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Influence of Tryptophan and Glycine Acids on Growth and Productivity of Fennel under Different Organic Fertilizer Levels

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I[n](#page-0-0)troduction

Foeniculum vulgare Mill., commonly referred to as fennel, is a medicinal and aromatic plant belonging to the family Umbelliferae (Apiaceae). Various parts of the fennel plant, including its leaves, stem, root, and particularly the fruits, possess aromatic properties and have been utilized in numerous applications (Badgujar et al.,

This research aimed to improve the growth, productivity, and volatile oil production of fennel (Foeniculum vulgare, Mill.) plants. An experiment was conducted to evaluate the effects of farmyard manure (FYM) and amino acid mixtures (tryptophan and glycine) at different concentrations on fennel plants and their interaction parameters. The highest plant height was recorded with the application of 40 $m³$ ha⁻¹ FYM. Significant effects due to the applied organics were observed on the number of branches plant-1, total dry weight plant-1, number of umbels plant-1, fruit yield, and volatile oil yield ha-1. When compared with the abundance of other amino acids under study, fennel plants sprayed with 300 ppm $T +$ glycine showed an increase in the above parameters. The best productivity and volatile oil was achieved in fennel plants grown with organic fertilizer $(40 \text{ m}^3 \text{ ha}^{-1})$, including foliar spray of amino acids at a concentration of 300 ppm. The gas chromatography (GC/MS) analysis of fennel oil showed that the main compounds were estragole, D-limonene, L-fenchone, and anethole, which had the highest proportions of volatile oil compositions compared to other compounds. This study concluded that using organic fertilizers with foliar application and amino acids had positive effects on fennel growth, productivity, and volatile oil compounds.

> 2014). The chemical constituents of fennel contribute to its medicinal properties (Kooti et al., 2015). Globally, fennel is employed to treat a wide array of ailments, including fever, flatulence, insomnia, renal disorders, cancer, stomachaches, liver discomfort, and abdominal pain (Choi and Hwang, 2004; Rather et al., 2012).

> Plant nutrients play a crucial role in enhancing

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plant productivity. Organic manure, when added to soil, is broken down by microbes into minerals, releasing nutrients that improve the soil's cation exchange capacity and water retention (Kramer and Boyer, 1995). The addition of farmyard manure (FYM) enhances the chemical and physical properties of soils. Due to its organic matter content, comprising macro- and microelements, amino acids, sugars, and organic acids, FYM promotes soil fertility (Hamza et al., 2006). Furthermore, Ibrahim et al. (2006) demonstrated that applying FYM at a rate of 16 m^3 per feddan increased vegetative growth and fruit yield per feddan in both fennel and caraway plants. Similarly, Abdelbaky et al. (2023) reported that among the seventeen identified components of fennel fruit essential oil, fenchone, transanethole, limonene, and estragole were highly abundant in FYM-treated plants. Al-Fraihat et al. (2011) indicated that the application of FYM resulted in maximum productivity in marjoram plants. More recently, Al-Fraihat et al. (2023a) found that high rates of organic fertilizers improved the growth, yield, and oil production of black cumin (Nigella sativa L.) and coriander (Coriandrum sativum L.).

Amino acids are vital in catalyzing secondary metabolic reactions in plants (Pratelli and Pilot, 2014). Tao et al. (2008) demonstrated that tryptophan is associated with the synthesis of camalexin, auxins, phenylpropanoids, phytoalexins, and other related natural compounds in plants. El-Awadi and Hassan (2010) observed significant increases in seed number per umbel, number of umbels per plant, and seed weight per umbel when the tryptophan concentration was set at 100 mg L-1. Glycine also plays a key role in plant growth and chlorophyll synthesis, facilitating the absorption of Zn, Fe, Cu, and Mn (Ghasemi et al., 2013). With a molar mass of 75 g mol-1, glycine is classified as a proteinogenic amino acid and a minor biological amino acid (Souri and Hatamian, 2019). The application of glycine at a concentration of 10 mg L-1 in nutrient solutions has been shown to enhance plant growth and the nutritional quality of coriander (Al-Fraihat et al., 2023c; Mohammadipour and Souri, 2019). Similarly, Ali and Sallam (2022) demonstrated that using glycine or tryptophan at a high concentration (150 ppm) significantly improved the essential oil percentage in caraway compared to the control. Al-Fraihat et al. (2023b) further indicated that the application of amino acids (L-tryptophan and glutamine) resulted in enhanced growth of rosemary (Rosmarinus officinalis L.).

