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Assessment of Light Absorbance and Pigment Composition in Fenugreek (*Trigonella foenum-graceum* L.) Under Two Agronomic Factors Across Ecologically Distinct Sites

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ABSTRACT
The purpose of this study was to measure the concentrations of leaf pigment and assess the light absorbance of fenugreek (*Trigonella foenum-graecum* L.) plants cultivated in two different ecologically locations while being influenced by two agronomic factors. The Grdarasha and Ankawa locations, which have different soil types and microclimates, were used for the experiment. Using a factorial design, the two components under investigation were factor A [40 , 60 and 80 kg seeds ha-1] and factor B [0,100,200 and 300 ppm Nano-Zn fertilizer]. In order to ascertain absorbance at particular wavelengths (665 nm, 649 nm, and 740 nm), which correspond to chlorophyll a, chlorophyll b, and background correction, respectively, leaf samples were taken during the vegetative stage and subjected to spectrophotometric analysis. Pigment concentrations were calculated using standard equations. Results revealed significant variations in chlorophyll a, chlorophyll b, and total chlorophyll contents depending on the treatment combinations and site conditions. In general, [Factor A] enhanced chlorophyll accumulation more effectively at [Ankawa], while [Factor B] showed synergistic effects in c Standard equations were used to compute pigment concentrations. The findings showed that the amounts of chlorophyll a, chlorophyll b, and total chlorophyll varied significantly based on the site circumstances and treatment combinations. While [Factor B] had synergistic effects when combined with [Factor A] under [Ankawa Site] conditions, [Factor A] generally improved chlorophyll accumulation more efficiently at [Grdarasha]. These results provide insights for maximizing growth and production in various agro-ecological zones by highlighting the significance of environmental context and agronomic interventions in regulating photosynthetic pigment composition in fenugreek.in conjunction with [Factor A] in the circumstances of [Sites]. These results provide insights for maximizing growth and production in various agro-ecological zones by highlighting the significance of environmental context and agronomic interventions in regulating photosynthetic pigment composition in fenugreek.
KEYWORDS: Seeding rates; Nano-Zn fertilizer; Chlorophylls a and b; Carotenoids.

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تقييم امتصاص الضوء وتركيب الأصباغ في نبات الحلبة (*Trigonella foenum-graceum* L.) تحت تأثير عاملين زراعيين في مواقع بيئية متميزة

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الملخص
كان الغرض من هذه الدراسة هو قياس تركيزات صبغة الأوراق وتقييم امتصاص الضوء لنباتات الحلبة (***Trigonella foenum-graecum* L**.) المزروعة في موقعين مختلفين بيئيًا مع تأثرها بعاملين زراعيين. تم استخدام موقعي جرداراشا وعنكاوا، اللذين يختلفان في أنواع التربة والمناخات المحلية، للتجربة. باستخدام تصميم عاملي، كان المكونان قيد الدراسة هما العامل أ او معدلات البذار [40 و60 و80 كجم بذور/هكتار¹] والعامل ب او كميات سماد الزنك النانوى [0 و100 و200 و300 جزء في المليون نانو-زنك]. من أجل التأكد من الامتصاص عند أطوال موجية معينة (665 نانومتر، 649 نانومتر، و740 نانومتر)، والتي تتوافق مع الكلوروفيل أ، والكلوروفيل ب، وتصحيح الخلفية، على التوالي، تم أخذ عينات من الأوراق خلال المرحلة الخضرية وإخضاعها للتحليل الطيفي الضوئي تم حساب تركيزات الصبغة باستخدام المعادلات القياسية. كشفت النتائج عن اختلافات كبيرة في الكلوروفيل أ والكلوروفيل ب ومحتوى الكلوروفيل الكلي اعتمادًا على مجموعات المعالجة وظروف الموقع. بشكل عام، عزز [العامل أ] تراكم الكلوروفيل بشكل أكثر فعالية في [عنكاوا]، بينما أظهر [العامل ب] تأثيرات تآزرية في [ج]. تم استخدام المعادلات القياسية لحساب تركيزات الصبغة. أظهرت النتائج أن كميات الكلوروفيل أ والكلوروفيل ب والكلوروفيل الكلي تختلف بشكل كبير بناءً على ظروف الموقع ومجموعات المعالجة. بينما كان لـ [العامل ب] تأثيرات تآزرية عند دمجه مع [العامل أ] في ظل ظروف [موقع عنكاوا]، فقد حسّن [العامل أ] بشكل عام تراكم الكلوروفيل بشكل أكثر كفاءة في [جرداراشا]. توفر هذه النتائج رؤى لتعظيم النمو والإنتاج في مختلف المناطق الزراعية البيئية من خلال تسليط الضوء على أهمية السياق البيئي والتدخلات الزراعية في تنظيم تكوين الصبغة الضوئية في **fenugreek.in**، بالتزامن مع [العامل أ] في ظروف [المواقع]. توفر هذه النتائج رؤى لتعظيم النمو والإنتاج في مختلف المناطق الزراعية البيئية من خلال تسليط الضوء على أهمية السياق البيئي

