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The Effect of Mycorrhizal Fungi Inoculation and Glycine Amino Acid Spraying on Some Physiological Traits and Chemical Content of Basil Plant (*Ocimum basilicum* L.)

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Abstract :

This study was conducted in the Tarmiyah region during the year 2024 to investigate the effect of root mycorrhizal fungi inoculation at two levels (1 and 2 g/kg soil), along with a control treatment involving planting in regular soil only, and the effect of glycine amino acid spraying at two concentrations (150 and 250 ppm), in addition to a control treatment involving spraying with distilled water only. The study aimed to assess some physiological traits and the chemical content of the basil plant (Ocimum basilicum). The statistical analysis was performed using a Completely Randomized Design (CRD). The results indicated that mycorrhizal fungi inoculation at both levels significantly improved the following traits: plant height (cm), number of branches per plant, dry weight of the plant (g), dry matter percentage (%), and chlorophyll a content (%), respectively. Moreover, the second level of mycorrhizal inoculation (250 ppm) showed superiority in the following traits: inflorescence length (cm), chlorophyll b content (%), phenol content (mg/100 g), flavonoid content (mg/100 g), alkaloid content (%), glycoside content (%), saponin content (%), and tannin content (%), respectively. Regarding the glycine amino acid treatment, spraying at the concentration of 250 ppm led to significant improvements in the following traits: number of branches per plant, dry weight of the plant (g), dry matter percentage (%), inflorescence length (cm), chlorophyll b content (%), phenol content (mg/100 g), flavonoid content (mg/100 g), alkaloid content (%), glycoside content (%), saponin content (%), and tannin content (%), respectively.

Keywords: Mycorrhiza, Glycine, Basil, Amino Acid.

تأثير التلقيح بفطريات المايكورايزا والرش بالحامض الاميني الكلايسين في بعض الصفات الفسلجية والمحتوى الكيميائي لنبات الريحان *Ocimum basilicum*

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مستخلص:

اجري هذا البحث في منطقة الطارمية خلال العام 2024 لدراسة تأثير كل من التلقيح بفطريات المايكورايزا الجذرية بمستويين هما (1 ، 2) غم/ كغم تربة بالإضافة الى معاملة المقارنة التي تضمنت الزراعة بالتربة العادية فقط، ودراسة تأثير الرش بالحامض الاميني الكلايسين بمستويين ايضاً هما ppm (150 ،250) بالإضافة الى معاملة المقارنة التي تضمنت الرش بالماء المقطر فقط في بعض الصفات الفسلجية والمحتوى لكيميائي لنبات الريحان . اجري التحليل الاحصائي باستعمال التصميم العشوائي الكامل CRD . اظهرت النتائج تفوق المعاملة بالفطر بكلا المستويين بكل من صفات (ارتفاع النبات (سم) ، عدد افرع النبات (فرع/ نبات) ، الوزن الجاف للنبات (غم) ، النسبة المئوية للمادة الجافة (٪) ، محتوى كلوروفيل a ٪) بالتتابع، في حين تفوقت المعاملة بالفطر بالمستوى الثانيppm (250) بكل من صفات (طول الشمراخ الزهري (سم)، محتوى كلوروفيل b (٪) ، محتوى الفينولات (mg/100gm) ، محتوى الفلافونويدات (mg/100gm) ، محتوى القلويدات (٪)، محتوى الكلوكوسيدات (٪)، محتوى السابونينات (٪) ومحتوى التانينات ٪) بالتتابع، اما المعاملة بالحامض الاميني الكلايسين فقد تفوق الرش بالمستوى ppm(250) بصفات (عدد افرع النبات (فرع/ نبات)، الوزن الجاف للنبات (غم) ، النسبة المئوية للمادة الجافة (٪) ، طول الشمراخ الزهري (سم)، محتوى كلوروفيل b (٪) ، محتوى الفينولات (mg/100gm) ، محتوى الفلافونويدات (mg/100gm) ، محتوى القلويدات (٪) ، محتوى الكلوكوسيدات (٪) ، محتوى السابونينات (٪) ومحتوى التانينات ٪) بالتتابع .

الكلمات المفتاحية : مايكورايزا ، كلايسين ، الريحان ، حامض اميني .

