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The Effect of Bio Fertilizer and Thermal Accumulation on the Flavonoid Content of Oat (*Avena sativa* L.) Grown in a Sandy Soil Texture

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ABSTRACT

This study investigates the effects of biofertilizers and thermal accumulation on the flavonoid content of oat (*Avena sativa* L.) cultivars grown in sandy soil. A factorial field experiment was conducted in Al-Musaib city (Babil province, Iraq) during the 2023–2024 growing season to evaluate the influence of three factors: oat cultivars (Shafa, Hamel, and Gazania); biofertilizer treatments including *Trichoderma harzianum* and *Azospirillum* spp.; and sowing dates (November 1, 15, and 30, 2023) to study thermal accumulation effects. The experiment followed a randomized complete block design with three replicates. Results showed significant effects of cultivar and biofertilizer treatments on most flavonoid compounds, except catechin which was not significantly affected. Notably, the Gazania cultivar exhibited the highest kaempferol, myricetin, and rutin contents, especially when treated with both fungal and bacterial biofertilizers. Hamel showed higher apigenin levels, particularly under bacterial stimulation and late sowing. The optimum sowing date for maximizing flavonoid content was November 15. The study highlights the potential of using specific biofertilizer treatments and sowing dates to enhance the nutritional and medicinal qualities of oats through flavonoid enrichment.

Keywords: Oats, Flavonoids, Thermal accumulation, Biofertilizer

INTRODUCTION

Oats (*Avena sativa* L.) are a member of the Poaceae family. Interest in them has grown in recent years due to their numerous beneficial effects on human health, particularly cardiovascular health and weight loss programs, as well as their ability to lower cholesterol levels, reduce high blood pressure, prevent cancer, and prevent Alzheimer's disease and neurological disorders [1]. Oats are currently considered one of the healthiest cereal grains for humans due to their numerous biologically active components, such as beta-glucan, which boosts the human immune system. They also contain significant amounts of antioxidants, such as phenolic compounds, flavonoids, and essential unsaturated fatty acids, such as linoleic acid [2].

Oats (*Avena sativa* L.) hold economic importance in Iraq primarily as animal feed, especially for livestock, due to their high protein and fiber content, while also serving in human nutrition (e.g., oatmeal) and soil improvement through crop rotation. Medicinally, oats are valued for their beta-glucans, which support heart health by reducing cholesterol, as well as for their anti-inflammatory properties and benefits in blood sugar management. Cultivation is mainly concentrated in northern Iraq (Erbil, Sulaymaniyah, and Duhok), where cooler climates and moderate rainfall (400–600 mm annually) favor growth, with sowing in October–November and harvesting in April–May. According to Iraq's Ministry of Agriculture, oats cover an estimated 5,000–10,000 hectares, though production remains limited compared to major crops like wheat and barley. Challenges include water scarcity, competition with more dominant cereals, and insufficient modern farming techniques. For precise data, official reports from the Ministry of Agriculture of Iraq or the Food and Agriculture Organization (FAO) should be referenced [3-5].

Interest in this crop remains limited in Iraq due to a lack of sufficient awareness of its nutritional, medicinal, and fodder importance. Therefore, its cultivation must be expanded by introducing new varieties, improving locally available varieties, adapting them to the country's prevailing environmental conditions, and increasing their production capacity by choosing the appropriate planting date, given its importance in light of climate change. This is due to global warming and high temperatures in recent years, which have been shown to impact seed germination, plant growth, and development, as well as all physiological processes [6].

Temperature is a significant factor influencing vital processes and stages of plant growth, development, and transition from one stage to another. It is also linked to other environmental factors, particularly sunrise and sunset hours and light, which directly and indirectly affect metabolism [7-9]. The use of biofertilizers has become important because they play an effective role in agricultural systems, especially plant growth-stimulating microorganisms. Their action is achieved through three main mechanisms: biofertilizers, plant stimulants, and biocontrol agents. These include the fungus Trichoderma harzianum and the bacteria Azospirillum spp. [10].

T. harzianum plays an important role in increasing the plant's ability to absorb water and nutrients from the soil. It increases plant growth after assisting germination, as a result of the fungal activity, which helps form deep roots to absorb water and nutrients, in addition to its role in secreting some hormones and growth-stimulating substances [11]. Most Azospirillum strains are characterized by possessing a set of mechanisms that contribute to improving plant growth through the secretion of several hormones, growth regulators, and enzymes, and their high efficiency in fixing atmospheric N, in addition to the secretion of many compounds that stimulate the growth and reproduction

of beneficial organisms [12, 13]. Given the high nutritional and medicinal value of this plant, this study aimed to identify the role of biofertilizers and thermal aggregation in the flavonoid content of oat varieties.