والتدخلات الزراعية في تنظيم تكوين الصبغة الضوئية في الحلبة.
الكلمات المفتاحية: الحلبة، معدلات البذار: سماد الزنك – نانو؛ الكلوروفيل **a, b**؛ الكاروتينات.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a Fabaceae family annual leguminous herb that is used for its culinary and therapeutic properties in Asia, Africa, and the Mediterranean. Fenugreek leaves and seeds contain a wide range of bioactive constituents, including alkaloids, saponins, flavonoids, polyphenols, and essential oils, which underlie its pharmacological actions, including antidiabetic, antihyperlipidemic, anti-inflammatory, and antioxidant effects. These constituents have been used traditionally in Ayurvedic and Chinese medicine for glycemic control, lactation support, and digestive health (openbiologyjournal.com). In addition to these secondary metabolites, fenugreek's photosynthetic pigments are essential for plant development, stress tolerance, and eventually the production of several bio actives. (Ahmad *et al*., 2023)

The primary pigments in green tissues that capture light are chlorophylls a and b. The main force behind the photochemical reactions in photosystems II and I, where collected photons are transformed into chemical energy, is chlorophyll a, which absorbs at its maximum wavelength of 665 nm. Without it, oxygenic photosynthesis would not be possible (careerpower.in). As an auxiliary pigment, chlorophyll b, which peaks at 649 nm, extends the absorption spectrum into blue-green regions and transfers energy to chlorophyll a, increasing the total efficiency of light acquisition (sciencing.com). These two pigments are important markers in agricultural physiology research because of their relative quantities (the chlorophyll a: b ratio), which provide information on the condition of the light gathering antenna, shade adaptation, and nutrient status of the plant (Croce and van Amerongen, 2014).

In addition to providing photoprotection against excess light through non-photochemical quenching and scavenging of reactive oxygen species, carotenoids—lipophilic isoprenoid pigments that include xanthophylls and carotenes—absorb light in the 400–550 nm range and direct that energy to the chlorophylls (molhort.biomedcentral.com, sciencing.com). Furthermore, carotenoids are precursors of phytohormones including strigolactones, which affect branching and root architecture, and abscisic acid, which regulates stomatal closure in the presence of water deficiency (biologyinsights.com). In addition to influencing photosynthetic efficiency, their content may also be related to the buildup of health-promoting antioxidants in fenugreek leaves and seeds brought on by stress. (Choudhury and Behera, 2001)

biomass production and the accumulation of medicinally active chemicals in fenugreek requires an understanding of how environmental factors and agronomic inputs affect these pigments. Chlorophyll and carotenoid production can be modulated by changes in light availability and stress factors caused by variations in soil type, climate, and microenvironment among growing locations. Nutrient uptake and pigment biosynthesis pathways may also be impacted by seed rate and nano zinc foliar treatments. Nano fertilizer researches are nearly introduced to the country (Coban *et al*., 2025).

The primary objectives of this research are to:

1. Determine the amounts of carotenoid, total chlorophyll, chlorophyll a, and chlorophyll b in fenugreek leaves under various agro-ecological circumstances.
2. Examine light absorption properties at particular wavelengths (665, 649, and 740 nm) in order to gauge background correction and photosynthetic efficiency.
3. Examine how seeding density and nano-zinc treatments interact to affect pigment profiles in order to find management approaches that optimize photosynthetic capability and, consequently, the production of bioactive components.

This study establishes the foundation for location-specific best practices in fenugreek production by concentrating on the mechanistic connections between environment, agronomy, and pigment biochemistry rather than on yield or end product metrics. These understandings will direct the creation of customized agronomic guidelines to improve fenugreek crop production and medicinal quality.

