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Impacts of Organic Fertilizer Along With Silicon and Zinc on Yield, Nutrient Uptake and Protein Content in Grain of Wheat

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ABSTRACT

The application of animal manure (AM) along with zinc (Zn) and silicon (Si) can help improve the growth, increase the yield and nutritional value of wheat grain. Hence, we investigated the effects of AM along with Zn and Si sources on yield components, grain yield, nutrient uptake and protein content in wheat grain. The experiment was conducted as a split plot in a randomized complete block design with three replications during the seasons of 2018-2019 and 2019-2020. The experimental treatments included the main plot assigned to AM application (control or non-application of AM and AM application), and the sub-plot assigned to Si and Zn fertilizers application (control, zinc sulfate, potassium silicate, ZnO-NPs, Si-NPs, potassium silicate + zinc sulfate, ZnO-NPs + potassium silicate, Si-NPs + zinc sulfate, and Si-NPs + ZnO-NPs). The results showed that the AM-treated plants had higher grain yield and greater protein accumulation when compared with untreated plants. Simultaneous foliar application of Si-NPs and ZnO-NPs on wheat plants produced the highest grain yields similar to co-application of potassium silicate and zinc sulfate as soil. The foliar application of nanoparticles had slightly better effects than soil application in terms of grain yield. The use of Si sources resulted in higher Si accumulation, and the application of Zn sources resulted in higher Zn and protein production in wheat grain. However, nanoparticles foliar application was superior to soil application in improving the nutrients uptake like Si and Zn and protein content in wheat grains. In general, the plants treated with co-application of AM + Si-NPs + ZnO-NPs showed higher grain yield compared with other experimental treatments. Overall, our results highlight that simultaneous foliar application of Si-NPs and ZnO-NPs plus AM has great potential in increasing yield and improving the nutritional quality of wheat grain.

Keywords: grain yield, grain protein, animal manure, nano-Si, nano-Zn, plant nutrition.

INTRODUCTION

Wheat (*Triticum aestivum* L.) has the largest area under rainfed cultivation in the world and provides about 20% of the calories, proteins, minerals and vitamins required by the world population (Halim et al., 2018). The main goals of an agricultural system include increasing production per unit area, reducing production costs, and achieving higher efficiency in the use of inputs, without harming soil, water, the environment, and crop quality (Mengistu et al., 2017). The poor soil organic matter in hot and dry regions, like Iran doubles the need to protect and improve soil organic matter to maintain soil health and achieve sustainable agriculture (Keshavarz Afshar et al., 2014). Thus, the application of organic fertilizers, in time increase the activity of beneficial soil microorganisms, provides nutrients like nitrogen (N), phosphorus (P), and potassium (K), and subsequently improves plant growth and yield (Madrid et al., 2007). The use of animal manure (AM) is an effective practical strategy for optimal crop production and wheat enrichment due to its role in improving soil fertility (Majidi and Shahbazi, 2020). Previous studies have shown that the application of AM manure improved the growth, grain yield, and nutrients uptake like N, K, and zinc (Zn) in wheat grain (Azad et al., 2022).

Zn is an essential element for plant growth, and its deficiency can cause an imbalance of nutrients in the plant and ultimately reduce the quantitative and qualitative yield of the crop (Abbasi et al., 2019). Zn deficiency in the soil can reduce yield by up to 40%, without the plant

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showing symptoms of Zn deficiency (Amanullah et al., 2020). Soil Zn deficiency may also lead to Zn deficiency in humans, especially in countries where diets are predominantly cereal-based (Cakmak and Kutman, 2017). Application of Zn fertilizer in Zn-poor soils is a suitable strategy for supplying Zn to the soil, which helps increase crop yield and improve Zn accumulation in plant tissue (Mahmoud Soltani et al., 2017). Shekari et al. (2015) reported that adding Zn to the soil increased the quantitative and qualitative yield of wheat crop. In another study, Seyed Hayat Gheyb et al. (2019) documented that the Zn application resulted in the highest number of grains per spike, thousand-grain weight, grain yield, and grain protein content. Kheyri (2024) documented that foliar application of ZnO-NPs has a great potential in increasing rice yield by enhancing yield attributes and also improving rice quality by enriching rice grain with Zn.