Introduction

Mycorrhiza refers to a group of fungi that form symbiotic associations with plant roots, resulting in mutual benefits for both the plant roots and the mycorrhizal fungi. This symbiosis enhances the external root surface area for nutrient absorption, facilitating the uptake of water, nutrients, and minerals from the soil, particularly phosphorus. Consequently, this interaction promotes plant growth, enhances resistance to soil-borne pathogenic microorganisms, and improves tolerance to high salinity levels and extreme temperatures. In return, the host plant supplies the fungi with essential energy compounds (Abdullah and Noor, 2013; Habibzadeh and Abedi, 2014). Sweet basil (Ocimum basilicum L.), a member of the Lamiaceae family, includes a wide range of leafy and aromatic plants distributed globally (Paton, 1992). Basil essential oil is known for its aromatic properties, and the composition and quantity of its volatile compounds vary depending on the geographical location of cultivation. The oil contains several bioactive compounds, such as linalool, estragole (methyl chavicol), geraniol, eugenol, methyl cinnamate, myrcene, and α-pinene, among others (Gaydou et al., 1989). In recent years, there has been increasing interest in adopting modern agricultural techniques, including foliar nutrition, to enhance plant growth. Among these advancements is the use of biofertilizers, such as phosphorus-solubilizing mycorrhizal fungi, to promote plant development.

Amino acids are the fundamental building blocks responsible for protein synthesis. These organic carboxylic acids consist of an amino group (NH_2), a carboxyl group (COOH), and an alkyl group (R), known as the side chain, all attached to the α-carbon atom. One such amino acid is glycine, which plays a crucial role in enhancing photosynthesis efficiency by promoting chlorophyll formation and encouraging vegetative growth. Furthermore, glycine contributes to pollination and fruit formation (Baqir et al., 2019).

Based on the aforementioned considerations, this study aims to investigate the physiological effects of mycorrhizal fungi and glycine amino acid on the physiological responses and chemical composition of basil plants.

Materials and Methods

Experimental Site and Design

The experiment was conducted in 2024 in the Al-Abayji district to study the effect of inoculation with two levels of arbuscular mycorrhizal fungi and two levels of the amino acid glycine on certain physiological traits and the chemical composition of basil plants. The experiment was designed using a Completely Randomized Design (CRD) with three replications .

Planting Procedure

Planting was carried out on March 1, 2024, using plastic pots with a height of 30 cm and a diameter of 30 cm. The experimental treatments were as follows:

1. Factor A (Mycorrhizal Inoculation):

This factor included two levels of arbuscular mycorrhizal fungi inoculation, in addition to a control treatment with regular soil (without inoculation), represented as (0, 1, 2) g/kg of soil.

2. Factor B (Glycine Application):

This factor involved foliar spraying with two concentrations of the amino acid glycine, along with a control treatment using distilled water only, represented as (0, 150, 250) ppm.

After the experiment concluded and the plants reached the maturity stage, the following parameters were measured:

Measured Traits

1. Plant Height (cm):

Plant height was measured from the soil surface to the highest point of each plant using a measuring tape.

2. Number of Branches (branches/plant):

The total number of primary .branches per plant was counted

3. Plant Dry Weight (g):

The plants were placed in perforated paper bags and dried in an electric oven at 70°C for 48 hours until a constant weight was achieved. The dry weight of each plant was then determined using a precision balance.

4. Percentage of Dry Matter (%):

The percentage of dry matter was calculated using the following formula:

$$\text{Percentage of Dry Matter} = \frac{\text{Dry Weight}}{\text{Fresh weight}} \times 100$$

5. Inflorescence Length (cm) :

The length of the floral spike was measured from its point of attachment to the stem to its terminal end using a measuring tape.

6. Chlorophyll Content (Chlorophyll a and Chlorophyll b):

The chlorophyll content in fresh leaves was estimated following the method described by Ranganna (1977). A random sample of 0.25 g of fresh leaves was collected, chopped, and ground using a ceramic mortar in the presence of 10 ml of 80% acetone. The extract was then separated using a centrifugal device operated at 500 rpm for 15 minutes.

After centrifugation, 1 ml of the supernatant was transferred to a test tube, and the volume was adjusted to 10 ml with acetone. The chlorophyll content was measured using a spectrophotometer at wavelengths of 663 nm and 643 nm. The device was initially calibrated with acetone as a blank, and readings were taken according to the specified wavelengths.

