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The effect of spraying with plant extracts and tryptophan on some growth characteristics and the content of *Vitex agnus-castus* L. plant and volatile oil.

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I. Abstract

This study was conducted at the College of Agriculture, University of Basrah, during the 2023-2024 season. It aimed to investigate the effects of different levels of the amino acid tryptophan, sage extract, and anise extract on vegetative growth indicators and the volatile oil content of the leaves of the chaste tree (Vitex agnus-castus L.). A Randomized Complete Block Design (R.C.B.D.) with three factors and three replicates was used. The means were compared using the Least Significant Difference (L.S.D.) test at a probability level of 0.05. The results showed that:

Treatment with the amino acid tryptophan led to the highest rates of fresh and dry weight, and also recorded an increase in volatile oil weight. It was also found that treatment with sage extract improved growth characteristics and the volatile oil content of the leaves. Similarly, the use of anise extract was observed to positively affect plant characteristics and its volatile oil content.

The results indicated that the combined treatments between the binary and tertiary factors significantly stimulated an increase in the rate of fresh and dry weight of the plant and the rate of volatile oil weight in the leaves.

The study concluded that the use of the amino acid tryptophan and plant extracts in the chaste tree stimulated the improvement of vegetative growth characteristics, which was reflected in a significant increase in the oil content of the leaves.

Keywords: Plant extracts, amino acids, Vitex agnus-castus

II. Introduction

Vitex agnus-castus, commonly known as chaste tree or monk's pepper, is considered one of the most important aromatic trees belonging to the group of medicinal plants widely known in the Middle East and Europe (Souto et al., 2020). It is also a deciduous plant that generally grows in humid places, valleys, and coastal areas (Dogan et al., 2011). Its original habitat is considered to be the Mediterranean region or the banks of rivers in Southern Europe. It is also found in tropical, subtropical, and temperate regions. In Iraq, it has been found in Baghdad, Erbil, Sulaymaniyah, Kirkuk, Mosul, and Khanaqin (Al Rawi, 1988).

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The fruits of the *Vitex agnus-castus* plant, which is cultivated in Iraq, contain the substance Casticin, which has therapeutic importance as an antioxidant and anticancer agent. It also has a bioactive effect against toxins. Shakir and Awad (2013) indicated that the use of Casticin extracted from the *Vitex agnus-castus* plant as a hormonal alternative works to increase the levels of estrogen and progesterone.

In a study conducted by Ibrahim et al. (2021), it was found that the fruits contain flavonoids such as Flavones and compounds that act as antioxidants. It also contains Vitexin, which works to scavenge free radicals and acts as a stimulant and antioxidant, playing an important role in the prevention of diseases (Souto et al., 2020). As for the seed oil, it has beneficial physiological effects in preventing cardiovascular diseases. The fatty acid Sterols in *Vitex agnus-castus* seed oil reduces total cholesterol and LDL cholesterol levels in humans by inhibiting cholesterol absorption from the intestines (Tapiero et al., 2003). Some studies indicate that the *Vitex agnus-castus* plant possesses certain hormonal properties through its estrogenic activity. Several in vitro laboratory studies have shown its ability to inhibit the growth of various types of cancers, such as breast cancer and lung cancer cells (Liu et al., 2001).

Given the medical and therapeutic importance of the *Vitex agnus-castus* plant due to its possession of many active chemical compounds, including essential oils, flavonoids, and glycosides, this study is important to determine the effect of some medicinal plant extracts on growth stimulation and the oil components in the plant.

III. Materials and Methods

The experiment was conducted in a shade house (covered with green netting) belonging to the College of Agriculture / University of Basrah / Karma-Ali site for the 2023-2024 season. The experiment was carried out as a factorial experiment to determine the effect of spraying with some plant extracts (sweet fennel and sage) and the amino acid tryptophan on some growth characteristics and its volatile oil content.

The experiment was conducted with three replicates and three factors: spraying with sweet fennel seed extract at three concentrations (0, 50, 100 $g.L^{-1}$), spraying with sage leaf extract at three concentrations (0, 25, 50 $g.L^{-1}$), and spraying with the amino acid tryptophan at three concentrations (0, 100, 200 $mg.L^{-1}$). The plants were sprayed with both factors three times, with two weeks between each spray.

Studied Traits

Plant Fresh Weight (g)

The fresh weight of the vegetative growth of the experimental unit plants was estimated using a sensitive balance of German origin, type KERN PCB, and the average was calculated.

Plant Dry Weight (g)

The dry weight of the vegetative growth was estimated after drying the samples in an electric oven, type Bender, of American origin, at a temperature of 70-75°C for 72 hours. They were then weighed several times until a constant weight was reached using a sensitive balance of German origin, type KERN PCB, and the average was calculated.