Silicon (Si) is the second most abundant element in the Earth's crust (Verma et al., 2020). Although Si is not recognized as an essential element for plants, it is considered a beneficial element for improving plant growth and productivity (Gaur et al., 2020). It has been documented that adding Si supplementation to plants improves growth and enhances plant tolerance to biotic and abiotic stresses (Hadi et al., 2016). It has been reported that the use of Si can have numerous beneficial impacts on plants (Verma et al., 2021). Salem et al. (2022) observed that the application of different sources of Si fertilizer significantly increased all agronomic traits of wheat. Rezabeighi et al. (2020) found that the application of Si can help increase the number of grains per spike, thousand-grain weight, biomass yield, grain yield, and harvest index in wheat. Saberiyan-Ranjbar et al. (2019) demonstrated that the Si application can play a vital role in improving the nutrients uptake in plants. Previous studies have shown that the application of Si supplementation can help improve the absorption of Si in the grain and subsequently increase the nutritional quality of rice (Kheyri, 2022).

The positive role of organic fertilizers in supplying nutrients and improving the physical, chemical and biological properties of the soil makes these fertilizers an ideal option for use in soils poor in organic matter. Also, the addition of Si and Zn to the wheat plants while ameliorating crop growth and yield, helps improve human health by increasing the bioavailability of these elements in grain. Thus, the present study aimed to evaluate the impacts of AM along with Si and Zn fertilizers on agronomic traits, grain yield, nutrient uptake and protein content in wheat grains.

MATERIAL AND METHODS

Experimental site

The field experiments were conducted during the seasons of 2018-2019 and 2019-2020 at the Research Farm of Islamic Azad University, Qaemshahr Branch, Mazandaran Province, Iran (36°28′N, 52°49′E; 30 m above sea level). The meteorological data of the experimental site during growth and development of wheat for both crop years are presented in Table 1. Soil sampling was performed at the experimental site at depths of 0-30 cm before the initiating experiment. The soil texture in the study site was Clay Loam. The results of soil physical and chemical properties are presented in Table 2.

Table 1. Meteorological data during the wheat vegetation period (2018-2019 and 2019-2020)

Months	Average temperature (°C)						TP (mm)		TSHM (h)	
	Min		Max		Average					
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Nov	12.3	11.3	20.5	19.8	16.4	15.5	57.8	228.4	127.4	128.0
Dec	9.1	6.4	16.9	15.8	13.0	11.1	65.7	28.0	98.3	140.4
Jan	4.8	5.5	15.7	15.3	10.2	10.4	128.3	44.9	133.8	132.0
Feb	5.6	3.8	15.0	15.5	10.3	9.7	106.5	129.2	132.2	156.4
Mar	6.7	7.3	17.1	16.5	11.5	11.5	170.7	103.4	127.6	106.1
Apr	10.6	9.8	18.8	18.2	14.7	14.0	78.2	80.8	131.5	121.4
May	15.3	14.8	25.8	25.0	20.6	19.9	51.1	33.4	200.6	182.5
Jun	21.4	20.3	31.7	31.7	26.5	26.0	1.0	4.0	272.4	297.0

Table 2. Physical and chemical characteristics of the experimental soil before planting

Years	2018-2019	2019-2020
Properties		
Soil texture	Clay Loam	Clay Loam
pH	8.3	7.8
EC, ds m^{-1}	0.68	0.62
Organic matter, %	1.5	1.4
Organic carbon, %	0.87	0.81
Total N, %	0.084	0.079
Available P, mg kg^{-1}	8	9
Available K, mg kg^{-1}	140	165
Available Zn, mg kg^{-1}	1.2	1.4

Experimental design and treatments

The experiment was carried out as a split plot in a randomized complete block design with three replications. The experimental treatments included the main plot assigned to AM application (control or non-application of AM and AM application), and the sub-plot assigned to Si and Zn fertilizers application (control, zinc sulfate, potassium silicate, ZnO-NPs, Si-NPs, potassium silicate + zinc sulfate, ZnO-NPs + potassium silicate, Si-NPs + zinc sulfate, and Si-NPs + ZnO-NPs).

Wheat planting and management

For land preparation, a moldboard plow and a disc were used in this experiment. Then, the experimental field was divided into three equal replications; each replicate had 18 plots. The plots size was 4.8 m^2. Each experimental plot consisted of 6 planting rows with row spacing of 20 cm and a length of 4 m. The wheat (Ehsan variety) was used as plant material in the present research. Planting was done manually at a density of 400 seeds m^2 (135-150 kg ha^{-1}) at the last week of November. The AM from the source of cow manure (12 t ha^{-1}) was mixed with soil at the planting time. The potassium silicate and zinc sulfate were applied as basal at the rate of 400 and 40 kg ha^{-1}, respectively. The Si-NPs and ZnO-NPs (produced by the US Research Nanomaterials, Inc) at a concentration of 50 mg L^{-1} was sprayed at the early, mid, and late stages of tillering and heading. The characteristics of the Si-NPs and ZnO-NPs applied in this study are presented in Table 3.

