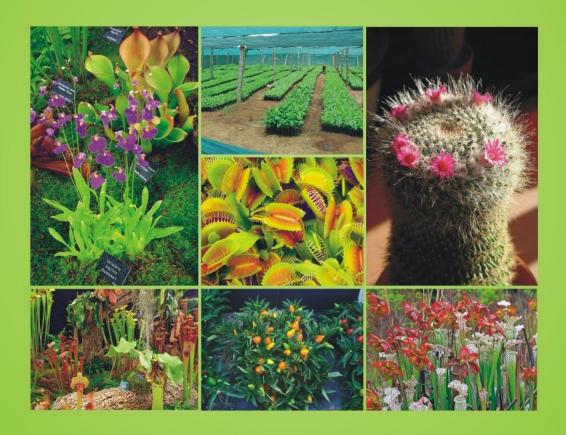




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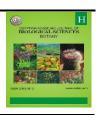
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# Yield Performance of Wheat under Various Sowing Dates and Nano and Biological **Fertilization**

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Wheat; sowing dates: Nano-bio fertilizers; yield; yield component.

#### **ABSTRACT**

Wheat (Triticum aestivum L.) is a staple food crop of global, regional, and national importance, particularly in Egypt, where it occupies a leading position in food security strategies. Two field experiments were conducted at the experimental farm of Saba Basha Agricultural Faculty, Alexandria Governorate, Egypt, during the 2022/2023 and 2023/2024 growing seasons to investigate the effect of nanobiofertilizer application and sowing date on productivity of wheat. Split plot design (SPD) was used for the experiment, with the three sowing dates dispersed among four replicates in the main plots, and Nano-biological fertilization in sub main

The results revealed significant differences due to both sowing date and fertilizer type across the two seasons. Early sowing on November 15 significantly improved plant height, spike length, number of spikes/m², grain number per spike, and overall yield components compared to later sowing dates. The combined application of nano NPK and biofertilizer led to superior performance in most traits due to enhanced nutrient availability, improved microbial activity, and better physiological responses. A significant interaction between sowing dates and fertilization treatments was observed, indicating the importance of integrating optimized sowing date with efficient nutrient management. Under Alexandria's environmental conditions, it is recommended to sow wheat in mid-November and apply a combination of nano and biofertilizers to maximize growth and yield. This integrated approach contributes to sustainable wheat production under climate variability and soil fertility challenges.

#### INTRODUCTION

Wheat (Triticum aestivum L.) is one of the most important staple food crops worldwide, playing a vital role in food security and nutrition. It ranks first among cereal crops in terms of cultivated areas and second after maize in global production. Wheat provides about 20% of the calories and protein consumed by the world's population, making it a cornerstone of food systems (FAOSTAT, 2023).

Globally, the total area under wheat cultivation reached approximately 219 million hectares in 2022, producing nearly 781 million metric tons, with an average yield of around 3.56 tons per hectare (FAOSTAT, 2023). The top wheat-producing countries include China, India, Russia, the United States, and France. Despite advancements in agronomy, breeding, and biotechnology, many regions still face yield stagnation or slow growth due to climatic stress, declining soil fertility, and disease pressures (Sharma et al., 2020). In Egypt, wheat is considered the most strategic cereal crop, forming the basis of the national food security policy. It is the primary component of the Egyptian diet, especially for bread.

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The growing wheat season extends from November to April under irrigation in the Nile Valley and Delta. However, Egypt's wheat self-sufficiency remains low, and the country is one of the largest wheat importers globally, primarily due to the gap between domestic production and consumption. As of 2023, the cultivated area of wheat in Egypt is estimated at around 1.4 million hectares, with an average yield of approximately 6.5 tons per hectare, placing Egypt among the top yield achievers globally (FAOSTAT, 2023). However, the production meets only about 45–55% of the national demand, leaving a substantial production-consumption gap that necessitates imports exceeding 10 million tons annually (Ghoneim *et al.*, 2021).

Several challenges hinder wheat production in Egypt, including limited arable land, water scarcity, climatic stress, and inefficiencies in agronomic practices. To bridge the production gap, strategies such as expanding cultivated land in new reclaimed areas, adopting high-yielding and stress-tolerant cultivars, and integrating precision agriculture technologies are being actively explored (Abdalla *et al.*, 2023).