MATERIALS AND METHDS

A field experiment was conducted in winter 2023–2024 at a private field near Musaib Project, Babil Governorate, Iraq, to assess the effects of biofertilizers and sowing dates on flavonoid content in oat grains. The soil was sandy textured, prepared by two perpendicular plowings, leveling, and fertilized based on local recommendations. The experiment employed a factorial design with three factors: three oat cultivars (Shafa, Hamel, and Gazania); three biofertilizer treatments—control (no biofertilizer), inoculation with *Trichoderma harzianum* (10 g per kg seeds), and inoculation with *Azospirillum* spp. (5 g per kg seeds); and three sowing dates (November 1, 15, and 30, 2023) to study thermal accumulation effects. Each treatment was replicated three times in plots measuring 2 × 2 m, arranged in a randomized complete block design.

Trichoderma harzianum inoculum was prepared by culturing the fungus on sterilized millet seeds and subsequently coating oat seeds with a spore suspension combined with adhesive agents to ensure uniform fungal adherence before sowing. *Azospirillum* spp. cultures were isolated and propagated under sterile conditions, then applied as a liquid bacterial suspension to the soil immediately after sowing. Standard crop management practices including irrigation, weeding, and pest control were maintained throughout the season.

Flavonoid compounds—kaempferol, apigenin, myricetin, rutin, and catechin—were extracted from harvested oat grains and quantified using high-performance liquid chromatography (HPLC) following established protocols. Statistical analysis was performed using Genestat software. Differences between treatment means were evaluated using least significant difference (LSD) tests at a 0.05 significance level.

Preparation of *Tricoderma* spp. Inoculum

The fungus was grown and propagated on *Panicum mellaceum* L. seeds after cleaning, washing them well with water and soaking them for 6 hours. The excess water was then filtered using filter paper. The seeds were then distributed into 100 g/bottle in glass bottles, the nozzles of which were sealed with cotton plugs. They were then autoclaved at 121°C and 1.5 kg/cm^2 for half an hour, then left to cool and sterilized again on the second day for another hour. The seeds were then inoculated with five discs of the fungus obtained from the Department of Biocontrol Technologies at the Technical College/Al-Musayyab in the PPA culture medium, on which the fungal colony grew, per bottle. They were then incubated in an incubator at 25 ± 2°C, taking care to shake the bottle by manual stirring every two days to ensure the fungus is distributed throughout the seeds to avoid clumping with the mycelium. This process continued for 14 days, after which the seeds bearing the fungus were used in the experiment. To mix *Trichoderma* with seeds of three oat cultivars, a seed coating method was employed. First, a Trichoderma spore suspension was prepared in sterile water or a suitable adhesive carrier (such as 1% carboxymethyl cellulose). The seeds of each oat cultivar were then evenly coated with the suspension at a recommended rate of 5 and 10 g of *Azospirillum* spp. and *Trichoderma*, respectivally, per kg of seeds, ensuring uniform distribution. The treated seeds were air-dried in shade for 30–60 minutes before sowing to prevent clumping and ensure optimal fungal adherence. This method enhances seed germination, early plant growth, and protection against soil-borne pathogens [14, 15].

Preparation of *Azosperillium* spp.

The study describes the isolation and cultivation of *Azospirillum* spp., a nitrogen-fixing bacterium used as a biofertilizer. Initially isolated at the University of Kufa, the bacteria were cultured in Al-Musayyab Technical College labs. A 10 ml bacterial culture was diluted in distilled water, and serial dilutions were performed under sterile conditions up to 10^{-5}. One milliliter of this dilution was transferred to nitrogen-free Nutrient Broth (Nfb) medium and incubated at 28°C for 3 days. Positive growth was indicated by a white ring (pellicle) formation below the surface after 24–48 hours. Bacterial inoculum from positive tubes was spread on Petri dishes with liquid culture medium and incubated again. A 5000 ml bacterial suspension was prepared for soil treatment by growing cultures in 250 ml flasks (1 ml each) at 28°C for 2–3 days. This process ensured sufficient bacterial quantity for experimental use [16]. The prepared bacteria were used as a biofertilizer. 20 ml of the pure liquid bacterial culture was injected into each pot for both experiments. Light irrigation was performed immediately after adding the bacterial culture, and the culture was left for five consecutive days to ensure homogeneity with the soil mixture in the planting pot and proliferation.