Table 3. The characteristics of the nanoparticles applied in this experiment

Nanoparticles	Purity percentage (%)	Particles size (nm)	True density (g cm^{-3})	SSA (g m^{-2})	Color
SiO_2	>99%	20-30	2.4	180-600	White
ZnO	>99%	10-30	5.606	20-60	Milky white

Weed control was carried out manually within the plots and between the plots using Tribenuron Methyl 75% DF and Puma super 75% EW for broadleaf and narrowleaf weeds, respectively. No pests or diseases were observed during the wheat growth period.

Sampling and measurement

At physiological maturity, the harvesting was done manually after removing the border rows. The determination of plant height and spike length was performed by measuring 10 plants in each plot. The number of spikelets per spike and number of seeds per spike was measured by counting of 15 spike. The 1000-grain weight was recorded by counting and weighing 10 samples of 100 seeds. The grain yield was determined by manually harvesting an area of 2 m^2 in the middle of each plot, and was recorded after adjusting the moisture content to 14%. The grain Si concentration by colorimetric method (Dallagnol et al., 2011) and grain Zn concentration by Atomic Absorption Spectrometry (AAS) method (Emami, 1996) were determined. The grain protein content was calculated by total N multiplied by 6.25 (Payegozar, 2008).

Statistical analysis

Statistical analysis of data was performed using SAS software ver. 9.2. Analysis of variance was conducted as a randomized completely block design with split plot arrangement combined over the years. Means values were compared by least significant difference (LSD) test at 5% probability level.

RESULTS AND DISCUSSION

Agronomic traits and grain yield

The results of combined analysis of variance indicated that the main effect of year was significant only on number of spikelets per spike. All the yield components and grain yield except 1000-grain weight were significantly affected by the main effects of animal manure (AM). Also, the simple effect of silicon + zinc (Si + Zn) was significant on plant height, number of spikelets per spike, number of seeds per spike and grain yield. The two-way interaction between AM and Si + Zn application ($P < 0.05$) was significant for grain yield (Table 4).

Table 4. Multivariate analysis of variance for agronomic traits and grain yield of wheat

Source of variation	df	Plant height	Spike length	No. of spikelets spike^{-1}	No. of seeds spike^{-1}	1000-grain weight	Grain yield
Year (Y)	1	12.67^{ns}	0.06^{ns}	1.11**	3.50^{ns}	5.11^{ns}	23290.70^{ns}
Replication (Y)	4	94.45	1.23	0.11	8.22	1.46	124348.41
Animal manure (AM)	1	784.08**	15.26**	19.79**	454.77**	4.67^{ns}	26349960.33**
Y×AM	1	2.08^{ns}	0.14^{ns}	2.32**	0.09^{ns}	10.29^{ns}	802556.48**
Error	4	51.80	0.42	0.06	9.52	2.57	78905.85
Si + Zn	8	81.81*	0.63^{ns}	0.54**	205.50**	1.12^{ns}	573765.79**
Y×Si + Zn	8	2.25^{ns}	0.13^{ns}	0.03^{ns}	9.70^{ns}	1.33^{ns}	14231.16^{ns}
AM×Si + Zn	8	31.12^{ns}	0.12^{ns}	0.07^{ns}	2.96^{ns}	2.13^{ns}	68843.33*
Y×AM×Si + Zn	8	5.04^{ns}	0.04^{ns}	0.11^{ns}	10.19^{ns}	0.93^{ns}	127960.98**
Error	64	30.70	0.42	0.11	12.18	3.57	25334.42
CV (%)	-	5.40	6.41	1.88	8.90	4.16	4.10

^{ns}, *, and ** are non-significant and significant at the 5 and 1% probability levels, respectively.