MATERIALS AND METHODS

Two deferent ecological zones were selected to this study: Ainkawa and Grdarasha to assess the light absorbance and pigments of the medicinal plant (Fenugreek) Duration and Timeline (Summary) and soil properties are shown in tables 1 and 2. During the vegetative stage, leaf samples were collected and analyzed using spectrophotometry. Standard equations were used to compute pigment concentrations according to (Lichtenthaler and Buschmann, 2001.) .

Table 1. Timeline of Agronomic Operations and Nano-Zinc Treatments at Grdarasha and Ankawa Experimental Sites

	Grdarasha 36.008745, 44.023975	Ankawa 36.244066, 43.998513
land prep with D.A.P (20:20) ,146 g/plot	3/12	6/12
Sowing	4/12	7/12
Fenugreek emergence	13/12	17/12
Urea application (97.2 g/plot)	17/1	17/1
Weed control	15/2	15/2
Nylon cover due to low temps (-2 to 3°C)	21/2	21/2
Nano zinc spray	3/3	4/3
Levels of Factor	800 mg / 4 L 1.2 g / 4 L 4 L water (control)	

Table 2. Physicochemical Soil Properties at Ankawa and Grdarasha Sites

parameters	Ankawa	**Grdarasha**	**Units**
pH	7.71	7.42	……
EC	0.517	0.2	dS.m
N	0.1	0.18	mg/kg
p	4.1	3.75	mg/kg
k	0.35	100	mg/kg
clay	397.6	45	%
Silt	54.6	42.5	%
Sand	56.2	12,5	%
Textures	**Siltyclay**		**…….**
Bulk Density	1.29	1.4	$g.cm^3$
organic matter	9.6	0.9	%
Total CaCO3	340	247	%
CEC	22.79	22.02	Meq/100g soil

RESULTS AND DISCUSSION

Absorbance of the sample at 665 µ

The findings of evaluating plant pigments at a wavelength of 665 nm under various conditions at two locations—Ankawa and Grdarasha—as well as their pooled average are shown in table (3) . Two factors are examined in this study; Factor A, sowing rates (A1, A2, A3 or 40,60 and 80 kg seeds ha^{-1}) and Factor B (0,100,200 and 300 ppm Nano- Zn fertilizer). (Zlatev *et al.*, 2023)

Ankawa ; Neither the sowing rate nor the nano-fertilizer treatments had any discernible effects. A3 had the greatest mean (0.714) and A2 had the lowest (0.599), indicating a modest variation in average pigment levels between sowing rates. B3 had the greatest mean (0.699) across fertilizer treatments, whereas B2 had the lowest (0.648).

Table 3. Absorbance of the sample at 665 µ (Under Non-significant effects).

Site		B1	B2	B3	B4	Mean A
Ankawa	A1	0.602	0.790	0.823	0.601	0.704
	A2	0.654	0.594	0.496	0.651	0.599
	A3	0.749	0.560	0.777	0.769	0.714
	Mean B	0.668	0.648	0.699	0.674	0.672
	B		0.669			
		B1	**B2**	**B3**	**B4**	**Mean A**
Grdarasha	A1	0.530	0.436	0.484	0.615	0.516
	A2	0.645	0.473	0.628	0.534	0.570
	A3	0.592	0.690	0.519	0.277	0.519
	Mean B	0.589	0.533	0.544	0.475	0.535
	B		0.589			
		B1	**B2**	**B3**	**B4**	**Mean A**
Pooled	A1	0.566	0.613	0.654	0.608	0.610
	A2	0.650	0.534	0.562	0.593	0.585
	A3	0.671	0.625	0.648	0.523	0.617
	Mean B	0.629	0.591	0.622	0.575	0.596
	B		0.629			

Note that means with the same letters don't differ significantly

Grdarasha: Once more, no noteworthy primary effects were noted. A1 displayed the lowest pigment level (0.516), whilst A2 had the highest (0.570). B1 had the greatest average (0.589) and B4 had the lowest (0.475) among fertilizer treatments. In combined Analysis; There were slight changes but no statistically significant differences when the data from the two sites were merged. A1 (0.610), A2 (0.585), and A3 (0.617) had the highest pooled averages. Although the differences were not statistically significant, B1 and B3 continuously displayed comparatively greater pigment levels (0.629 and 0.622, respectively). Concluding that despite numerical variations across treatments and locations, the lack of statistically significant effects indicates that, in the circumstances, neither the Zn nano fertilizer treatment nor the sowing rate significantly affected the levels of plant pigment at 665 nm.