Physical Characteristics of the Volatile Oil

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Extraction of Volatile Oil

The volatile oil was extracted from the leaves of the *Vitex agnus-castus* plant using water-steam distillation, according to the British Pharmacopoeia (1968). A Clevenger apparatus was used, and the oil was then transferred to a glass vial with a tight lid and stored at 4°C until examination and diagnosis of the quantity and quality of the volatile oils.

Refractive Index of the Volatile Oil

The refractive index of the oil produced from all treatments was determined using a Refractometer of the Abble Refractometer Universal Type at room temperature (25°C), according to the method described by Guenther (1972).

Weight of the Volatile Oil

The weight of the oil for each treatment was determined by weighing the sample with the vial, and then subtracting the weight of the vial from the total weight. The oil weight was calculated as follows:

Weight of Volatile Oil = Weight of Sample – Weight of Vial

Statistical Analysis

The experiment was conducted as a split-plot design based on a randomized complete block design with three replicates. The plant extract treatments were considered the main plot factor, and the amino acid treatments were considered the subplot factor. The data were statistically analyzed according to the adopted design using GenStat software (2007). The arithmetic means were compared using the Least Significant Difference (LSD) test at a significance level of ($p \leq 0.05$), based on the method described by Al-Rawi and Khalaf Allah (2000).

IV. Results and Discussion

Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Fresh Weight of the Vegetative Growth of *Vitex agnus-castus* (g)

Table (1) shows the effect of treatments with tryptophan, sage, and sweet fennel, and their interactions, on the fresh weight of the vegetative growth. It is observed that the treatment with the amino acid tryptophan significantly affected this trait, with the concentration of 200 mg/L^{-1} outperforming other treatments and giving the highest value of 132.91 g, compared to the lowest value of 123.38 g. Similarly, the treatment with sage extract significantly affected the fresh weight, with the concentration of 25 mg/L^{-1} giving the highest average of 131.17 g, compared to the lowest value of 124.77 g. The study also showed that the treatment with sweet fennel extract at a concentration of 100 g/L^{-1} gave the highest average of 132.33 g, with a significant difference from the 50 g concentration and the control treatment.

The study also indicates that the dual interaction between tryptophan and sweet fennel significantly affected the fresh weight. The treatment with 200 mg/L^{-1} tryptophan and 100 g/L^{-1} sweet fennel gave the highest average of

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140.55 g, compared to the lowest average of 122.74 g. The interaction between tryptophan and sage at a concentration of 100 mg/L⁻¹ tryptophan with 25 g/L⁻¹ sage recorded 140.96 g, compared to the lowest value of 114.19 g. As for the interaction between sweet fennel and sage, it also had a significant effect, with the treatment of 100 g/L⁻¹ sweet fennel and 50 g/L⁻¹ sage giving 137.34 g, compared to the lowest value of 122.74 g. The triple interaction significantly affected the fresh weight, with the treatment of 200 mg/L⁻¹ tryptophan, 100 g/L⁻¹ sweet fennel, and 50 g/L⁻¹ sage giving the highest value of 147.25 g, compared to the lowest value of 107.56 g.

Table (1): Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Fresh Weight of the Vegetative Growth of *Vitex agnus-castus* (g)

Tryptophan mg.l⁻¹	Sweet fennel g L⁻¹	sage concentration g L⁻¹			Tryptophan and sweet fennel interaction
		0	25	50	
0	0	105.24	120.52	142.58	122.78
	50	121.85	114.50	137.55	124.63
	100	133.43	107.56	127.22	122.74
100	0	122.52	148.20	111.88	127.53
	50	131.23	137.62	115.21	128.02
	100	126.52	137.07	137.56	133.72
200	0	118.55	138.94	118.75	125.41
	50	128.34	137.02	132.94	132.77
	100	135.25	139.14	147.25	140.55
RLSD 0.05=		8.7			5.07
Sage average		124.77	131.17	130.10	Tryptophan average
RLSD 0.05=		2.9			
Tryptophan and sage interaction					
0		120.17	114.19	135.78	123.38
100		126.76	140.96	121.55	129.76
200		127.38	138.37	132.98	132.91
RLSD 0.05=		5.07			2.9
sage and sweet fennel interaction					sweet fennel average
0		122.78	122.78	122.78	125.24

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50	**124.63**	**124.63**	**124.63**	**128.47**
100	**122.74**	**122.74**	**137.34**	**132.33**
RLSD 0.05=	**5.07**			**2.9**

Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Vegetative Dry Weight of *Vitex agnus-castus* (g)

Table (2) shows the effect of treatments with tryptophan, sage, and sweet fennel, and their interactions, on the dry weight of the vegetative growth. It is observed that the treatment with the amino acid tryptophan did not significantly affect this trait. Similarly, the treatment with sage extract did not show a significant effect. The study also indicated that the treatment with sweet fennel extract did not have a significant effect.