This study aimed to evaluate the effects of varying farmyard manure rates and the concentration of amino acids (tryptophan and glycine) on the growth, fruit yield components, and essential oil production of fennel plants (Foeniculum vulgare Mill.) (Shahhoseini et al., 2020).

Material and Methods

A field experiment was conducted over two consecutive seasons, 2020–2021 and 2021–2022, at the experimental farm in the Ajloun region of Jordan. The aim was to investigate the effects of organic fertilizers, specifically farmyard manure (applied at rates of 0, 20, 30, and 40 m^3 ha⁻¹), on fennel growth, yield, and volatile oil production. Additionally, the experiment assessed the impact of amino acid mixtures, such as tryptophan and glycine (applied at concentrations of 0, 100, 200, and 300 parts per million), as well as their interactions on these variables.

Before planting, the physical and chemical properties of the experimental soil were evaluated using methods outlined by Black (1965) and Page et al. (1982). Soil samples were taken from three random locations at a depth of 0–30 cm. The soil analysis was conducted at the National Center for Agricultural Research, Department of Laboratories in Jordan. The results of the soil analysis are presented in Table 1, followed by the nutritional composition of the analyzed fennel fruits, as shown in Table 2.

Experimental design and tested treatments

This experiment followed a randomized complete block design (RCBD) with three replicates, using a split-plot distribution. The main plots were farmyard manure rates $(0, 20, 30, 40, 40, m³$ ha⁻¹. A mixture of amino acids tryptophan and glycine at concentrations (0, 100, 200, and 300 ppm) were assigned as sub-plot. Fennel fruits were cultivated on 5 November of the bathing season. Amino acid treatments were sprayed at regular interval times of 45, 60, and 90 d from the sowing date.

Each sub-plot 3.0×3.0 m contained 5 rows each one 60 cm apart. The spacing between plants was 30 cm. After 33 d following the initial planting, the plants underwent thinning, producing two plants hill⁻¹. Data recorded in the first week of May included plant height (cm), number of branches plant-1, fresh and dry weights (g). It also included number of umbels plant⁻¹, fruit yield plant⁻¹ (g), and fruit yield ha⁻¹ (kg). All of them in addition to volatile oil percentage were calculated in line with to Guenther (1961). Then, volatile oil yield plant- 1 (mL), and volatile oil yield ha -1 (L) were computed. Before planting, mature cattle manure was put into the soil and mixed in. This was done to get the soil prepared for farming.

Table 1. Experimental soil analysis of the research area.												
		ppm			$Mg L^{-1}$		$\frac{0}{0}$					
рH	EC (ds m ⁻¹) K		Ca	Mg			Cl Na HCO3 O.M		Clav	Silt	Sand	Texture
8.38	0.52	473	2.82	3.18 3.4 0.8			-1.6	3.4	52.0	29.3	18.7	Clay

Table 2. Chemical analysis of farmyard manure in 2020-2021 and 2021-2022.

Volatile oil insulation

To extract volatile oil, fruits from each treatment were gathered over both seasons and weighed. Then, 100 g of each duplicate was handled for three h during hydrodistillation (HD) using a Clevenger-type apparatus (Clevenger, 1928). Volatile oil content was calculated as a relative percentage (v/w). Likewise, total EO was calculated as mL 100 plants-1 using dry weight. VOs taken out from A. abrotanum were gathered during the two seasons of each handling and dried on anhydrous sodium sulfate for chemical identification.

Gas chromatography and mass spectrometry (GC–MS) analysis

The Agilent 8890 GC System was leveraged for the analysis of the samples. It was linked to an Agilent 5977B GC/MSD mass spectrometer, which was fitted with an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The oven was initially intended to operate at 50 °C, with a 5 °C min-1 increase in temperature from 50 to 220 °C. At a rate of 20 °C min⁻¹, the temperature was then raised once more, from 220 °C to 280 °C. The temperature was kept at 280 °C for five min. The carrier gas, helium, was made use of at a flow rate of 1.0 mL min-1. A gas chromatograph (GC) was used to analyze 1 μL of the dissolved volatile oil (30 μL of essential oil mL-1 of diethyl ether). The split ratio of the GC was set to 1:50. At injection, the temperature was

230 °C. Using electron impact (EI) mode, which has a scan range of m/z 39 to 500 amu, mass spectra were obtained at an energy of 70 electron volts (eV) (Araghi, 2019; Taghizadeh et al., 2021). An experimental method was used on a split-plot design with RCBD. Gomez and Gomez's (1984) methodology was followed in the evaluation of the data. The statistical software package, Statistics Version 9, was utilized to compare the derived means (Analytical Software, 2008).