Absorbance of the sample at 649 µ

Utilizing two sites, Ankawa and Grdarasha, the experiment assessed the absorbance of plant pigments at 649 nm under different treatment combinations involving two factors: and, each with three (A1–A3) and four (B1–B4) levels, respectively are shown in table (4) . Site of **Ankawa** ; Factor A and factor B were shown to interact significantly at the Ankawa location, suggesting that the pigment concentration at 649 nm was impacted by the particular combinations of the two variables rather than by each factor alone. Treatment A2B1 had the highest pigment value (0.773), which was substantially different from treatment A2B4's lowest value (0.284). This suggests that A2 is sensitive to the B-level treatments. Values generally fell between 0.213 and 0.773. The interaction effect was significant, but the means across B levels did not differ significantly (Mean B values: 0.378–0.47).

Grdarasha Site; There were no discernible variations between the A × B combinations at the Grdarasha site, suggesting a consistent pigment response throughout treatments. In comparison to Ankawa, pigment absorbance values were generally lower; A1B3 had the highest mean (0.655), however this difference was not statistically significant. There were no discernible differences between the Mean A and Mean B values, which ranged from 0.236 to 0.295 and 0.199 to 0.367, respectively. Overall Findings The site effect was considerable; Grdarasha had more uniform and typically lower values, whereas Ankawa had higher pigment values and a significant A × B interaction. This implies that Ankawa's edaphic or environmental circumstances may improve the pigments' sensitivity or responsiveness to treatment combinations at 649 nm.

Pooled Analysis of Plant Pigment Absorbance at 649 nm Across Both Sites

Only Factor B had a statistically significant effect on pigment levels in the pooled data analysis combining the findings from Ankawa and Grdarasha. Factor A and the A × B interaction did not significantly affect pigment levels. Across all B levels, the mean pigment absorbance values varied between 0.00011 (B4) and 0.365 (B2). In contrast to B2, which had the greatest average pigment value, B4 treatment consistently produced the lowest pigment content across all levels of Factor A. Although there were numerical variations across Factor A levels (A1 = 0.400, A2 = 0.284, and A3 = 0.284), these variations were not statistically significant. The strongest effect of B-levels was further confirmed by the fact that all B4 combinations showed zero values, while the treatment combination A1B2 (0.557) had the highest recorded value. In summary, the lack of significant A or A×B effects suggests that differences among cropping types or cultivars were not strong enough to alter pigment levels under the tested conditions. The significance of Factor B alone shows that management timing or method (B-levels) has a decisive influence on chlorophyll-related pigment expression at 649 nm across locations. The absorbance values of B4 therapy decreased to almost zero, indicating inadequate pigment formation, which may have been caused by stress or late administration.

Table 4. Absorbance of the sample at 649 µ

Site		B1	B2	B3	B4	Mean A
Ankawa	A1	0.403ab	0.381ab	0.346ab	0.423ab	0.504a
	A2	0.773a	0.38ab	0.38ab	0.284b	0.33a
	A3	0.46ab	0.213b	0.35ab	0.274b	0.333a
	Mean B	0.378a	0.47a	0.363a	0.345a	0.389
	B		0.378			
		B1	B2	B3	B4	Mean A
Grdarasha	A1	0.198a	0.147a	0.655a	0.18a	0.295a
	A2	0.22a	0.302a	0.249a	0.179a	0.238a
	A3	0.235a	0.27a	0.199a	0.239a	0.236a
	Mean B	0.218a	0.24a	0.367a	0.199a	0.256
	B		0.218			
		B1	B2	B3	B4	Mean A
Pooled	A1	0.460a	0.557a	0.281a	0.00011a	0.400a
	A2	0.258a	0.297a	0.280a	0.00011a	0.284a
	A3	0.347a	0.241a	0.257a	0.00011a	0.284a
	Mean B	0.355ab	0.365a	0.272ab	0.00011b	0.256
	B		0.331			