The active compounds content of oat grains were estimated in the laboratories of the Ministry of Science and Technology/Department of Environment and Water. The oat grain extract was prepared according to the method used by Hu [17] to estimate some flavonoid compounds, including catechin, kaempferol, apigenin, myricetin, and rutin. The detection and separation of these compounds was carried out according to the method described by Ngamsuk *et al.* [18] using high-performance liquid chromatography (HPLC).

Statistical Analysis

After collecting and tabulating the data, it was analyzed statistically using the ready-made statistical analysis program Genestat, and the differences between the arithmetic means were compared on the basis of the least significant difference (LSD) and at a probability level of 0.05 [19].

RESULTS

Kaempferol Content of Grains

Table (1) shows that cultivar significantly affected the content of the medicinal compound Kaempferol in grains. Gazania plants significantly outperformed the other two cultivars, achieving the highest average of 20.82 mg ml-1, a significant difference from the other two cultivars. Hamel plants, on the other hand, recorded the lowest average of 17.90 mg ml^{-1}. Biofertilizer also had a significant effect on this trait. The fungal addition treatment significantly outperformed the control treatment alone, without significantly differing from the

bacteria addition treatment. They yielded averages of 19.76 and 19.49 mg ml-1 for both, respectively. This contrasts with the control treatment, which achieved the lowest average of 18.99 mg ml-1. Planting date had no significant effect on this trait (Tables 2 – 5).

Table 1 Effect of cultivar, biofertilizer, and heat accumulation on the Kaempferol content of oat grains

Cultivar	Bio fertilizers	Heat Accumulation			Cultivar × Bio fertilizers
		11/1	11/15	11/30	
Shafa	Con.	19.15	20.45	17.15	18.92
	Tri.	18.92	20.25	21.25	20.14
	Azos.	17.85	19.78	20.75	19.46
Gazania	Con.	21.75	18.45	20.32	20.17
	Tri.	21.15	22.85	19.75	21.25
	Azos.	21.25	20.33	21.58	21.05
Hamel	Con.	18.30	17.10	18.20	17.87
	Tri.	18.90	15.90	16.40	17.07
	Azos.	18.10	18.90	19.30	18.77
L.S.D. *0.05*		2.72			1.24
Cultivar x Heat Accumulation					Cultivar
Shafa		18.64	20.16	19.72	19.51
Gazania		21.38	20.54	20.55	20.82
Hamel		18.43	17.30	17.97	17.90
L.S.D. *0.05*		1.24			0.42
Heat Accumulation x Bio fertilizers					Bio fertilizers
Con.		19.73	18.67	18.56	18.99
Tri.		19.66	19.67	19.13	19.49
Azos.		19.07	19.67	20.54	19.76
L.S.D. *0.05*		1.24			0.42
Heat Accumulation		19.49	19.33	19.41	
L.S.D. *0.05*		N. S.			

Table 2 The main Effect of cultivar, biofertilizer, and heat accumulation on the Kaempferol Apigenin, Myricetin, Rutin and Catechin content of oat grains

Cultivar	Kaempferol mg ml^{-1}	Apigenin mg ml^{-1}	Myricetin mg ml^{-1}	Rutin mg ml^{-1}	Catechin mg ml^{-1}
Shafa	19.51 b	19.51b	20.38795b	21.78478b	22.76509b
Gazania	20.82 a	20.82a	21.7569a	23.24752a	24.29366a
Hamel	17.90 c	17.9c	18.7055c	19.98706c	20.88648c
L.S.D. *0.05*	0.42				
Bio fertilizers					
Control	18.99 b	18.99b	19.84455b	21.20415b	22.15834b
Tricoderma	19.49 a	19.49a	20.36705a	21.76245b	22.74176
bAzosperillium	19.76 a	19.76a	20.6492a	22.06393a	23.05681a
L.S.D. *0.05*	0.42				
Sowing date					
1 November	19.49 a	19.49a	20.36705a	21.76245a	22.74176a
15 November	19.33 a	19.33a	20.19985a	21.58379a	22.55506a
30 November	19.41 a	19.41 a	20.28345a	21.67312a	22.64841a
L.S.D. *0.05*	ns				