The chlorophyll content was calculated using the following equations:

- Chlorophyll a:
- Chlorophyll b:

Where the constants (2.9, 5.10, 12.25, and 21.5) are specific coefficients used in chlorophyll estimation.

7. Estimation of Bioactive Compounds

The analysis was conducted in the laboratories of the Ministry of Science and Technology - Environment and Water Directorate, using a Shimadzu 2010 gas chromatography system, manufactured in Japan. The system was equipped with a flame ionization detector (FID) and utilized a capillary column of type DM-5Ms, with the following dimensions: 30 m × 0.25 µm × 0.25 mm.

The injection and detector temperatures were set at 280°C and 340°C, respectively. The column temperature was programmed to increase gradually from 100°C to 300°C at a rate of 10°C per minute. Inert nitrogen gas was used as the carrier gas at a pressure of 100 kPa, following the method described by Hcini (2013).

This method was employed to estimate the content of various bioactive compounds, including:

- Phenolic compounds
- Flavonoids
- Alkaloids

- Glycosides
- Saponins
- Tannins

The concentrations of these bioactive compounds were determined based on their retention times on the separation column, using the following sample concentration formula:

$$\text{Concentration of the compound} = \frac{\text{Concentration of the standard solution} \times \text{sample area}}{\text{Standard solution area}} \times \frac{\text{Dilution factor}}{\text{Sample weight}}$$

Statistical Analysis

The results were analyzed using a simple Completely Randomized Design (CRD) following the method outlined by Al-Rawi and Khalaf Allah (1980), with the assistance of the SAS statistical analysis software. The significance of differences among treatments was evaluated using Duncan's Multiple Range Test (DMRT) at a 0.05 probability level.

Results and Discussion

Plant Height:

The results presented in Table 1 demonstrate that the second and third levels of mycorrhizal fungi inoculation (1 and 2 g/kg) significantly increased plant height, recording 51.65 cm and 51.22 cm, respectively, compared to the control treatment, which exhibited the shortest plant height of 38.15 cm. This increase can be attributed to the enhanced nutrient uptake efficiency resulting from mycorrhizal colonization. The expansion of the hyphal network facilitates greater absorption of nutrients, thereby promoting plant growth. These findings are consistent with those reported by Ali et al. (2007).

Regarding the foliar application of the amino acid glycine, no significant difference was observed between the two application levels and the control treatment.

However, the interaction between the two factors revealed a significant effect. The highest plant height of 58.93 cm was recorded for the interaction between the second level of mycorrhizal inoculation and the control treatment (distilled water) for glycine application.

Table (1) Plant height (cm)

Factor A	A * B			B / A
	b3	b2	b1	
38.15 b	40.30 de	41.96 c-e	32.20 e	a1
51.65 a	46.86 b-d	49.16 a-d	58.93 a	a2
51.22 a	53.63 ab	47.40 b-d	52.63 a-c	a3
	46.93 a	46.17 a	47.92 a	Factor B

Number of Plant Branches

The results presented in Table 2 indicate that the second and third levels of mycorrhizal fungi inoculation significantly increased the number of primary branches per plant, recording 8.61 and 8.72 branches per plant, respectively, compared to the control treatment, which showed the lowest number of branches (7.16 branches per plant). This increase can be attributed to the enhanced nutrient absorption efficiency facilitated by mycorrhizal colonization. The extended hyphal network significantly increases the root absorption area, and the efficiency of nutrient uptake by hyphae surpasses that of root hairs. This improvement positively impacted the plant's metabolic processes, ultimately leading to an increase in branching. These findings align with the results reported by Ali (2007).

Moreover, foliar application of the amino acid glycine at the second level resulted in the highest number of branches, reaching 9.44 branches per plant, while no significant difference was observed between the control treatment and the first level of glycine application, both of which produced fewer branches per plant.

Regarding the interaction between the two factors, the combination of first-level mycorrhizal inoculation and second-level glycine application produced the highest number of branches, with 10.50 branches per plant, compared to the interaction between the control treatments of both factors, which resulted in the lowest number of branches (4.83 branches per plant).