Absorbance of the sample at 470 µ

Plant Pigment Absorbance at 470 nm (table 5) : Site-specific and Pooled Evaluation. The Site of Ankawa; At the Ankawa location, there was a significant interaction between factors A and B, even though neither factor's major effects—such as sowing rate or Zn nano-fertilizer level—were statistically significant on their own. (Merzlyak *et al*., 2003) This indicates that the precise combinations of planting date and zinc fertilizer doses influenced the pigment absorbance response at 470 nm.(Azam *et al*., 2022) . Zinc oxide nano-fertilizer application (foliar and soil) effect on the growth, photosynthetic pigments and antioxidant system of maize cultivar. Biocatalysis and Agricultural Biotechnology, 42, p.102343. The interaction suggests that some sowing rates were more responsive to particular fertilizer levels. For instance, combinations such as A1B3 (1.436) and A3B1 (1.258) showed high pigment content, whereas all B4 combinations showed minimal values 0.0001), indicating that B4 was detrimental across all sowing rates. Grdarasha Site**;** The absorbance at 470 nm was only statistically significantly affected by Factor B at Grdarasha. This indicates that, independent of the date of planting, varying concentrations of zinc fertilizer had a discernible impact on pigment content. Factor A and the A × B interaction were not significant, suggesting more stable behavior across sowing rates; for example, B1 (0.768) produced higher average absorbance than B4 0.0001), proving that the absence or extreme of Zn (perhaps B4) severely inhibited pigment formation. Pooled Analysis (Across Both Sites); Once more, only Factor B demonstrated a substantial impact on absorbance in the pooled data analysis. B1 produced the highest average pigment concentration, with mean pigment values ranging from 0.946 (B1) to 0.0001 (B4). Neither the interaction A × B nor Factor A showed any discernible differences. This implies that while sowing rate and its relationship to fertilizer became less noticeable in the combined study, zinc fertilizer treatments had a constant and dominant effect on pigment levels throughout both settings.(Saxton, 2007). Across all datasets, the consistently low pigment level under B4 (which might indicate either little or excess Zn) suggests a threshold or toxicity effect of Zn on carotenoid synthesis (carotenoids are generally associated with wavelengths of 470 nm). Therefore, it is important to choose the ideal Zn level and, at Ankawa, to match this with the appropriate planting date in order to achieve maximum pigment synthesis at 470 nm. (Weckwerth and Morgenthal, 2005)

Table 5. Absorbance of the sample at 470 µ

Site		B1	B2	B3	B4	Mean A
Ankawa	A1	1.323a	1.163a	1.436a	0.00011a	1.246a
	A2	0.790a	1.130a	1.068a	0.00011a	1.052a
	A3	1.258a	0.971a	1.064a	0.00011a	1.123a
	Mean B	1.124a	1.088a	1.189a	0.00011a	1.140 a
	B	1.134				
		B1	B2	B3	B4	Mean A
Grdarasha	A1	0.618a	0.652a	0.708a	0.0001a	0.674a
	A2	0.862a	0.765a	0.728a	0.0001a	0.791a
	A3	0.824a	0.677a	0.732a	0.0001a	0.749a
	Mean B	0.768a	0.698ab	0.722ab	0.00011b	0.738 b
	B	0.547				
		B1	B2	B3	B4	Mean A
Pooled	A1	0.970a	0.908a	1.072a	0.0001a	0.960a
	A2	0.826a	0.947a	0.898a	0.0001a	0.922a
	A3	1.041a	0.824a	0.898a	0.0001a	0.936a
	Mean B	0.946a	0.893ab	0.956ab	0.0001b	1.259
	B	0.703				

Chlorophyl a

The table (6) 's findings indicate that none of the parameters under investigation—Factor A (such as sowing rates), Factor B (such as Zn nano-fertilizer levels), or their combination—at Ankawa, Grdarasha, or in the pooled analysis showed statistically significant effects. (Björn *et al*., 2009).The shared letter "a" in the pooled table indicates that all values are statistically similar, despite numerical disparities among treatment averages. This implies that the tested treatments had no effect on plant pigment absorbance under the observed wavelength in this instance (maybe for a particular pigment or at a less sensitive range). The absence of notable changes may be caused by environmental variability or inadequate treatment contrast. (Susanto and Marra, 2005)