Results

Plant growth parameters

The results presented in Table 3 demonstrate that fennel growth and development were significantly higher at all farmyard manure (FYM) application rates (20, 30, and 40 m^3 ha⁻¹) in both seasons, compared to the control. The highest values for plant height, number of branches per plant, total fresh weight, and total dry weight per plant were observed at the highest FYM rate (40 m³ ha-1) during the 2020–2021 and 2021–2022 seasons. The application of organic fertilizers not only provided essential nutrients for plant nutrition but also contributed to the long-term sustainability of agricultural ecosystems (Mącik et al., 2020; Shaji et al., 2021). Organic fertilizers, derived from natural sources, supply necessary nutrients to plants.

Similarly, Massoud et al. (2019) reported that the growth of Petroselinum sativum (as measured by plant height, fresh weight, and dry weight) was enhanced with increasing rates of FYM at six tons per feddan, reaching maximum values. In contrast, Abd El-Kafie et al. (2020) found that coriander plants fertilized with organic fertilizers

exhibited greater plant height and branch number compared to those fertilized with synthetic fertilizers such as FYM.

FYM = Farmyard manure (20, 30 and 40 m³ ha⁻¹) and AM = Amino acids (100, 200, and 300 ppm).

According to Table 3, applying an amino acid mixture (tryptophan and glycine) at a high concentration (300 ppm) to fennel plants led to a significant improvement in plant height (123.58 and 126.42 cm). It also increased the number of branches plant-1 (10.92 and 13.67 branches),

total fresh (169.58 and 177.83 g plant⁻¹), and dry weights $(40.50$ and 44.98 g plant⁻¹). They contrasted with the control and the other concentrations being investigated in the first and second seasons, respectively. Generally, increasing amino acid concentrations gradually increased fennel growth parameters in both seasons. Moreover, certain amino acids applied topically may have positive impacts on vegetative development and yield (Sadak et al., 2015; Shams et al., 2016). Likewise, compared to the control, Soliman et al. (2023) indicated that spraying glycine at 200 and 400 ppm concentrations considerably boosted the vegetative growth of cumin plants, as measured by plant height, branch number plant-1, and fresh and dry weights of cumin.

Table 3 showed that spraying fennel plants thrice season-1 with amino acids at 300 ppm concentration + 40 m^3 ha⁻¹ of FYM recorded increased values. These increased values in plant growth parameters contrasted to other composite coefficients used in study coefficients.

Plant height, branch count, and fresh and dry weights plant-1 were all gradually increased in both seasons by increasing amino acid concentrations under any rate of fiber wasting management. As mentioned before, plant growth is generally enhanced by the use of amino acids and organic fertilizer separately. Yet, when combined, their impacts can be even more pronounced, producing the tallest fennel plants, the most branches plant-1, and the heaviest fresh and dry weights plant-1.

Yield and its constituents

Table 4 show that using FYM fertilization treatments at a high rate $(40 \text{ m}^3 \text{ ha}^{-1})$ significantly gave the highest values in umbels number plant-1 (29.42-33.25 umbels). It also the highest values in fruit yield fennel plant⁻¹ (39.33-42.50 g plant⁻¹) and fruit yield ha⁻¹ (3186.7-3400.0 kg ha⁻¹); being contrasted to control and the lowest rates in both seasons, respectively. Likewise, all FYM rates (20, 30, and 40 $m³$ ha⁻¹) significantly increased yield components of fennel (Foeniculum vulgare Mill) contrasted to control during the two seasons. Following El-Sayed et al. (2017), the use of 100% organic fertilizer resulted in the greatest umbel count, umbel diameter, and umbel number dill plant-1. Moreover, when contrasted with other treatments for the dried ripe fruits, the 100% organic fertilizer treatment produced the finest results in terms of fruit weight (g plant-1) and fruit output (t fed-1). Kumar et al. (2019) showed the maximum values for several different order umbels, seed yield, and biological yield of Daucus carota plants. They were recorded with FYM at 20 t ha⁻¹ in both years.

Table 4 showed increases in several umbels plant- 1 (4.00-3.47 branches) and fruit yield plant⁻¹ $(3.67-4.34$ g plant⁻¹). They also show the recorded increases in fruit yield ha⁻¹ (293.3-346.6 kg ha⁻¹). This happened when fennel plants fertilized with 40 m^3 ha⁻¹ in the first and second season, respectively. All FYM fertilization rates significantly increased yield components of fennel in contrast to the control during the two seasons. Furthermore, increasing FYM rates gradually increased fennel yield parameters in both seasons.