Table (2) Number of plant branches (branch/plant)

Factor A	A * B			B / A
	b_3	b_2	b_1	
7.16 b	8.33 bc	8.33 bc	4.83 e	a_1
8.61 a	10.50 a	6.38 d	8.50 b	a_2
8.72 a	9.50 ab	7.16 cd	9.50 ab	a_3
	9.44 a	7.44 b	7.61 b	**Factor B**

Plant Dry Weight

The results presented in Table 3 reveal that the first and second levels of mycorrhizal fungi inoculation significantly increased the plant dry weight, recording 2.17 g and 2.19 g, respectively, compared to the control treatment, which exhibited the lowest dry weight of 1.45 g. This increase can be attributed to the enhanced absorption of essential nutrients such as nitrogen, phosphorus, and potassium facilitated by mycorrhizal colonization, ultimately promoting vegetative growth and increasing dry weight (Kartani and Al-Taie, 2011).

Furthermore, the second level of amino acid glycine application resulted in the highest plant dry weight, reaching 2.48 g, while the control treatment recorded the lowest dry weight of 1.78 g. This improvement can be explained by the role of glycine in enhancing cellular capacity to absorb water and dissolved nutrients from the growth medium, thereby promoting vegetative growth and subsequently increasing plant dry weight.

The interaction between the two factors demonstrated a significant effect. The combination of the first level of mycorrhizal inoculation and the second level of glycine application produced the highest dry weight of 2.72 g per plant. In contrast, the interaction between the control treatments of both factors resulted in the lowest dry weight, measuring 0.60 g. This increase can be attributed to the synergistic effect of mycorrhizal fungi and glycine in enhancing branch formation, which in turn increases the number of leaves, ultimately contributing to higher plant dry weight.

Table (3) Plant dry weight (g)

Factor A	A * B			B / A
	b_3	b_2	b_1	
1.45 b	2.21 c	1.80 d	0.60 f	a_1
2.17 a	2.72 a	1.31 e	2.49 b	a_2
2.19 a	2.53 b	1.80 d	2.25 c	a_3
	2.48 a	1.64 c	1.78 b	Factor B

Percentage of Dry Material

The results presented in Table 4 indicate that the first and second levels of mycorrhizal fungi inoculation significantly increased the percentage of dry matter, recording 14.92% and 14.90%, respectively, compared to the control treatment, which exhibited a lower dry matter percentage of 12.91%.

Similarly, the second level of amino acid glycine application resulted in the highest dry matter percentage, reaching 14.83%, compared to both the control treatment and the first level of glycine application, which recorded 13.85% and 14.06%, respectively. However, the difference between these two treatments was not statistically significant.

Regarding the interaction between the two factors, the combinations of the second level of mycorrhizal inoculation with both the first and second levels of glycine and the control treatment with the second level of glycine produced the highest dry matter percentages of 15.15%, 15.16%, and 15.41%, respectively. In contrast, the interaction between the control treatments of both factors resulted in the lowest dry matter percentage, measuring 10.64%.

The significant increase in plant dry weight and the number of branches due to mycorrhizal inoculation and glycine application contributed to the higher dry matter percentage, which reflects the enhanced efficiency of the photosynthetic process.

Table (4) Percentage of dry material (%)

Factor A	A * B			B / A
	b_3	b_2	b_1	
12.91 b	14.17 b	13.91 b	10.64 c	a_1
14.92 a	15.15 a	14.12 b	15.50 a	a_2
14.90 a	15.16 a	14.13 b	15.41 a	a_3
	14.83 a	14.06 b	13.85 b	**Factor B**

Inflorescence Length

The results presented in Table 5 show that the second level of mycorrhizal fungi inoculation significantly increased the inflorescence length, recording the highest value of 14.38 cm, while the control treatment produced the shortest inflorescence length of 10.55 cm. This increase can be attributed to the enhanced plant height resulting from improved water and nutrient uptake facilitated by mycorrhizal colonization, ultimately promoting the growth of longer inflorescences.

Regarding the amino acid glycine application, the second level of foliar spraying resulted in the longest inflorescence, measuring 14.22 cm, whereas the first level of glycine application produced a shorter inflorescence length of 11.22 cm.