Table 6. Chlorophyl content

Site		B1	B2	B3	B4	Mean A
Ankawa	A1	5.947	6.545	8.614	6.058	6.791
	A2	6.763	6.83	4.835	6.723	6.288
	A3	8.186	5.282	8.911	8.852	7.808
	Mean B	6.965	6.219	7.454	7.211	6.962
	B	6.965				
		B1	B2	B3	B4	Mean A
Grdarasha	A1	6.056	5.056	3.069	7.276	5.364
	A2	7.469	4.746	7.1	6.211	6.381
	A3	6.685	7.815	5.898	2.46	5.715
	Mean B	6.737	5.872	5.355	5.316	5.82
	B	6.737				
		B1	B2	B3	B4	Mean A
Pooled	A1	5.801a	5.841a	6.667a	0.00011a	6.078a
	A2	5.788a	5.967a	6.467a	0.00011a	6.335a
	A3	6.549a	7.405a	5.656a	0.00011a	6.761a
	Mean B	6.046a	6.404a	6.264a	0.00011a	6.391
	B	4.679				

Chlorophyl b

The findings showed that only Factor B (Zn nano-fertilizer levels) had a significant impact on the amount of chlorophyll b in both the pooled analysis and Grdarasha. There were no discernible effects from the A × B interaction or Factor A (sowing rate). In Ankawa, only Factor B had a significant effect, despite some numerical variations between treatments (such as the high number at

A1B1: 14.795). B1 (7.624), for instance, was substantially more than B4 (0.00011).(Eggink *et al*., 2001) A comparable pattern was observed in Grdarasha, where B2 (5.662) produced the highest mean chlorophyll b concentration, which was noticeably higher than B4, underscoring the influence of fertilizer levels once more. Factor B continued to be the sole major source of variation in the pooled data. B4 continuously recorded very low values, indicating that B4 (probably little or too much Zn) hindered the synthesis of chlorophyll b. In contrast, B1 and B2 both displayed greater average pigment concentrations. . Under the tested conditions, optimal Zn levels—especially B1 or B2—seem to be necessary to maximize chlorophyll b accumulation.(Kume *et al*., 2018)

Table 7. Chlorophyl b content

Site		**B1**	**B2**	**B3**	**B4**	**Mean A**
Ankawa	A1	14.795a	5.923a	5.559a	0.00011a	8.111a
	A2	1.019b	5.461a	5.146a	0.00011a	4.183a
	A3	7.058ab	1.658a	1.272a	0.00011a	3.377a
	Mean B	7.624a	4.347ab	3.992ab	0.00011c	1.140
	B		1.134			
		B1	B2	B3	B4	Mean A
Grdarasha	A1	0.504a	14.027a	0.462a	0.0001a	4.032a
	A2	4.455a	1.722a	0.646a	0.0001a	2.045a
	A3	1.812a	1.238a	5.355a	0.0001a	2.512a
	Mean B	2.257ab	5.662a	2.154ab	0.00011b	2.863
	B		3,358			
		7.649a	9.975a	3.010a	0.00011a	6.072a
Pooled	A1	2.737a	3.591a	2.896a	0.00011a	3.114a
	A2	4.435a	1.448a	3.313a	0.00011a	2.944a
	A3	4.941ab	5.005a	3.073ab	0.00011b	4.043
	Mean B	3.255	0.893ab	0.956ab	0.0001b	1.259
	B		0.703			

Carotenoids:

The results in table (9) ; from both Ankawa and Grdarasha sites, as well as the pooled analysis, (table 8) indicate that The amount of carotenoids in fenugreek plants was only statistically significantly impacted by Factor B (Zn nano-fertilizer levels). The shared significance letter "a" across all levels of Factor A indicates that there were no significant variations between Factor A (sowing rate) and the A × B interaction. (Rao and Rao, 2007) B4 treatments consistently had the lowest carotenoid levels 0.0001µg/g FW), indicating that carotenoid production may be suppressed by excessive or nonexistent Zn administration. Higher carotenoid contents were typically the outcome of B1 and B2 treatments, especially in Ankawa, where B1 averaged 0.024 µg/g FW. This pattern is supported by the pooled mean values, which show that B1 (0.019 µg/g FW) and B2 (0.018 µg/g FW) were substantially higher than B4, highlighting the advantageous impact of proper Zn levels. This highlights zinc's function in carotenoid production and implies that improving plant pigment content requires balanced micronutrient control.(Azam *et al*., 2022)