Means with the same letter are not significantly different from each other according to LSD (P>0.05)

Table 3 The Cultivar × Bio fertilizers interaction Effect on the Kaempferol Apigenin, Myricetin, Rutin and Catechin content of oat grains

Cultivar	Bio fertilizers	Kaempferol mg ml^{-1}	Apigenin mg ml^{-1}	Myricetin mg ml^{-1}	Rutin mg ml^{-1}	Catechin mg ml^{-1}
Shafa	Control	18.92 bc	19.77 bc	21.125bc	22.076	11.281bc
	Tricoderma	20.14 ab	21.04bc	22.488c	23.500	12.008c
	Azosperillium	19.46 bc	20.33bc	21.728c	22.706	11.603cd
Gazania	Control	20.17 ab	21.077 ab	22.52bc	23.535	12.026a
	Tricoderma	21.25 a	22.206 a	23.727 a	24.79 ab	12.670a
	Azosperillium	21.05 a	21.99a	23.504 a	24.562a	12.551a
Hamel	Control	17.87 d	18.674 d	19.953 d	20.851a	10.655de
	Tricoderma	17.07 d	17.838 d	19.06d	19.918	10.178e
	Azosperillium	18.77 c	19.614 d	20.95d	21.901	11.191e
L.S.D. *0.05*		1.24	2.36	5.6	5.4	6.7

Means with the same letter are not significantly different from each other according to LSD (P>0.05)

Table 4 The Cultivar × Sowing date interaction Effect on the Kaempferol Apigenin, Myricetin, Rutin and Catechin content of oat grains

Cultivar	Sowing date	Kaempferol mg ml^{-1}	Apigenin mg ml^{-1}	Myricetin mg ml^{-1}	Rutin mg ml^{-1}	Catechin mg ml^{-1}
Shafa	1 November	18.64 cd	19.47 cd	20.81 bc	21.74 b	11.11 bc
	15 November	20.16 bc	21.06 d	22.51 bc	23.52 b	12.02 bc

	30 November	19.72 c	20.60 d	22.01 bc	23.01 b	11.75 bc
Gazania	1 November	21.38 a	22.34 a	23.87 a	24.94 a	12.74 a
	15 November	20.54 ab	21.46 a	22.93 ab	23.96 a	12.24 ab
	30 November	20.55 ab	21.47 a	22.94 ab	23.97 a	12.25 ab
Hamel	1 November	18.43 cd	19.25 bc	20.57 c	21.50 bc	10.98 cd
	15 November	17.30 d	18.07 c	19.31 c	20.18 bc	10.31 c
	30 November	17.97 d	20.86 bc	22.29 c	23.30 bc	11.90 cd
L.S.D. *0.05*		1.85	4.5	6.5	5.9	8.5

Means with the same letter are not significantly different from each other according to LSD (P>0.05)

Table 5 The Bio fertilizers × Sowing date interaction Effect on the Kaempferol Apigenin, Myricetin, Rutin and Catechin content of oat grains

Bio fertilizers	Sowing date	Kaempferol mg ml^{-1}	Apigenin mg ml^{-1}	Myricetin mg ml^{-1}	Rutin mg ml^{-1}	Catechin mg ml^{-1}
Control	1 November	19.73 ab	20.61 ab	22.03 a	23.02ab	11.76a
	15 November	18.67 bc	19.51 ab	20.84ab	21.78bc	11.13a
	30 November	18.56 bc	19.39 ab	20.72ab	21.65bc	11.06a
Tricoderma	1 November	19.66 ab	20.54 ab	21.95a	22.94ab	11.72a
	15 November	19.67 ab	20.55 ab	21.96a	22.95ab	11.72a
	30 November	19.13 bc	19.99 ab	21.36a	22.32ab	11.40a
Azosperillium	1 November	19.07 bc	19.92 ab	21.29a	22.25ab	11.37a
	15 November	19.67 ab	20.55 ab	21.96 a	22.95ab	11.72a
	30 November	20.54 a	21.46 a	22.93 a	23.96a	12.24a
L.S.D. *0.05*		1.35	3.8	4.9	6.8	7.5

Means with the same letter are not significantly different from each other according to LSD (P>0.05)