Sowing date is a critical agronomic factor that significantly influences wheat growth, development, and final yield. Choosing the optimal sowing date helps wheat plants escape environmental stresses during sensitive growth stages, such as flowering and grain filling. Under changing climatic conditions, particularly rising temperatures and shifting rainfall patterns, the selection of appropriate sowing dates has become even more crucial. Delayed sowing often results in reduced tillering, shortened grain filling periods, and lower yields due to increased heat stress during critical phenological stages (Asseng *et al.*, 2015). On the other hand, early sowing may expose crops to frost damage in some regions or misalign growth with rainfall availability. Studies in arid and semi-arid environments, including North Africa and the Middle East, have emphasized that adjusting sowing dates is a key adaptation strategy to mitigate the negative impacts of climate change on wheat productivity (Zhao *et al.*, 2017). Moreover, integrating sowing date optimization with climate-resilient varieties and resource-efficient practices can substantially enhance wheat resilience under variable weather conditions (Kassie *et al.*, 2019).

Nano-fertilizers, especially nano-formulations of essential nutrients like nitrogen (N), phosphorus (P), and potassium (K), have emerged as promising alternatives to conventional fertilizers in modern agriculture. In wheat cultivation, nano-NPK fertilizers have demonstrated significant potential to improve nutrient uptake, enhance physiological efficiency, and boost grain yield while reducing environmental losses. Studies have shown that nano-sized particles improve the surface area and solubility of nutrients, allowing for more efficient absorption by plant roots and leaves (Raliya *et al.*, 2016). When applied at lower doses compared to conventional fertilizers, nano-NPK has been reported to enhance photosynthetic activity, chlorophyll content, and biomass accumulation in wheat (Subramanian *et al.*, 2015). Furthermore, nano-fertilizers can help in minimizing nutrient leaching and volatilization, thus improving fertilizer use efficiency (FUE) and sustainability in wheat production systems (Solanki *et al.*, 2015). Several field trials have demonstrated that wheat treated with nano-NPK formulations achieved higher grain yields and better stress tolerance, especially under water-limited or nutrient-deficient conditions (Zulfiqar *et al.*, 2020).

Biofertilizers are environmentally friendly microbial inoculants that promote plant growth by enhancing the availability of nutrients in the rhizosphere. They include beneficial microorganisms such as *Azotobacter*, *Azospirillum*, *Rhizobium*, *Bacillus*, and phosphate-solubilizing bacteria, which play a vital role in nitrogen fixation, phosphorus solubilization, and growth hormone production. In wheat production, the use of biofertilizers has been shown to improve root development, chlorophyll content, and grain yield, particularly when integrated with reduced doses of chemical fertilizers (Mahdi *et al.*, 2010; Mohamed *et al.*, 2024). Biofertilizers also enhance soil microbial activity, improve nutrient uptake efficiency,

and contribute to the sustainability of agroecosystems by reducing reliance on synthetic inputs (Vessey, 2003). Several studies have confirmed that biofertilization is an effective strategy to improve wheat yield and stress tolerance, especially under low-input or environmentally stressed conditions.

This study aims to investigate the effects of sowing dates and fertilizer types specifically biofertilizers and nano-NPK on the grain yield of wheat (*Triticum* aestivum L.) under changing climatic conditions. Sowing date is a critical factor that influences wheat growth and productivity, especially in the context of temperature fluctuations and rainfall variability caused by climate change. Meanwhile, biofertilizers and nano-fertilizers offer promising alternatives to traditional chemical inputs by improving nutrient efficiency and supporting sustainable crop production. The research seeks to evaluate how each of these factors, individually and interactively, affect wheat yield, with the goal of identifying the most effective combination of sowing date and fertilization strategy for optimizing productivity and resource use efficiency.

#### **MATERIALS AND METHODS**

# 2.1. Study Area:

Two field experiments were conducted at the experimental farm of Saba Basha Agricultural Faculty, Alexandria Governorate, Egypt, during the 2022/2023 and 2023/2024 growing seasons to investigate the effect of nano-biofertilizer application and sowing date on wheat productivity. The experimental site is located at approximately 31°12′N latitude and 29°58′E longitude, with a Mediterranean climate range.

The preceding crop was rice in both growing seasons, which provided favorable soil conditions for wheat cultivation. Soil samples from the experimental sites were collected at a depth of 0-30 cm before the initiation of each experiment to determine the physical and chemical properties according to the standard methods described by Chapman and Pratt (1978). The soil analysis results are presented in Table 1.