The results indicated that the plant height was significantly positive influenced by the AM application. In the present research, when the AM was added to the soil, the plant height increased by 5.12% compared with control or non-application of AM (Table 5). Organic fertilizers increase the growth of aerial parts and dry matter production by improving the physical and chemical properties of the soil, creating a suitable environment for root growth, and providing nutrients needed by the plant (Saiedinejad et al., 2022). Our results are confirmed by Azad et al. (2022) who found that the AM (cow manure) application significantly enhanced the plant height of wheat compared with control plants.

Our findings showed that in all treatments where Zn fertilizer was used in both zinc sulfate and Zn-NPs forms, either individually or in combination with Si sources, the plant height increased significantly compared with the control plants. In this study, the highest plant height was observed in treatments of zinc sulfate (104.9 cm), Zn-NPs (104.9 cm), potassium silicate + zinc sulfate (105 cm) and Si-NPs + zinc sulfate (104.9 cm), although there was no significant difference with the potassium silicate + Zn-NPs (102.5 cm) and Si-NPs + Zn-NPs (102.7 cm). However, the application of Si sources (potassium silicate or Si-NPs) alone could not produce a significant change in wheat plant height (Table 5). Raeesi Sadati et al. (2021) documented that Zn application increased stem length and internode length in wheat plants by increasing indole acetic acid production. Our results are in agreement with Shahmardan et al. (2022) and Raeesi Sadati et al. (2021) who reported that the zinc sulfate as soil application and ZnO-NPs as foliar application caused a significant increase in plant height in rapeseed and wheat plants, respectively. Narimani et al. (2024) attributed the enhance in wheat plant height by foliar application of ZnO-NPs to an improvement in chlorophyll index and an increase in dry weight and root volume. In similar results, Sarto et al. (2014) found that the plant height of wheat did not respond positively to Si fertilization.

Table 5. Mean comparison of main effects of Y, AM and Si+Zn on agronomic traits and grain yield of wheat

Experimental treatments	Plant height (cm)	Spike length (cm)	No. of spikelets $spike^{-1}$	No. of seeds $spike^{-1}$	1000-grain weight (g)	Grain yield $(kg\ ha^{-1})$
Year						
2018-2019	102.20a	10.10a	18.10b	39.03a	45.62a	3863a
2019-2020	102.88a	10.15a	18.31a	39.39a	45.18a	3893a
AM						
Control (non-application of AM)	99.85b	9.75b	17.78b	37.16b	45.19a	3384b
Application of AM	105.24a	10.50a	18.63a	41.26a	45.61a	4372a
Si+Zn						
Control	99.91b	9.61b	17.69b	31.82d	45.84a	3399g
Zinc sulfate (ZS)	104.91a	10.15a	18.09a	35.66c	45.29a	3748f
Potassium silicate (PS)	99.33b	10.24a	18.30a	35.68c	45.81a	3799ef
ZnO-NPs	104.91a	10.26a	18.15a	39.03b	45.06a	3876def
Si-NPs	98.75b	10.05ab	18.35a	39.03b	45.31a	3906cde
PS + ZS	105.00a	10.09ab	18.36a	42.25a	45.51a	4059ab
PS + ZnO-NPs	102.50ab	10.23a	18.30a	42.46a	45.09a	4012bc
ZS + Si-NPs	104.91a	10.05ab	18.31a	42.77a	45.61a	3961bcd
Si-NPs + ZnO-NPs	102.66ab	10.45a	18.30a	44.19a	45.08a	4145a

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$.
AM: animal manure; ZnO-NPs: zinc oxide nanoparticles, Si-NPs: silicon dioxide nanoparticles.

As shown in Table 5, the spike length increased by 7.14% by the application of AM compared with the control plants, indicating a positive impact of AM on improving plant growth. The results of the present work are in line with recent findings by Chen et al. (2024) during the 2022-2023 crop season, as these researchers documented that adding AM (cow manure) to the soil had significant impacts on increasing wheat spike length, similar to the application of NPK chemical fertilizers. Hammad et al. (2011) documented that the application of AM along with other organic fertilizers significantly increased the spike length in wheat plants when compared with control plants.

The results suggested that the application of different treatments of Si and Zn in both individual and combined forms resulted in ameliorated spike length when compared with the control treatment. However, the highest spike length (10.45 cm) was achieved when Si and Zn were foliar sprayed on the wheat plants in the form of nanoparticles. The lowest spike length was observed with an 8.03% reduction in control conditions or non-application of Si and Zn (Table 5). Our findings are further strengthened by the findings of Pezeshk et al. (2023), who reported that the application of Si via either foliar application or soil application increased the spike length in wheat. In another study, Kheyri et al. (2019b) observed that the use of Si and Zn in combination or alone via nanoparticles or soil application improved panicle length in rice when compared with a treatment without Si and Zn. Narimani et al. (2024) found that the application of Zn nanoparticles resulted in a 13.4% increase in spike length compared with the control treatment.