Concerning the interaction between the study factors, the combination of the first level of mycorrhizal inoculation with the control treatment of glycine produced the longest inflorescence, measuring 15.50 cm. In contrast, the interaction between the control treatments of both factors resulted in the shortest inflorescence length, measuring only 8.0 cm.

Table (5) Flower stalk length (cm)

Factor A	A * B			B / A
	b_3	b_2	b_1	
10.55 c	14.33 bc	9.33 e	8.0 f	a_1
13.44 b	14.16 c	10.66 d	15.50 a	a_2
14.38 a	14.16 c	13.66 c	15.33 ab	a_3
	14.22 a	11.22 c	12.94 b	**Factor B**

Chlorophyll a Content

The results presented in Table 6 indicate that the first and second levels of mycorrhizal fungi inoculation significantly increased Chlorophyll a content, recording 23.35% and 23.15%, respectively, compared to the control treatment, which exhibited the lowest chlorophyll a content of 18.29%. This increase can be attributed to the role of mycorrhizal fungi in enhancing the absorption of essential nutrients, particularly magnesium, which is a key component of chlorophyll structure, thereby increasing the plant's chlorophyll a content.

Regarding the foliar application of glycine, the first level of application resulted in the highest chlorophyll a content, reaching 22.64%, while no significant difference was observed between the control treatment and the second level of glycine application, both of which recorded the lowest chlorophyll a content. This outcome can be explained by the fact that glycine acts as a fundamental building block for chlorophyll synthesis within the plant. It enhances chlorophyll concentration, thereby optimizing the photosynthetic process. Additionally, glycine functions as a biostimulant, promoting nutrient uptake and enhancing nutrient use efficiency. It also serves as a chelating agent for essential micronutrients such as iron, zinc, copper, magnesium, and calcium, facilitating their absorption and translocation within the plant, ultimately increasing chlorophyll content (Kandi et al., 2016).

Regarding the interaction between the two factors, the combination of the first level of mycorrhizal inoculation and the first level of glycine application resulted in the highest chlorophyll a content, measuring 24.68%, while the interaction between the control treatments of both factors produced the lowest chlorophyll a content of 17.42%.

Table (6) Chlorophyll a content (%)

Factor A	A * B			B / A
	b_3	b_2	b_1	
18.29 b	18.12 e	19.33 d	17.42 e	a_1
23.35 a	23.42 b	24.68 a	21.96 c	a_2
23.15 a	22.10 c	23.90 ab	23.45 b	a_3
	21.21 b	22.64 a	20.94 b	Factor B

Chlorophyll b Content

The results presented in Table 7 indicate that the second level of mycorrhizal fungi inoculation produced the highest chlorophyll b content, reaching 12.32%, while the control treatment exhibited the lowest content, measuring 9.94%.

Regarding the foliar application of the amino acid glycine, the second level of glycine application resulted in the highest chlorophyll b content, reaching 12.22%, compared to the control treatment, which recorded the lowest chlorophyll b content of 9.87%.

Concerning the interaction between the two factors, the combination of the first level of mycorrhizal inoculation and the first level of glycine application resulted in the highest chlorophyll b content, measuring 13.76%. In contrast, the interaction between the first level of mycorrhizal fungi and the control treatment of glycine yielded the lowest chlorophyll b content, measuring 7.79%.

Table (7) Chlorophyll b content (%)

Factor A	A * B			B / A
	b_3	b_2	b_1	
9.94 c	12.43 b	8.42 de	8.99 d	a_1
11.46 b	12.83 b	13.76 a	7.79 e	a_2
12.32 a	11.42 c	12.72 b	12.82 b	a_3
	12.22 a	11.63 b	9.87 c	Factor B

Content of Bioactive Chemical Compounds

The results presented in Tables 8, 9, 10, 11, 12, and 13 demonstrate that the second level of mycorrhizal fungi inoculation produced the highest content of the bioactive compounds (phenols, flavonoids, alkaloids, glycosides, saponins, and tannins), recording 155.16 mg/100 g and 118.92 mg/100 g, and 25.80%, 21.51%, 24.53%, and 12.48%, respectively. In contrast, the control treatment exhibited the lowest content, measuring 134.25 mg/100 g and 95.02 mg/100 g, and 14.83%, 9.10%, 11.36%, and 5.05%, respectively.

