion method for saffron corms, which did not differ significantly from the other biological factor treatments.

The same table shows the significant superiority of plant height with treatment (T-22) using the immersion and watering methods (8.75 and 7.40 cm), which differed significantly from the bacterial biological factor, while the shortest height was with the control treatment at 3.42 cm.

Floral growth characteristics

The results of the statistical analysis in **Table 2** show the significant superiority of biological fertilizers (fungal and bacterial) in the number of days required for the emergence of flower buds using both methods of application., immersion the corms and watering the soil in which they were planted. The best result in terms of the fewest number of days was obtained with treatment (T-22) using the watering method (89.77 days).With regard to the number of days required for the first flower to open, the same table shows significant differences between the biological treatments, the best of which was the biological resistance treatment (T-22) by immersion the corms, with an average of 125.6 days, while the comparison treatment recorded the highest number of days required for the first flower to open, at 135.0 days. This is due to the fact that *Trichoderma* fungi colonize plant roots and promote plant growth by increasing nutrient absorption (Oyesola et al.,2024). This is consistent with the findings of (Mahmood et al., 2023; Janowska et al., 2020).

With regard to the wet weight of the stigma, significant differences were observed between the biological treatments, with the fungal biological fertilizer treatment (T-22) and the two methods of application 0.088 and 0.085 g, respectively, and the biological factors differed from the comparison treatment and reached 0.021 g. As for the dry weight of the stigma, significant differences were observed between the biological fertilizer treatments (fungal and bacterial) and the comparison treatment, with the best weight being that of the fungal biological fertilizer treatment (T-22) using the watering method, weighing 0.009 g, and the lowest weight being that of the comparison treatment, weighing 0.002 g (Mahmood et al.,2023; Janowska et al., 2020).

Estimation of Peroxide Enzyme (unit/minute/gram fresh weight).

The results of the estimation of peroxidase enzyme units in saffron leaves **Table 3** showed that it plays an important role in the oxidation of phenolic substances and in the formation of toxic substances for pathogens as protection for the saffron plant from infection. There were

Table 1 - Effect of biological factors on vegetative growth characteristics of *Crocus sativus L.*

Treatments	Number of days for vegetative bud emergence (day)	plant height (cm)
Comparison treatment	26.65 a	3.42 c
Watering the planted soil (T-22)	22.24 b	7.40 a
Immersing the corms (T-22)	17.65 c	8.75 a
Watering the planted soil B. subtilis	22.45 b	5.10 b
Immersing the corms B. subtilis	20.55 b	5.35 b

Values with similar letters for each factor or its interactions individually do not differ significantly according to Dunkin's multiple range test under the 5% probability level.

Table 2 - Effect of biological factors on the flowering growth characteristics of *Crocus sativus L.*

Treatments	Number of days required for the flower bud to appear (day)	Number of days required for the first flower to bloom (day)	Wet weight of stigmas/g	dry weight of stigmas /g
Comparison treatment	89.77 b	135.0 a	0.021c	0.002 b
Watering the planted soil (T-22)	96.55 a	129.56 b	0.085 a	0.009 a
Immersing the corms (T-22)	96.62 a	125.6 c	0.088 a	0.007 a
Watering the planted soil B. subtilis	91.77 b	130.2 b	0.052 b	0.005 a
Immersing the corms B. subtilis	90.77 b	126.1 c	0.056 b	0.007 a

Values with similar letters for each factor or its interactions individually do not differ significantly according to Dunkin's multiple range test under the 5% probability level.

Table 3 - Effect of biological factors on the number of Peroxides enzyme units in *Crocus sativus L.* leaves.

Treatments	Peroxides (unit/minute/gram fresh weight)
Comparison treatment	0.011 d
Watering the planted soil (T-22)	0.076 b
Immersing the corms (T-22)	0.096 a
Watering the planted soil B. subtilis	0.073 b
Immersing the corms B. subtilis	0.076 b

Values with similar letters for each factor or its interactions individually do not differ significantly according to Dunkin's multiple range test under the 5% probability level.

Table 4 - Effect of biological factors on the characteristics of corms yield of *Crocus sativus L.*

Treatments	number of corms	wet weight of corms(g)	number of creams	wet weight of creams(g)
Comparison treatment	4.66 b	0.34 c	1.11 c	0.19 c
Watering the planted soil (T-22)	7.66 a	0.91 b	4.00 ab	0.35 ab
Immersing the corms (T-22)	7.33 a	1.17 ab	5.00 ab	0.36 ab
Watering the planted soil B. subtilis	7.33 a	1.17 ab	3.00 b	0.34 ab
Immersing the corms B. subtilis	7.66 a	1.46 a	5.33 a	0.42 a

Values with similar letters for each factor or its interactions individually do not differ significantly according to Dunkin's multiple range test under the 5% probability level.

significant differences between the biological agents, with the fungal biological control agent (T-22) treatment outperforming the others by 0.096 unit/minute/gram fresh weight, which was significantly different from the other treatments.

Characteristics of corm yield

The results of the statistical analysis in **Table 4** show that there are no significant differences between the biological treatments (fungal and bacterial) and the two methods of use in terms of the number of corms per saffron plant, which differed significantly from the comparison treatment, which had 4.66 corms. As for the wet weight of saffron corms, the same table shows that there are significant differences between the biological treatments, the highest being the bacterial biological fertilizer treatment using the immersion method at 1.46 g, and the lowest being the control treatment. As for the number of creams, the lowest was with the comparison treatment, which was 1.11 creams, and the highest was with the bacterial biological treatment using the immersion method, which was 5.33 creams. This did not differ significantly from the fungal fertilizer treatment (T-22) and the two methods of use, but it differed significantly from the bacterial fertilizer treatment for pots planted with saffron. From the same table, it can be seen that there were no significant differences between the bacterial and fungal biological treatments in the wet weight of the creams. The best results were obtained with the bacterial biological fertilizer using the immersion method for saffron corms, reaching 0.42 g, while the comparison treatment achieved the lowest wet weight for creams and differed significantly from the bacterial and fungal biological treatments by an average of 0.19 g (Mahmood et al., 2024 Sisodia et al., 2015).

Conclusion

The study showed a clear positive effect of bioagents (*Trichoderma harzianum* and *Bacillus subtilis*) in improving the growth and productivity of the crocus plant (*Crocus sativus L.*) under greenhouse conditions. Treatment with T-22 mushrooms for soaking corms was superior in reducing the number of days of vegetative and floral bud appearance, increasing plant height, splash and dry miasm weight, and peroxidase enzyme activity, and increasing the number and weight of corms and creams compared to the comparison treatment. The study recommends using these agents as an environmentally safe alternative to improve saffron production.

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