Table 4 showed that all combination treatments between FYM fertilization at 40 m³ ha-1 and a mixture of amino acids (tryptophan and glycine) caused significant augments. These augments exemplified the number of umbels fennel plant-1, fruit yield plant⁻¹, and ha^{-1} , contrasted to unfertilized plants. Furthermore, the yield components of the fennel plant were increased because of all combination treatments between FYM fertilization rates and amino acids contrasted to that of FYM fertilization alone. Gendy et al. (2015) indicated that foliar application of 100 ppm Tryptophan resulted in a visible increase in yield components of fenugreek (number of pods plant-1, seed yield plant-1, and seed yield ha-1. In addition, Lemma (2022) revealed that the highest fruit number plant-1; dry sepals yield every ha, and biological yield every ha was recorded with 15 t of FYM ha-1.

Volatile oil production

Table 5 clarified that FYM rate treatments significantly increased fennel volatile oil production in contrast to the control in 2020/2021 and 2021/2022. The highest values in Foeniculum vulgare volatile oil percentage (2.256-2.419%), volatile oil yield plant-1 (0.900- 1.029 mL plant⁻¹), and volatile oil yield ha⁻¹ (71.980-82.320 L ha⁻¹) were obtained from 40 m^3 FYM ha-1. These were in contrast to the control and the other rates during the study in the first and second seasons, respectively. Also, Kamal et al., 2023; El-Sayed et al., 2017 found that using 100% organic fertilizer resulted in an elevated proportion of dill essential oil. Also, it caused the highest essential oil content (mL plant-1) and essential oil production (L ha-1) when contrasted to other treatments. This was observed across two growing seasons using dried ripe fruits. In line with Kamal et al. (2023), organic fertilizer influenced coriander's volatile oil. Additionally, applying 20 $m³$ of compost feddan⁻¹ raised the proportion of volatile oil in both seasons, while unfertilized crops had the least significant values. Table 5 results show exposing fennel plants to amino acids mixture (tryptophan and glycine) concentrations significantly increased volatile oil percentage, volatile oil yield plant-1 (mL), and ha $¹$ (L), compared to the control (unsprayed plants)</sup> in 2020/2021 and 2021/2022 seasons. In addition, the highest values in fennel volatile oil production (2.149 and 2.318%, 0.794 and 0.925 mL plant⁻¹ as well as 63.483 and 73.996 L ha⁻¹) were produced in response to 300 ppm of amino

acids concentration contrasted to the control, with other concentrations during the study. Generally, a progressive increase in this concern was brought about by an increase in the concentrations of amino acids during both seasons.

 $FYM = Farmyard$ manure (20, 30 and 40³ ha⁻¹) and $AM = Amino$ acids (100, 200, and 300 ppm).

Table 5 revealed that increasing amino acid concentrations from 200 to 300 ppm under each FYM rate gradually increased fennel volatile oil percentage; yield plant-1; yield feddan-1 in both seasons. The highest values in this regard were

(2.337- 2.480%, 0.981-1.108 mL plant-1 in conjunction with 78.490 and 88.630 L hat ¹). They were achieved by FYM at 40 m^3 ha⁻¹ + mixture of amino acids at 300 ppm contrasted to other combinations under consideration in both

seasons, respectively.

In addition, fennel plant volatile oil yield increased as a percentage and plant -1 and ha -1 when FYM fertilization and different amino acid concentrations were used alone. Together, these factors may have maximized the effects of the individual treatments, resulting in higher volatile oil yield plant-1 and ha-1.

 $\text{FYM} = \text{Farmyard manure}$ (20, 30 and 40^3 ha^{-1}) and $\text{AM} = \text{Amino acids}$ (100, 200, and 300 ppm).

Volatile oil components

The results of fennel oil gas chromatographic/mass spectrometry (GC/MS) indicated that it contains twelve different components (Table 6). When comparing the chemical compound values in the oil, we find that, in contrast to other compounds, the aromatic oil compounds with the largest percentages were enethral, limonene, phenol, and anethole.

Table 6. Influence of farmyard manure rate, amino acid concentration and their combinations on volatile oil