This increase can be attributed to the positive effects of mycorrhizal fungi in enhancing plant growth by secreting growth-regulating substances and improving the ability of soil microorganisms to fix atmospheric nitrogen. Mycorrhizae also play a significant role in enhancing the plant's ability to efficiently absorb nutrients from supplemented soil sources. Consequently, this increased nutrient uptake stimulates the biosynthesis of bioactive compounds in plant tissues, leading to their higher accumulation.

Regarding the foliar application of glycine, the second level of glycine application resulted in the highest content of bioactive compounds, recording 155.70 mg/100 g and 119.40 mg/100 g, and 26.25%, 22.45%, 26.14%, and 13.75%, respectively. In contrast, the control treatment for glycine application exhibited the lowest content, measuring 134.43 mg/100 g and 94.09 mg/100 g, and 14.86%, 9.62%, 11.31%, and 4.54%, respectively. This increase is attributed to the role of glycine in stimulating the plant's physiological and metabolic processes, particularly photosynthesis, which enhances the accumulation of bioactive compounds. These findings align with those reported by Salama and Yousef (2015).

Concerning the interaction between the two factors, the combination of the second level of mycorrhizal inoculation and the second level of glycine application produced the highest content of bioactive compounds, recording 166.50 mg/100 g and 130.10 mg/100 g, and 32.50%, 30.10%, 34.80%, and 18.86%, respectively. In contrast, the interaction between the control treatments of both factors resulted in the lowest content of bioactive compounds, measuring 125.83 mg/100 g and 85.60 mg/100 g, and 11.40%, 6.50%, 8.86%, and 2.73%, respectively.

Table (8) Phenols content (mg/100 gm)

Factor A	A * B			B / A
	b_3	b_2	b_1	
134.25 c	140.46 f	136.46 g	125.83 i	a_1
147.24 b	160.13 b	149.53 d	132.06 h	a_2
155.16 a	166.50 a	153.60 c	145.40 e	a_3
	155.70 a	146.53 b	134.43 c	Factor B

Table (9) Flavonoids content (mg/100 gm)

Factor A	A * B			B / A
	b_3	b_2	b_1	
95.02 c	101.60 f	97.86 g	85.60 i	a_1
109.28 b	126.50 b	112.50 d	88.86 h	a_2
118.92 a	130.10 a	118.86 c	107.80 e	a_3
	119.40 a	109.74 b	94.09 c	Factor B

Table (10) Alkaloids content (%)

Factor A	A * B التداخل			B / A
	b_3	b_2	b_1	
14.83 c	17.40 f	15.70 g	11.40 i	a_1
21.65 b	28.86 b	22.40 d	13.70 h	a_2
25.80 a	32.50 a	25.40 c	19.50 e	a_3
	26.25 a	21.16 b	14.86 c	Factor B

Table (11) Glucoside content (%)

Factor A	A * B التداخل			B / A
	b_3	b_2	b_1	
9.10 c	11.40 f	9.40 g	6.50 i	a_1
17.04 b	25.86 b	17.40 d	7.86 h	a_2
21.51 a	30.10 a	20.10 c	14.50 e	a_3
	22.45 a	15.63 b	9.62 c	Factor B

Table (12) Saponins content (%)

Factor A	التداخلA * B			B / A
	b_3	b_2	b_1	
11.36 c	13.83 f	11.40 g	8.86 i	a_1
19.82 b	29.80 b	19.80 d	9.86 h	a_2
24.53 a	34.80 a	23.60 c	15.20 e	a_3
	26.14 a	18.26 b	11.31 c	Factor B

Table (13) Tannins content (%)

Factor A	A * B التداخل			B / A
	b_3	b_2	b_1	
5.05 c	6.60 f	5.83 g	2.73 i	a_1
9.50 b	15.80 b	9.20 d	3.50 h	a_2
12.48 a	18.86 a	11.20 c	7.40 e	a_3
	13.75 a	8.74 b	4.54 c	Factor B

Conclusions:

1- There is a significant effect of the second level of Mycorrhiza fungi (2 g/kg of soil) on most physiological traits.

2- There is a physiological effect of the second level (ppm 250) of the amino acid glycine.

3- A significant response to the content of active chemical groups with increasing levels of the two factors.

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