The table data recorded a significant difference in the same trait due to the effect of the two-way interaction of the experimental factors, as the interaction of the variety with the biological treatment achieved a significant difference in the grain content of the compound Kaempferol, as the Gazania variety treated with fungi recorded the highest average of 21.25 mg ml-1, thus outperforming most treatments, while the lowest average was found when the Hamel variety was treated with fungi with an average of 17.07 mg ml-1. As for the interaction of the variety with the planting date, a significant effect was found in that, as significant differences were recorded when the Gazania variety was planted on the 1/11 date, which gave the highest average of 21.38 mg ml-1, while the lowest average was recorded when the Hamel variety was planted on the second date, reaching 17.30 mg ml-1. Significant differences were also found in the effect of planting date and biofertilizer. The treatment with the addition of Azosperillium bacteria at the third date (November 30) recorded the highest average of 20.54 mg ml-1, compared to 18.56 mg ml-1 recorded when the biofertilizer was not added at the third date. When combining the three experimental factors, the treatment of Gazania cultivar plants, not treated with the biofertilizer and planted at the first date (November 11), achieved the highest average of 21.75 mg ml-1, outperforming most other treatments. The lowest average was recorded in the triple interaction treatment (Hamel cultivar plants with the addition of Trichoderma fungi at the second date (November 15), which reached 15.90 mg ml-1. Apigenin Content of Grains

The results of Table 6) indicate that cultivar significantly influences the apigenin content of grains. Hamel plants significantly outperformed the other two cultivars, achieving the highest average of 16.40 mg ml-1, with a significant difference from the other two cultivars under the experimental conditions. Meanwhile, Shafa plants recorded the lowest average for this trait, 15.20 mg ml-1. Biofertilizer treatments had a significant impact on this trait. The Azosperillium treatment significantly outperformed the other treatments, with an average of 16.87 mg ml-1, in contrast to the no-additive treatment (the control), which achieved the lowest average of 15.12 mg ml-1. Planting date had no significant effect on this trait. The data in the same table showed a significant difference in the effect of the two-way interaction of the experimental factors, as the interaction of the cultivar with the biological treatment achieved a significant difference in the grain content of apigenin. The treatment of Hamel cultivar plants treated with bacteria recorded the highest average of 18.77 mg ml-1, outperforming most of the other treatments, while the lowest average was found in the second interaction treatment (Shafa cultivar plants not treated with biological treatment) with an average of 14.55 mg ml-1. As for the two-way interaction between the cultivar and planting date, a significant effect was also found, as significant differences were recorded in Hamel cultivar plants with the 11/30 date, which gave the highest average of 16.97 mg ml-1, while the lowest average was recorded when Shafa plants were planted with the first date 11/1, reaching 14.45 mg ml-1. Significant differences were also found in the effect of planting date and biofertilizer. The treatment with Azosperillium bacteria added at the second date recorded the highest average of 16.83 mg ml-1, compared to 14.87 mg ml-1 for the treatment without adding the biofertilizer at the second date.

The combined three-way interaction of the study factors produced a significant superiority in the aforementioned trait. The highest average of 19.80 mg ml-1 was found in the three-way interaction between Hamel cultivar plants, biologically treated with bacteria, and planted at the third date (November 30). This outperformed most other three-way interaction treatments. The lowest average was recorded in the three-way interaction (Shafa cultivar plants, not biologically treated and planted at the third date (November 30)), reaching 13.85 mg ml-1.

Table 6 Effect of cultivar, biofertilizer, and heat accumulation on oat grain apigenin content

Cultivar	Bio fertilizers	Heat Accumulation			Cultivar × Bio fertilizers
		11/1	11/15	11/30	
Shafa	Con.	14.15	15.65	13.85	14.55
	Tri.	14.55	15.25	17.15	15.65
	Azos.	14.65	15.68	15.85	15.39
Gazania	Con.	17.75	14.75	16.12	16.21
	Tri.	15.85	17.75	14.75	16.12
	Azos.	16.12	15.60	17.60	16.44
Hamel	Con.	14.68	14.20	14.90	14.59
	Tri.	15.50	15.80	16.20	15.83
	Azos.	17.30	19.20	19.80	18.77
L.S.D. *0.05*		3.42			2.07
Cultivar x Heat Accumulation					Cultivar
Shafa		14.45	15.53	15.62	15.20
Gazania		16.57	16.03	16.16	16.25
Hamel		15.83	١٦.40	16.97	16.40
L.S.D. *0.05*		2.07			1.03
Heat Accumulation x Bio fertilizers					Bio fertilizers
Con.		15.53	14.87	14.96	15.12
Tri.		15.30	16.27	16.03	15.87
Azos.		16.02	16.83	17.75	16.87
L.S.D. *0.05*		2.07			1.03
Heat Accumulation		15.62	15.99	16.25	
L.S.D. *0.05*		N. S.			