The results presented in Table 5 indicated that the number of spikelets per spike was higher in the second year (18.31) than first year (18.10) of the experiment. In our study, the application of AM increased the number of spikelets per spike by 4.56%, when compared with control treatment. Organic fertilizers increase crop growth and yield by improving the soil chemical characteristics, such as the uptake of essential nutrients, along with ameliorating the physical and biological properties of the soil, such as soil water holding capacity, soil aggregate stability, soil porosity, population and activity of beneficial soil organisms (Rayne and Aula, 2020). AM gradually releases nutrients into the soil; thus, the plant can benefit from proper nutrition at different stages of growth (Wan et al., 2021). The use of AM increases tillering, the number of fertile spikes, and the number of spikes m^{-2} by saving soil moisture and supplying the nutrients needed by wheat (Moshatati et al., 2019). These researchers demonstrated that the application of AM at rates of 15 and 30 t ha^{-1} increased the number of spike m^{-2} in wheat by 11.9 and 18.8%, respectively, when compared with control plants. Similar findings were confirmed by Hammad et al. (2011), who reported an increase in number of spikelets per spike when wheat plants received organic fertilizers like AM.

According to the results of this study, the application of all Si and Zn treatments, whether alone or in combination, enhanced the number of spikelets per spike compared with the control treatment. There was no significant difference in the spikelets number per spike between the Si and Zn treatments. The lowest number of spikelets per spike with an average of 17.69 spikelets was observed in the control treatment (Table 5). Previous studies confirm the results of the present work that Si plays a vital role in improving wheat plant growth (Maghsoudi and Emam, 2016). Narimani et al. (2024) found that foliar application of Zn and Si nanoparticles plus putrescine can help improve wheat growth and yield.

In the present study, the number of seeds per spike responded significantly to the AM application. When the wheat plants received the AM, the seeds number per spike enhanced by 9.93% compared with plants that did not receive AM treatment (Table 5). The nutrients released from organic fertilizers cause better leaf growth and improved photosynthesis, resulting in increased production of assimilable substances (Dubey et al., 2022). Moradi and Shahbazi (2023) observed that the grain number per spike enhanced by 11.8% when the AM (sheep manure) was added to the soil.

Our findings indicated that the application of Si and Zn fertilizers in both nanoparticles and soil application forms, either individually or in combination, improved the number of seeds per spike when compared with the control treatment. However, the results showed that the highest seeds number per spike was obtained with the combined treatments of Si and Zn in both nanoparticles and soil application forms. The application of potassium silicate + zinc sulfate, potassium silicate + ZnO-NPs, zinc sulfate + Si-NPs and Si-NPs + ZnO-NPs increased the number of seeds per spike by 24.7, 25, 25.6, and 28%, respectively, compared with the control. We also found that foliar application of nanoparticles on wheat plants had better impacts on improving the number of seeds per spike compared with soil application of this elements, such that the separate application of Si and Zn nanoparticles enhanced the seeds number per spike by 8.6% compared with soil application of Si and Zn. No significant difference was observed between Si and Zn in either nanoparticles or soil form in terms of the number of seeds per spike (Table 5). Zn plays a prominent role in carbohydrate metabolism, controlling growth hormones, increasing enzyme activity, and seed production and maturation (Laware and Raskar, 2014). Application of Si helps increase photosynthesis and carbohydrate production by improving chlorophyll content, increasing Rubisco enzyme activity, and enhancing leaf number and area (Savvas and Ntatsi, 2015). Foliar application of ZnO-NPs, by improving chlorophyll content and increasing photosynthesis rate, enhances the duration of the grain filling period and subsequently increases the number of seeds per spike in wheat (Babaie et al., 2020). Our findings are further strengthened by the findings of Mohammadi Kale Sarlo and Seyed Sharifi (2022), who reported that the addition of Si and Zn fertilizers to the wheat plants increased the number of seeds per spike compared with control treatment. Narimani et al. (2024) reported that plants treated with Si-NPs and ZnO-NPs showed a 10.4 and 8% increase in the number of seeds per spike, respectively, when compared with untreated plants. In another study, Kheyri et al. (2019a) documented that the application of Zn and Si fertilizers in both nanoparticle and soil application forms had significant positive effects on increasing the number of filled grains per panicle in rice plants compared with the treatment without application of Zn and Si.