**Table 1:** Some physical and chemical properties of the experimental soil in both seasons.

Soil	Seasons							
Properties	2022/2023	2023/2024						
Mechanical analysis								
Sand	16.00							
Silt	39.70	15.00						
	39.70	40.50						
Clay	44.30	44.50						
Soil texture	Clay loam	Clay loam						
pH (1:1)	8.20	8.15						
	Chemical proper	ties						
EC (1:1) dS/m	3.60	3.70						
S	oluble cations (1:2) (cn	nol/kg soil)						
K <sup>+</sup>	1.55	1.60						
Ca++	14.17	14.20						
Mg++	10.34	10.40						
Na <sup>+</sup>	14.55	15.00						
s	oluble anions (1:2) (cn	nol/kg soil)						
CO-3+HCO-3	2.90	3.00						
CL-	21.00	21.50						
SO-4	16.70	17.01						
Calcium carbonate (%)	6.20	6.40						
Total N (%)	1.20	1.35						
P (mg/kg)	3.40	3.35						
Organic matter	1.40	1.60						

# 2.2. Experimental Design and Crop Management:

A split-plot design with three replications was employed for both seasons. Each experimental plot measured 10.5 m<sup>2</sup> (3.5 m × 3.0 m), providing adequate space for accurate measurements and minimizing border effects. The wheat variety Sakha 95 was selected for its adaptability to local conditions and consistent performance. Seeds were sown at a rate of 60 kg/feddan (one feddan = 4200 m<sup>2</sup>), which is equivalent to approximately 143 kg/ha. The treatments were randomly distributed as follows:

Main plots (Sowing date): (November 15, December 1, December 15.

Sub-plots (Nano-biofertilizer treatments): (Control (No additional fertilizer), Nano fertilizer (Nano NPK), Biofertilizer (Biovet) and Nano + Bio (Mixed application).

According to the previously mentioned treatments, nano-particles of N, P, and K were applied at a rate of 2 g each per liter of water, along with 20 g of Biovet compound per liter. Foliar spraying was carried out at this rate twice, at 45 and 60 days after sowing wheat during both growing seasons. The structure of Biovet is shown in Table 2.

This factorial arrangement resulted in 12 treatment combinations (3 sowing date × 4 fertilizer treatments), each replicated three dates, for a total of 36 experimental units per season.

Prior to planting and during soil preparation, mono calcium superphosphate (containing 15.5% P<sub>2</sub>O<sub>5</sub>) was incorporated into the soil at a rate of 24 kg P<sub>2</sub>O<sub>5</sub> per feddan. Urea (46.5% N) was applied as the nitrogen fertilizer at a total rate of 70 kg N per feddan, divided into two equal portions: the first applied at sowing and the second just before the first irrigation. All other agronomic practices were conducted in accordance with the guidelines set by the Ministry of Agriculture and Land Reclamation of Egypt. Additional agricultural practices were implemented in compliance with the guidelines provided by Egypt's Ministry of Agriculture.

Table 2: Composition of BioVit Fertilizer

Element	Potassium	Organic	Amino	Mn	Zn	Fe	Beneficial
	(K)	Carbon	Acids	(mg/L)	(mg/L)	(mg/L)	Bacterium
Value	9%	30%	15%	11.3	483.9	71.0	Bacillus subtilis

## 2.3. The Studied Characters:

- 1. Plant height at harvest (cm): was measured from the soil surface to the top of the plant using 20 plants randomly selected from each plot at harvest date.
- 2. Grain yield (t/fed): determined by harvesting specific area (m<sup>2</sup>) from each plot in terms of kg and converted to t/fed.
- 3. Biological yield: determined from the harvested area (m<sup>2</sup>) of each plot in terms of kg and converted to t/fed.
- 4. Straw yield (t/fed): calculated by separating straw and spikes of biological yield then weight the yield of the straw in kg/m<sup>2</sup> and converted to t/fed.
- 5. Spike length (cm): measured from the base to the tip of the spike using a ruler. 20 randomly selected spikes from each subplot were measured.
- 6. Grains number/spike: counted as an average number of grains of twenty random spikes samples from each plot.
- 7. Spikes number/m<sup>2</sup>: counted as the number of fertile tillers/m<sup>2</sup> from each plot at harvest

8. Harvest index (HI %) estimated according to the following equation: Harvest index (HI %) = 
$$\frac{\text{Grain yield}}{\text{Biological yield}} x 100$$

# 2.4. Statistics analysis:

All collected data were submitted to analysis of variance using the describe method by Gomez and Gomez (1984). All statistical analyses were carried out using the analysis of variance approach and the CoStat computer software program (CoStat, 2005). To compare the treatment means, the least significant difference (LSD at 0.05 level of probability) was utilized.