Myricetin Content of Grains

The results of the statistical analysis shown in Table 7 showed that the cultivar had a significant effect on the myricetin content of grains. Gazania plants significantly outperformed the cultivar, achieving the highest average of 22.16 mg ml-1, compared to Shafa plants, which achieved the lowest average of 19.84 mg ml-1. The significant effect continued with biofertilizer, as both the fungal and bacterial treatments achieved the highest average of 21.37 and 21.29 mg ml-1, without any significant differences. This contrasts with the control treatment, which recorded the lowest average of 20.05 mg ml-1. Planting date had no significant effect on this indicator.

Table 7 Effect of cultivar, biofertilizer, and heat accumulation on the myricetin content of oat grains

Cultivar	Bio fertilizers	Heat Accumulation			Cultivar × Bio fertilizers
		11/1	11/15	11/30	
Shafa	Con.	19.15	19.85	18.85	19.28
	Tri.	19.28	19.85	20.15	19.76
	Azos.	20.15	20.05	21.25	20.48
Gazania	Con.	22.35	20.55	21.38	21.43
	Tri.	22.85	25.15	21.35	23.12
	Azos.	23.12	20.78	21.88	21.92
Hamel	Con.	20.23	18.80	19.30	19.44
	Tri.	20.50	21.30	21.90	21.23
	Azos.	20.70	21.30	22.40	21.47
L.S.D. *0.05*		2.48			1.28
Cultivar x Heat Accumulation					Cultivar
Shafa		19.53	19.92	20.08	19.84
Gazania		22.77	22.16	21.54	22.16
Hamel		20.48	20.47	21.20	20.71
L.S.D. *0.05*		1.28			0.64
Heat Accumulation x Bio fertilizers					Bio fertilizers
Con.		20.58	19.73	19.84	20.05
Tri.		20.88	22.10	21.13	21.37
Azos.		21.32	20.71	21.84	21.29
L.S.D. *0.05*		1.28			0.64
Heat Accumulation		20.93	20.85	20.94	
L.S.D. *0.05*		N.S			

Significant differences were found due to the effect of bilateral interactions between the experimental factors. The interaction of the cultivar with the biological treatment had a significant difference in the grain content of apigenin. The Gazania cultivar treated with fungi recorded the highest average of 23.12 mg ml-1, outperforming most treatments. The lowest average was found in the Shafa cultivar, which was not biologically treated, with an average of 19.28 mg ml-1. A significant effect was also found when the cultivar was interacted with the planting date. Significant differences were recorded for the Gazania cultivar with the 1/11 date, which gave the highest average of 22.77 mg ml-1, while the lowest average was recorded when the Shafa cultivar was planted with the first date, reaching 19.92 mg ml-1. Significant differences were also found in the effect of planting date and biofertilizer. The treatment with Trichoderma fungi added at the

second date recorded the highest average, 22.10 mg ml-1, compared to 19.73 mg ml-1 recorded by the treatment without biofertilizer at the second date. The interaction of the three study factors demonstrated significant superiority, with the Gazania variety, biotreated with fungi and planted at the second date (November 15), achieving the highest average, 25.15 mg ml-1, outperforming most other treatments. The lowest average was recorded in the triple interaction treatment (the Hamel variety, not biotreated and planted at November 15), reaching 18.80 mg ml-1.