The study also showed that the dual interaction between tryptophan and sweet fennel did not have a significant effect on this trait. Likewise, the interaction between tryptophan and sage did not show a significant effect. However, the study indicated that the interaction between sage and sweet fennel significantly affected the dry weight, with the treatment of 25 g sage combined with the control treatment for sweet fennel giving the highest value of 96.33 g, compared to the lowest value of 81.37 g. Similarly, the triple interaction between tryptophan, sage, and sweet fennel significantly affected the dry weight, with the treatment of 100 mg L^{-1} tryptophan and 25 g L^{-1} sage combined with the control treatment for sweet fennel giving the highest value of 111.70 g, compared to the lowest value of 76.41 g.

Table (2): Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Vegetative Dry Weight of *Vitex agnus-castus* (g)

Tryptophan mg.l^{-1}	**Sweet fennel g L^{-1}**	**sage concentration g L^{-1}**			**Tryptophan and sweet fennel interaction**
		0	**25**	**50**	
0	**0**	**76.41**	**81.46**	**92.90**	**83.59**
	50	**83.94**	**85.01**	**89.43**	**86.12**
	100	**96.33**	**82.90**	**86.06**	**88.43**
100	**0**	**86.42**	**111.70**	**79.96**	**92.69**
	50	**86.56**	**95.30**	**80.92**	**87.60**
	100	**89.61**	**83.18**	**100.10**	**90.96**
200	**0**	**83.03**	**95.83**	**80.68**	**86.51**
	50	**93.17**	**85.33**	**85.48**	**87.99**
	100	**80.98**	**78.03**	**84.16**	**81.05**

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RLSD 0.05=	14.83			NS
Sage average	86.27	88.75	86.63	Tryptophan average
RLSD 0.05=	NS			
Tryptophan and sage interaction				
0	85.56	83.12	89.46	86.05
100	87.53	96.73	86.99	90.42
200	85.73	86.40	83.44	85.19
RLSD 0.05=	NS			NS
sage and sweet fennel interaction				sweet fennel average
0	81.95	96.33	84.51	87.60
50	87.89	88.55	85.27	87.24
100	88.97	81.37	90.10	86.82
RLSD 0.05=	8.56			NS

Based on the aforementioned results, the effect of spraying with the amino acid tryptophan on vegetative growth characteristics could be attributed to its important and fundamental role in protein synthesis, which is crucial for the formation of Porphyrins and nucleotides. It also acts as a stimulant for hormones and a group of coenzymes (Ismail, 2011). This improvement in vegetative growth characteristics due to spraying with the amino acid tryptophan may be because it acts as a growth promoter and is the precursor in the biosynthesis of indole-3-acetic acid (IAA). Its multiple biosynthetic pathways, with three out of four pathways being dependent on the amino acid tryptophan, further demonstrate its significant role in plant growth and development (Mano et al., 2012).

Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Refractive Index of the Volatile Oil

Table (3) shows that the tryptophan factor significantly affected the refractive index of the oil. The refractive index increased at the highest tryptophan concentration, recording 1.44 at 200 mg L^{-1}, while the lowest value was 1.38 in the control treatment. Similarly, sage also affected the refractive index, with the 50 g L^{-1} treatment outperforming others and giving the highest value of 1.42, and the lowest value of 1.40 in the control treatment. As for the sweet fennel seed extract, it also significantly affected the refractive index, with the 100 g L^{-1} treatment giving the highest value of 1.44, compared to the lowest value of 1.39 at the 50 g L^{-1} concentration. The binary treatments also significantly affected the refractive index in the tryptophan-sweet fennel, tryptophan-sage, and sweet fennel-sage combinations. The triple interaction between the treatments also significantly affected the refractive index of the oil, with the treatment of 100 mg L^{-1} tryptophan, 50 g L^{-1} sweet fennel, and the control treatment for sage giving the highest value of 1.49, compared to the lowest value of 1.33.

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Table (3): Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Refractive Index of the Volatile Oil

Tryptophan mg.l^{-1}	Sweet fennel g L^{-1}	sage concentration g L^{-1}			Tryptophan and sweet fennel interaction
		0	25	50	
0	0	1.34	1.33	1.47	1.33
	50	1.33	1.33	1.47	1.33
	100	1.33	1.33	1.48	1.48
100	0	1.47	1.33	1.37	1.48
	50	1.49	1.38	1.38	1.37
	100	1.47	1.39	1.38	1.38
200	0	1.33	1.47	1.48	1.38
	50	1.33	1.47	1.48	1.47
	100	1.47	1.47	1.48	1.48
RLSD 0.05=		0.001			0.001
Sage average		1.40	1.41	1.42	Tryptophan average
RLSD 0.05=		0.0005			
Tryptophan and sage interaction					
0		1.38	1.38	1.38	1.38
100		1.39	1.42	1.41	1.41
200		1.43	1.43	1.47	1.44
RLSD 0.05=		0.001			0.0005
sage and sweet fennel interaction					sweet fennel average
0		1.38	1.38	1.44	1.40
50		1.38	1.39	1.45	1.39
100		1.42	1.40	1.42	1.44
RLSD 0.05=		0.001			0.0005

Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Weight of the Volatile Oil (g)

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We observe from the table 4 that spraying with the amino acid tryptophan had a significant effect, with the treatment at a concentration of 200 mg L⁻¹ outperforming others and yielding the highest value of 0.35 g, compared to the lowest value of 0.29 g. Similarly, sweet fennel seed extract significantly affected the oil weight, with the treatment at a concentration of 100 g L⁻¹ giving the highest value of 0.34 g, compared to the lowest value of 0.28 g. As for sage leaf extract, it also had a significant effect, with the treatment at a concentration of 25 g L⁻¹ giving 0.31 g, compared to the lowest value of 0.30 g.

The binary interaction between tryptophan and sweet fennel significantly affected the oil weight, with the treatment of 200 mg L⁻¹ tryptophan combined with 50 g L⁻¹ sweet fennel giving the highest value of 0.40 g, compared to the lowest value of 0.23 g. The interaction between tryptophan and sage also had a significant effect, with the treatment of 200 mg L⁻¹ tryptophan combined with 50 g L⁻¹ sage giving the highest value of 0.37 g, compared to the lowest value of 0.27 g.

The interaction between sweet fennel and sage significantly affected the oil weight, with the treatment of 100 g/L⁻¹ sweet fennel combined with 50 g L⁻¹ sage giving the highest value of 0.35 g, compared to the lowest value of 0.26 g.The triple interaction also had a significant effect, with the treatment of 200 mg/L⁻¹ tryptophan, 50 g L⁻¹ sweet fennel, and 25 g L⁻¹ sage giving the highest value of 0.45 g, compared to the lowest value of 0.21 g.

Table (4): Effect of Spraying with Extracts and Tryptophan, and Their Interactions on the Weight of the Volatile Oil (g)

Tryptophan mg.l⁻¹	Sweet fennel g L⁻¹	sage concentration g L⁻¹			Tryptophan and sweet fennel interaction
		0	25	50	
0	0	0.24	0.23	0.24	0.24
	50	0.21	0.23	0.24	0.23
	100	0.41	0.39	0.38	0.39
100	0	0.34	0.32	0.32	0.33
	50	0.23	0.28	0.29	0.27
	100	0.23	0.24	0.24	0.24
200	0	0.28	0.24	0.29	0.27
	50	0.36	0.45	0.39	0.40
	100	0.38	0.37	0.42	0.39
RLSD 0.05=		0.044			0.025
Sage average		0.30	0.31	0.31	Tryptophan average
RLSD 0.05=		0.02			
Tryptophan and sage interaction					

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0	**0.29**	**0.28**	**0.29**	**0.29**
100	**0.27**	**0.28**	**0.28**	**0.28**
200	**0.34**	**0.35**	**0.37**	**0.35**
RLSD 0.05=	**0.025**			**0.02**
sage and sweet fennel interaction				**sweet fennel average**
0	**0.29**	**0.26**	**0.29**	**0.28**
50	**0.27**	**0.32**	**0.31**	**0.30**
100	**0.34**	**0.33**	**0.35**	**0.34**
RLSD 0.05=	**0.025**			**0.02**

This improvement in oil characteristics may be due to the increase in vegetative parts when sprayed with the amino acid. This is because of its significant and major role in enhancing biochemical and physiological processes. These amino acids contribute to protein synthesis and carbohydrate formation through chlorophyll synthesis and stimulation of photosynthesis, leading to plant growth. This positively reflects on increasing the number of oil-producing leaves and enhancing secondary metabolite production as the specialized oil glands become filled due to their expansion and increased number. This is attributed to its role in cell division and increasing cell number (Abdel Hamid et al., 1993). Major elements play a significant role in plant growth through their active involvement in the photosynthesis process, which positively reflects in increased plant height, number of leaves, and leaf area. Additionally, they are crucial in protein synthesis and activation of metabolic enzymes (Johnson and Mirza, 2020). This increase in vegetative characteristics leads to an increase in volatile oil.

As for the plants affected by spraying with sweet fennel seed extract, this could be attributed to it being a source of proteins and amino acids, which play a role in increasing the ability of root cells to absorb mineral nutrients and water from the soil, thus increasing vegetative growth. This increase subsequently leads to an increase in the oil content of the plant (Sharma-Natu and Ghildiyal, 2005).

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