#### RESULTS AND DISCUSSION

Table 3, the results revealed significant differences in plant height due to both sowing dates and fertilizer treatments across the two growing seasons. The highest plant heights were recorded with the application of nano NPK combined with Biovet, reaching 98.78 cm and 96.22 cm in the first and second seasons, respectively. Early sowing on November 15 also resulted in taller plants compared to later dates. These increases can be attributed to the more favorable environmental conditions available during early sowing, in addition to the enhanced nutrient absorption and metabolic stimulation induced by nano and bio-fertilizers. According to Kekeli *et al.* (2025), nano-fertilizers improve nutrient use efficiency and promote vegetative growth through controlled nutrient release and better root interaction. Similarly, Hafez *et al.* (2025) found that combining nano and biofertilizers significantly improved wheat growth parameters.

Spike length also showed a notable response to both sowing date and fertilization. The combined application of nano NPK and Biovet led to the longest spikes across both seasons, particularly when sowing occurred in mid-November or 1<sup>st</sup> December. This may be due to the improved nutrient availability and hormonal balance provided by the fertilizers, which enhance cell division and elongation in the developing spike (Youssef *et al.*, 2020). Furthermore, the application of amino acid-rich organic inputs such as Biovet may also contribute to better assimilating partitioning toward reproductive organs (Farid *et al.*, 2023).

In terms of the number of spikes per square meter, the highest values were observed with the Biovet-only treatment. This suggests that biofertilizers enhance tillering by improving soil microbial activity, nutrient cycling, and root architecture. These results are supported by Patra (2017), who reported increased spike density in wheat due to the application of plant growth-promoting rhizobacteria (PGPR) and organic matter inputs. Early sowing also had a positive influence, likely due to the extended vegetative phase allowing more date for tiller development.

The number of grains per spike showed some variation and was less consistently affected by fertilizer treatments. However, early sowing generally supported higher grain numbers, possibly due to more favorable temperature and moisture conditions during flowering and grain filling stages. Grain number is highly sensitive to environmental stress during anthesis (Foulkes *et al.*, 2011), which may explain the variability across treatments and seasons. Though nano and bio-fertilizers can improve reproductive success indirectly, the environmental conditions during flowering appear to have a dominant influence.

The significant interaction between sowing dates and fertilization treatments underscores the importance of optimizing both management practices to enhance wheat productivity. For example, sowing on December 1 with nano NPK yielded high values in multiple traits, indicating that slight delays in sowing can be compensated with improved fertilization strategies. This aligns with the findings of Prasad (2017) and Verma *et al.* (2023), who emphasized the role of nutrient formulations in maintaining crop performance under sub-optimal planting dates.

In conclusion, integrating nano-fertilizers with bio-based products like Biovet proved highly effective in enhancing growth and yield components in wheat. These strategies offer promising avenues for improving crop productivity in the face of climatic

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variability and soil fertility challenges, as supported by numerous recent studies (Kekeli *et al.*, 2025; Farid *et al.*, 2023).

**Table 3.** Plant height (cm), spike length (cm), number of spikes/m<sup>2</sup>, and number of grains/spike of wheat (*Triticum aestivum* L.) cv. Sakha 95 as affected by sowing dates and nano-biofertilizer treatments, and their interaction in both seasons