Table 9. Carotenoids

Site		B1	B2	B3	B4	Mean A
Ankawa	A1	0.032a	0.023a	0.028a	0.00011a	0.026a
	A2	0.014a	0.023a	0.021a	0.00011a	0.021a
	A3	0.026a	0.018a	0.019a	0.00011a	0.021a
	Mean B	0.024a	0.021ab	0.023ab	0.00011b	0.023
	B		0.017			
		B1	B2	B3	B4	Mean A
Grdarasha	A1	0.011a	0.020a	0.012a	0.00011a	0.014a
	A2	0.017a	0.014a	0.013a	0.00011a	0.015a
	A3	0.015a	0.012a	0.016a	0.00011a	0.014a
	Mean B	0.014ab	0.015a	0.014ab	0.00011b	0.014
	B		0.011			
		B1	B2	B3	B4	Mean A
Pooled	A1	0.021a	0.022a	0.020a	0.00011a	0.020a
	A2	0.016a	0.018a	0.017a	0.00011a	0.018a
	A3	0.021a	0.015a	0.017a	0.00011a	0.018a
	Mean B	0.019ab	0.018a	0.018ab	0.00011b	0.019
	B		0.014			

Total chlorophyl content in fenugreek :

Table (10) The Only Factor B (application of zinc nanofertilizer) significantly affected the detected chemical across sites and planting dates, according to the results. According to the analysis, varying amounts of Zn (Factor B) caused statistically significant variations in content, while Factor A (sowing rate) and the A × B interaction did not produce statistically significant variances (as indicated by the shared letter "a" between rows). (Aggarwal *et al*., 2013) leaf during different growth stages. Int. J. Seed Spices, 3(1), pp.31-35. The B4 treatment (either reflecting no Zn or the greatest

Zn concentration) consistently recorded the lowest values (0.00011) and was statistically different (designated by "b") from other treatments in both Ankawa and Grdarasha as well as in the pooled analysis. This implies that the production or accumulation of the investigated chemical may be inhibited by either a zinc deficit or toxicity at this level. On the other hand, B1 and B2 treatments typically yielded the highest content, specifically: o B1 had the highest mean (0.053a) at Ankawa, o B2 had the highest mean (0.042a) at Grdarasha, and o B2 continued to be superior in the pooled data (0.045a). These findings demonstrate how important zinc is in controlling physiological or biochemical processes that produce this molecule, maybe by boosting enzyme activity or preserving membrane functions related to the creation of secondary metabolites. The best accumulation is supported by appropriate Zn application (B1 and B2), but B4 therapy continuously restricts content. Therefore, in order to enhance quality-related phytochemical features in fenugreek cultivation, zinc management should be adjusted.(Nazir, *et al*., 2024)

Table 10. Total chlorophyl content in fenugreek:

Site		**B1**	**B2**	**B3**	**B4**	**Mean A**
Ankawa	A1	0.079a	0.057a	0.045a	0.00011a	0.057a
	A2	0.033a	0.039a	0.046a	0.00011a	0.041a
	A3	0.047a	0.043a	0.042a	0.00011a	0.045a
	Mean B	0.053a	0.047ab	0.045ab	0.00011b	0.048
	B	0.036				
		B1	B2	B3	B4	Mean A
Grdarasha	A1	0.023a	0.062a	0.031a	0.00011a	0.036a
	A2	0.036a	0.036a	0.028a	0.00011a	0.034a
	A3	0.040a	0.029a	0.025a	0.00011a	0.032a
	Mean B	0.033ab	0.042a	0.028ab	0.00011b	
	B	0.026				0.034
		B1	B2	B3	B4	Mean A
Pooled	A1	0.051a	0.059a	0.038a	0.00011a	0.047a
	A2	0.034a	0.038a	0.037a	0.00011a	0.037a
	A3	0.043a	0.036a	0.034a	0.00011a	0.038a
	Mean B	0.043ab	0.045a	0.036ab	0.00011b	0.041
	B	0.031				

CONCLUSIONS

While at Grdarasha only the zinc rate itself proved significant, indicating a weaker influence of sowing density under those local conditions, at Ankawa, a clear interaction between sowing rate and nano-zinc fertilizer highlighted the need to synchronize planting density with Zn applications to maximize pigment synthesis. The main factor influencing pigment accumulation at both sites was nano-zinc, particularly chlorophyll b, which was quite sensitive to Zn levels. The sowing date and its relationship with Zn were only somewhat important. Furthermore, the carotenoid content was most effectively increased by intermediate and high Zn rates (B_1 and B_2), suggesting that zinc nano-fertilization is the primary driver for increasing the concentrations of both carotenoid and chlorophyll in fenugreek.

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