Rutin Content of Grains

The results in Table 8 show a significant difference in the grain content of the medicinal compound rutin depending on the cultivar. Gazania plants recorded a significant superiority, with the highest average of 36.00 mg ml-1, in contrast to Hamel plants, which recorded the lowest average of this compound, 33.47 mg ml-1. Biofertilizer had no significant effect on this indicator. Planting dates also had a significant effect on this trait. The second and third planting dates were optimal for achieving the highest average rutin content, 34.86 and 35.19 mg ml-1, respectively, significantly outperforming the first planting date, which recorded the lowest average of 34.14 mg ml-1. Significant differences were found due to the effect of the bilateral interactions of the experimental factors. The interaction of the cultivar with the biological treatment had a significant difference in the grain content of the medicinal compound Apigenin, as the treatment of Gazania cultivar plants treated with both fungal and bacterial fertilizers achieved the highest average of (36.20 and 36.18) mg ml-1, respectively, thus outperforming most of the other bilateral interaction treatments. While the lowest average was found when treating Hamel cultivar plants treated with fungi, with an average of 33.03 mg ml-1. A significant effect was also found when the cultivar was interacted with the planting date, as significant differences were recorded for Gazania cultivar plants with the first date 1/11, which gave the highest average of 36.63 mg ml-1, while the lowest average was recorded when planting Hamel cultivar plants with the first date, reaching 31.78 mg ml-1. Significant differences were also found in the effect of planting date and the biofertilizer method. The Azosperillium bacteria treatment, applied on the third planting date (November 30), recorded the highest average for this trait, 36.19 mg ml-1, compared to 33.32 mg ml-1 recorded by the bacteria treatment applied on the first planting date (November 1).

The triple interaction caused significant differences in this trait, as the Gazania cultivar, treated with fungi and planted on the second planting date (November 15), had the highest average of 37.85 mg ml-1, outperforming most other treatments under the experimental conditions. The lowest average was recorded in the triple interaction treatment (the Hamel cultivar, treated with bacteria and planted on November 1), reaching 30.50 mg ml-1.

Table 8 Effect of cultivar, biofertilizer, and heat accumulation on the rutin content of oat grains

Cultivar	Bio fertilizers	Heat Accumulation			Cultivar × Bio fertilizers
		c0	c1	c2	
Shafa	Con.	34.55	36.05	32.15	34.25
	Tri.	34.25	35.15	36.75	35.38
	Azos.	33.25	35.05	35.35	34.55
Gazania	Con.	37.45	33.85	35.55	35.62
	Tri.	36.25	37.85	34.50	36.20
	Azos.	36.20	35.33	37.03	36.18
Hamel	Con.	33.44	35.20	33.80	34.15
	Tri.	31.40	32.90	34.80	33.03
	Azos.	30.50	32.40	36.80	33.23
L.S.D. *0.05*		2.84			1.42
Cultivar x Heat Accumulation					Cultivar
Shafa		34.02	35.42	34.75	34.73
Gazania		36.63	35.68	35.69	36.00
Hamel		31.78	33.50	35.13	33.47
L.S.D. *0.05*		1.42			0.71
Heat Accumulation x Bio fertilizers					Bio fertilizers
Con.		35.15	35.03	33.83	34.67
Tri.		33.97	35.30	35.35	34.87
Azos.		33.32	34.26	36.39	34.66
L.S.D. *0.05*		1.42			N. S.
Heat Accumulation		34.14	34.86	35.19	
L.S.D. *0.05*		0.71			

Catechin Content of Grains

The content of the active medicinal compound catechin in grains had no significant effect under the experimental conditions and for all three oat cultivars planted at different planting dates. Arithmetic differences were found between the treatments used, but these differences did not reach the level of significance, as shown in the statistical analysis results shown in Table 9. The experimental treatments did not significantly affect the content of this compound in grains.

Table 9 Effect of cultivar, biofertilizer, and heat accumulation on the catechin content of oat grains

Cultivar	Bio fertilizers	Heat Accumulation			Cultivar × Bio fertilizers
		11/1	11/15	11/30	
Shafa	Con.	5.08	5.75	6.07	5.63
	Tri.	5.35	5.57	5.93	5.62
	Azos.	4.77	5.60	5.73	5.37
Gazania	Con.	5.07	5.64	5.91	5.54
	Tri.	5.98	6.18	5.98	6.05
	Azos.	5.77	5.80	6.19	5.92
Hamel	Con.	4.83	5.37	5.77	5.32
	Tri.	5.07	5.37	6.07	5.50
	Azos.	4.97	5.23	5.30	5.17
L.S.D. *0.05*		N.S.			N.S.
Cultivar x Heat Accumulation					Cultivar
Shafa		5.07	5.64	5.91	5.54
Gazania		5.61	5.87	6.03	5.84
Hamel		4.96	5.32	5.71	5.33
L.S.D. *0.05*		N.S.			N.S.
Heat Accumulation x Bio fertilizers					Bio fertilizers
Con.		4.99	5.59	5.91	5.50
Tri.		5.47	5.71	5.99	5.72
Azos.		5.17	5.54	5.74	5.48
L.S.D. *0.05*		N.S.			N.S.
Heat Accumulation		5.21	5.61	5.88	
L.S.D. *0.05*		N.S.			