Treatments		Plant height (cm)		Spike length (cm)		Number of spikes/m2		Number of grains/spike	
		2022/2023	2023/2024	2022/2023	2023/2024	2022/2023	2023/2024	2022/2023	2023/2024
A) Sowing date									
15 <sup>th</sup> Novembe	r	98.25a	95.08a	10.43 a	10.21 a	157.0c	210.8 в	53.3 a	60.5 a
1 <sup>st</sup> December		92.67b	90.50ъ	9.79 ъ	9.58 ъ	198.0ъ	205.3 ъ	51.8 a	53.3 a
15 <sup>th</sup> December		98.33 с	88.42c	9.54 b	9.42 b	215.4 a	224.0 a	49.2 b	57.0 a
					nd biofertilizer				
Nano (NPK)	+ Biovet	98.78 a	96.22a	10.60 a	10.44 a	186.9b	199.9 с	50.9 a	37.7 c
Nano (NPK)		97.67a	94.896	10.46 a	10.33 a	185.0ъ	217.4 ъ	50.3 a	69.3 a
Biovet		90.89 ъ	89.11c	9.56 b	9.28 ъ	229.3a	250.2 a	51.0 a	61.3 ab
Control		86.33 c	85.11d	9.05 c	8.88 c	159.3c	185.9 d	53.4 a	59.3 b
Interaction (A x B)		*	*	*	*	*	*	*	*
Sowing date (A)	Nano + Bio (B)		Interaction						
	Nano + Biovet	97.33 c	94.33 c	10.67 a	10.16 bcd	174.3de	194.3de	47.0d	43.7 c
15th Nov.	Nano (NPK)	95.66 d	92.66 d	10.17 b	10.50 ab	152.0 fg	213.7 bcd	52.3 b	72.7 a
15 1101.	Biovet	90.66 g	88.66 e	9.67 c	9.00 f	164.7 ef	235.0 bc	53.3 b	66.0 ab
	Control	87.00 h	86.33 fg	8.67 e	8.66 fg	137.0 g	200.0 cde	60.7 a	59.7 a b
	Nano + Biovet	104.66 a	101.00 a	11.00 a	10.83 a	137.0 de	169.0e	58.7 a	41.7 cd
1 <sup>st</sup> Dec.	Nano (NPK)	103.33 b	99.00 Ъ	11.04 a	10.66 ab	192.7 cd	214.3 bcd	47.0 d	65.0 ab
	Biovet	95.33 de	93.00 d	9.83 bc	9.66 de	259.7 a	243.7 ad	51.6 bc	52.7 bc
	Control	89.66 g	87.33 f	9.83 bc	9.66 de	166.7 ef	194.3 de	49.6 bcd	54.0 bc
15 <sup>th</sup> December	Nano + Biovet	94.33 ef	93.33 cd	10.17 b	10.33 abc	213.3 ъ	236.3 abc	47.0 d	28.0 d
	Nano (NPK)	94.00 f	93.00 d	10.17 b	9.83 cd	210.3 bc	224.3 b cd	51.7 bc	70.3 a
	Biovet	86.66 h	85.66 g	9.17 d	9.17 ef	263.7 a	272.0 a	48.0 cd	65.3 ab
	Control	82.33 i	81.66 h	8.67 e	8.33 g	174.3 d e	163.3 e	50.0 bcd	64.3 ab
<ul> <li>Values sharing the same letter within the same column are not significantly different.</li> </ul>									

Table 4, the results clearly demonstrate that sowing dates and the application of nano and biofertilizer treatments had significant effects on wheat productivity (*Triticum aestivum* L. cv. Sakha 95) during both growing seasons. Early sowing, particularly in mid-November, was associated with enhanced grain yield, straw yield, biological yield, and harvest index. This can be attributed to more favorable environmental conditions during critical growth stages, leading to better vegetative development and grain filling. In contrast, delayed sowing to mid-December resulted in substantial reductions in all measured parameters, likely due to exposure to terminal heat stress or shortened growth duration, which adversely affects photosynthetic efficiency and assimilates partitioning (Bahalkeh *et al.*, 2021; Fazily *et al.*, 2021).

In terms of fertilization, the combined application of nano NPK fertilizers with the biofertilizer Biovet significantly improved plant performance compared to individual applications or the untreated control. The enhanced performance is likely due to improved nutrient availability and uptake efficiency, as well as the beneficial effects of biofertilizers on root development, microbial activity, and plant hormone stimulation (Tarafdar 2021; Deshmukh *et al.*, 2023). Nano fertilizers alone also showed high effectiveness, reflecting their role in promoting plant metabolism and enhancing resilience under varying environmental conditions.

The interaction between sowing date and fertilization treatments was statistically significant, emphasizing that maximum productivity can be achieved through the integration of optimal sowing timing with efficient nutrient management. Early sowing combined with nano-biofertilizer treatments yielded the best outcomes, whereas late sowing without fertilization severely limited plant growth and productivity. These findings highlight the

#### Morphological and Microsatellite-Based Molecular Diversity in Egyptian Germplasm Collection

importance of precise agronomic management under changing climatic conditions to ensure sustainable wheat production (Verma *et al.*, 2022; Rai and Avila-Quezada., 2024).