		R T	Treatments							
$\bf No$	Component Name		control	$FYM 30 m3 h$ a $1 + AM$ 200 ppm	FYM 30 m ³ $ha^{-1} + AM$ 300 ppm	FYM 40 m ³ $ha^{-1} + AM$ 200 ppm	FYM 40 m^3 ha ⁻¹ + AM 300 ppm			
1	α -Pinene	6.316	1.04	0.91	1.42	1.15	1.11			
$\boldsymbol{2}$	β -Phellandrene	7.281	0.24	0.30	0.31	0.29	0.33			
3	β -Myrcene	7.691	0.30	0.35	0.41	0.36	0.43			
4	α -Phellandrene	8.078	$---$	----	0.23	---	0.21			
5	p -Cymene	8.609	0.30	0.28	0.37	0.33	0.39			
6	D-Limonene	8.725	11.35	12.90	13.28	11.94	13.26			
7	Eucalyptol	8.811	0.56	0.48	0.58	0.46	0.55			
8	$trans-β-Ocimene$	8.933	0.61	0.68	0.83	0.75	0.86			
9	γ -Terpinene	9.533	0.35	0.35	0.40	0.44	0.42			
10	L-Fenchone	10.382	4.34	4.12	4.30	4.87	4.28			
11	Estragole	13.559	78.3	76.27	75.50	77.54	75.44			
12	Anethole	15.852	2.6	3.39	2.18	1.95	2.24			
Number of vehicles selected			11	11	12	12	12			
Total % of compounds identified			100	100	100	100	100			

components of Foeniculum vulgare (mixed samples of 2020/2021).

 $FYM = Farmyard$ manure (20, 30, and $40³$ ha⁻¹) and AM = amino acids (100, 200, and 300 ppm).

The uppermost average was recorded in the Estragole compound (78.30) in control, followed by FYM at 40 m^3 ha⁻¹ + 200 ppm amino acids (AM), which recorded (77.54). Then FYM at 30 at $m³$ ha⁻¹ + 200 ppm amino acids (AM), which was (76.27) followed. The highest average was for the compound D-Limonene (13.28) in FYM at 30 at $m³$ ha⁻¹ + 300 ppm amino acids. FYM at 30 at $m³$ ha^{-1} + 200 ppm amino acids, which recorded (12.90), followed. The highest percentages were for the compound L-Fenchone (4.87) in treatment FYM at 40 m^3 ha⁻¹ + 200 ppm amino acids. The highest relative to the anethole compound (3.39) in treatment FYM at 30 at m^3 ha⁻¹ + 200 ppm amino acids (AM). This implies the treatments had a discernible impact on raising the concentrations of some of the fundamental fennel oil constituents. Additionally, the treatments clearly affect several chemicals. This complies with the findings of Mahfouz et al. (2007) that using fertilizers increased the proportion of oil and aromatic oil components. This is explained by the integration of the two forms of fertilization and how it affects the availability of every component. Nutrients for the plant and this outcome support the findings of (DARWISH et al., 2011) that fertilizers increase the amount of chemicals present in fennel plants.

Discussion

The registered results of this investigation demonstrated the potential of organic manures to increase dill chemical constituents, yield components, and rate of plant growth. Numerous researchers have noted the significant functions that organic manures play in biological and physiological processes, which may account for the increase in these earlier features. Franz (1973) showed that augmenting the content of organic matter in soils caused an increase in dehydrogenase activity. In addition, organic manure holds moisture and maintains sufficient pore spaces to permit good air circulation and excessive water drainage. Schachtschable (1979) and Bohn et al. (1985) concluded that organic matter is a main source of N, P, S and contains high content of B and Mo. Also, it is considered as a source of energy for Azotobacter growth. Organic manure minimizes the loss of nutrients by leaching (Saber, 1997) and it caused an increase in microbial activities in the root zone when supplied to the soil (Taiwo et al., 2002). Mashali (1997) demonstrated that the incorporation of organic manure in the soil improves the permeability of soil and releases carbon dioxide and certain organic acids during decomposition. Amino acids serve as fundamental components of proteins and the precursors for several other compounds that play vital roles in plants. Amino

acids participate in the production of other chemical molecules, including alkaloids, plant hormones enzymes, and terpenoids that regulate diverse plant functions (Glawischnig et al., 2000; Ibrahim et al., 2010). Soliman et al. (2023) concluded that using glycine at 200 and 400 ppm as a foliar spray on cumin plants significantly affected the volatile oil percentage, associated with volatile oil yield plant⁻¹ and ha⁻¹.

Tryptophan is a necessary amino acid that plants need for their growth because it plays a role in the production of natural auxins called indole acetic acid (IAA). It affects the yield of plants (Yassen et al., 2010). Also, many plants and horticultural crops, in particular, depend heavily on glycine for proper nutritional management. Because of its simple technique of synthetic production, there is interest in mixing it with minerals to make chelates, which will help plants absorb and transport nutrients more efficiently (Souri and Hatamian, 2019).

Conclusion

The findings suggest that 40 $m³$ of FYM acre⁻¹ in conjunction with a 300 ppm foliar spray of an amino acid combination is advised to enhance fennel plant growth, yield components, and volatile oil production.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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