DISCUSSION

The experimental factors varied in demonstrating a significant response in the content of medically active flavonoid compounds in oat grains, as shown in the results of Tables (1, 6-9). All active compounds were present except for catechin, which did not respond significantly to the study factors. The apparent response in the significant levels of compounds may be due to the variation in oat cultivars. Gazania plants were distinguished by their significant levels of kaempferol, myricetin, and rutin in their grains, while Humel plants were superior in their apigenin content. This may be due to the genetic differences in the cultivar's ability to express itself in its growing environment, demonstrating significant levels of flavonoids, including variations in weather conditions, soil quality, and tissue content of essential elements [20, 21]. While it has been conducted [22] that the interaction between the variety and the environment can express genetic potential through the combination of genetic and environmental factors, as the field and qualitative characteristics of all varieties differ, taking into account the effect of treatments on them. Therefore, organizations working in the field of oat plant breeding and improvement usually resort to introducing new varieties or developing those using different breeding methods and testing them in many locations in order to obtain approved varieties with good qualitative characteristics and also characterized by high productivity. As for the effect of biofertilizer, the addition of Trichoderma fungi improved the moral index of both Myricetin and Rutin. This may be explained by its positive effect on the plant due to its properties and advantages, as it enters the element cycle in the soil by supplying and fixing N, P, and S elements [23]. It also works to convert insoluble nitrogen into soluble nitrogen to facilitate its absorption by the roots, such as the transformation of nitrates into ammonia, with its role in the formation of bacterial nodules. It has the ability to dissolve many poorly soluble nutrients such as zinc, iron, and copper due to its containing biochemical compounds produced in large quantities responsible for dissolving the elements [24]. All of these elements are positively reflected in increasing the grain content of secondary metabolites, including flavonoids. As for the effect of Azosprillium spp. bacteria, which significantly affected the grain content of Apigenin compound. This may be attributed to the effect of this type of bacterial fertilizer in supplying the plant with the nutrients it needs, which contribute to encouraging growth and development and facilitating their absorption by the root system through its contribution in converting forms not ready for absorption into forms easy for absorption, in addition to its ability to provide the necessary growth regulators for growth. It also contributes to fixing atmospheric nitrogen through its symbiotic and non-symbiotic coexistence with the roots of the host plant [25], in addition to its fertilizer of the photosynthesis process, which increases the amount of carbohydrates, and this is directly reflected in the increase of medically effective compounds [26, 27]. It has been also mentioned [28] that adding bacterial fertilizer increases alkaloids due to its ability to increase the plant's content of the nitrogen element, which stimulates the increase in alkaloid production. There was also a significant difference between the planting dates of oat cultivars in showing the significant status of the studied flavonoid compounds, but the best was the second planting date of 15/11, which is considered the ideal date for planting oat seeds and was considered in our study as a comparative treatment with the other two dates (early and late by 15 days) from the appropriate date. This may be explained by the difference in temperature ranges during November and their role in the growth and maturity of the plant and their effective contribution to the vital and physiological processes that the plant carries out during its life cycle, including photosynthesis, which positively affects the increase of nutrients from which secondary metabolites are built. In addition, the cultivars differ in their thermal requirements according to their growth stage [29-31]. In addition, the role of temperature in filling and vitality of the grains [32]. Heat can also cause environmental stress on plant growth, resulting in an increase in the defensive compounds the plant builds during the growth phase, including flavonoids. This results in an impact on protein and fat content, a decrease in the rate of photosynthesis, and a loss of cell membrane integrity [33]. This is what actually occurred in the significant increase in Kaempferol and Apigenin, depending on the cultivar.

CONCLUSION

We conclude from the study that the experimental factors had a positive impact on increasing and enhancing the flavonoid content of oat grains. Gazania plants showed the highest significant response according to the studied indicators, in contrast to Shifa plants, which recorded the lowest results. In the same context, biofertilizer treatments with fungi and bacteria also had a significant effect. However, the study indicators did not respond to a specific planting date that increased all the studied compounds. The results varied according to planting dates, which affected the thermal accumulation of the effective temperatures at the start of planting.

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