**Table 4.** Grain yield (t/fed), straw yield (t/fed), biological yield (t/fed), and harvest index (HI %) of wheat (*Triticum aestivum* L.) cv. Sakha 95 as affected by sowing dates and nano-biofertilizer treatments, and their interaction in both seasons

Treatments		Grain yield (t/fed)		Straw yield (t/fed)		Biological yield (t/fed)		Harvest index (HI %)	
		2022/2023	2023/2024	2022/2023	2023/2024	2022/2023	2023/2024	2022/2023	2023/2024
C) Sowing date									
15 <sup>th</sup> N	November	2.45 a	2.43 a	2.43 a	2.45 a	4.88 a	4.88 a	50.20 a	49.80 ъ
1st D	ecember	2.19 b	2.24 b	2.24 b	2.19 b	4.43 b	4.43 b	49.44 ъ	50.56 a
15sth [	December	1.92 c	1.91 c	1.91 c	1.92 c	3.83 c	3.83 c	50.13 a	49.87 ъ
				D) Nano a	nd biofertilizer				
Nano (N	PK) + Biovet	2.47 a	2.37 a	2.37 a	2.47 a	4.84 a	4.84 a	51.03 a	48.97 ъ
Nan	o (NPK)	2.42 a	2.30 ab	2.30 ab	2.42 a	4.72 a	4.72 a	51.27 a	48.73 ъ
E	Biovet	2.30 b	2.23 b	2.23 b	2.30 b	4.53 ъ	4.53 b	50.77 b	49.23 ъ
	ontrol	1.56 c	1.87 c	1.87 c	1.56 c	3.43 с	3.43 c	45.48 b	54.52 a
Interac	tion (A x B)	*	*	*	*	*	*	*	*
Sowing date	Nano + Bio				Inte	raction			
	Nano + Biovet	2.79 a	2.76 a	2.55 ab	2.49 ab	5.34 ab	5.25 a	52.25 ab	52.57 a
15th Nov.	Nano (NPK)	2.78 a	2.74 a	2.43 b	2.36 b	5.21 b	5.10 b	53.36 ab	53.73 a
10 1101.	Biovet	2.11 b	2.00 bc	2.16 c	2.05 c	4.27 d	4.05 e	49.46 efg	49.38 bc
	Control	1.26 f	0.81 f	1.61 e	2.04 c	2.87 f	2.85 g	43.90 g	28.30 h
	Nano + Biovet	2.76 a	2.73 a	2.67 a	2.51 ab	5.43 a	5.24 a	50.80 ab	52.06 ab
1st Dec.	Nano (NPK)	2.76 a	2.71 a	2.66 a	2.51 ab	5.42 a	5.22 a	50.89 a	51.92 abc
1 500.	Biovet	1.97 c	1.92 c	2.68 a	2.64 a	4.65 c	4.56 c	42.40cd	42.14 e
	Control	1.43 e	0.90 e	1.80 d	2.06 c	3.22 f	2.96 g	44.37 cde	30.35 g
	Nano + Biovet	2.08 bc	2.03 b	2.18 c	2.10 с	4.26 d	4.12 d	48.79 bc	49.15 c
15 <sup>th</sup> December	Nano (NPK)	2.09 bc	2.05 b	2.17 с	2.04 c	4.26 d	4.09 de	49.11 bc	50.07 bc
	Biovet	1.61 d	1.57 d	2.07 c	2.00 c	3.68 e	3.57 f	43.77 fg	43.93 d
	Control	1.05 g	0.78 f	1.26 f	1.50 d	2.31 g	2.28 h	45.40 def	34.04 f
<ul> <li>Values sharing the same letter within the same column are not significantly different.</li> </ul>									

# **CONCLUSION**

Based on the findings of this study, it is recommended that wheat (*Triticum aestivum* L. cv. Sakha 95) should be sown in mid-November to maximize productivity under the prevailing climatic conditions. Furthermore, the integrated use of nano NPK fertilizer combined with the biofertilizer Biovet is highly effective in enhancing grain yield, biological yield, and harvest index. This integrated nutrient management approach not only improves nutrient uptake and plant performance but also contributes to sustainable and environmentally friendly wheat production. Future research should explore the long-term effects of nano-biofertilizer combinations across different wheat genotypes and agroecological zones to confirm their broader applicability and optimize fertilization schedules under climate change scenarios.

# **Declarations:**

**Ethical Approval:** No animal model(s) or human participants were involved in the current study, so no ethical considerations are required.

**Competing Interests:** The authors declare no conflict of interest.

**Author's Contributions**: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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