In this research, no significant difference was observed in terms of 1000-grain weight between the use and non-use of AM, as well as between different Si and Zn treatments. The grain weight is influenced by the genetic composition of the wheat plants (Zarea, 2024). Chen et al. (2024) reported that no significant difference was observed between cow manure, pig manure, and chemical fertilizer treatments in terms of thousand-grain weight of wheat. Similar findings were confirmed by Narimani et al. (2024), who reported that the foliar application of Si and Zn nanoparticles individually or in combination failed to produce a significant change in the 100-grain weight of wheat compared with the treatment without nanoparticles.

The results in present study showed that the grain yield significantly increased in response to AM application. The AM-treated plants produced significantly higher grain yield (22.6%) than non-treated plants (Table 5). As it is shown in Table 5, the wheat plants treated with Si-NPs + ZnO-NPs (4145 kg ha^{-1}) had higher grain yield than other treatments, although there was no significant difference with the simultaneous application of potassium silicate + zinc sulfate (4059 kg ha^{-1}). We found that the greatest yields were observed when Si and Zn were applied simultaneously, either as soil or as nanoparticles. Although the application of Si and Zn in the form of nanoparticles had slightly better impacts than soil application in terms of grain yield, the difference was not significant. Also, application of both Si and Zn fertilizers resulted in similar grain yield.

Interaction between AM × Si+Zn for grain yield (Table 6), illustrated that the highest grain yield (4784 kg ha^{-1}) was obtained by co-application of AM + Si-NPs + ZnO-NPs, followed by AM + potassium silicate + zinc sulfate (4525 kg ha^{-1}). The lowest grain yield with a 36.8% reduction was observed in control plants. This augmentation in yield could be attributed to greater nutrient accumulation and higher agronomic traits resulting from the co-application of AM, Si, and Zn in the present study. Our results demonstrated that in the absence of AM, co-application of Si and Zn as soil application had similar performance to foliar application of nanoparticles in terms of grain yield, but when AM was added to the soil, the use of the nanoparticles sources had more positive effects on improving grain yield than soil application. Our findings showed that adding AM to Si and Zn sources can have positive effects on improving grain yield. The application of AM helps to absorb nutrients and subsequently increase crop yield by neutralizing acid and regulating soil pH (Sun et al., 2015). Increased wheat grain yield following the use of AM can be due to increased organic matter, improved soil fertility, enhanced soil water holding capacity, and gradual release of nutrients (Zemikhael and Dechassa, 2018). Jalili (2017) suggested that the application of AM (20 t ha^{-1}) was an ideal option to enhance the quantitative and qualitative yield of wheat. Si supplementation increases the photosynthesis rate and crop yield (Zargar et al., 2019) by Si deposition in the leaf, increasing leaf strength, and improving chlorophyll content in the leaf surface (Maghsoudi et al., 2013). Salem et al. (2022) documented the positive effects of potassium silicate in increasing wheat grain yield. Zn application increases crop yield by increasing vegetative growth, improving photosynthetic system (Abbasi et al., 2019) and enhancing nutrients uptake like N, P and K (Kuchak Dezfuli et al., 2019). Similarly, Ma et al. (2017) observed that the Zn foliar application significantly increased the grain yield in wheat plants. In another study, Damary et al. (2017) reported that Zn application by both foliar and soil application methods resulted in improved grain yield, although soil application had more positive effects in increasing yield than foliar application.

Table 6. Mean comparison of interactions between AM and Si + Zn on grain yield and grain protein content of wheat

		Grain yield (kg ha^{-1})	Grain protein (%)
Control (non-application of AM)	Control	3021g	7.39h
	Zinc sulfate (ZS)	3284f	10.02fg
	Potassium silicate (PS)	3296f	8.13gh
	ZnO-NPs	3371ef	11.86c-f
	Si-NPs	33326f	8.29gh
	PS + ZS	3592de	11.70c-f
	PS + ZnO-NPs	3534def	12.66b-e
	ZS + Si-NPs	3521def	11.17def
	Si-NPs + ZnO-NPs	3506ef	12.00c-f
Application of AM	Control	3777d	11.42def
	Zinc sulfate (ZS)	4211c	14.78a
	Potassium silicate (PS)	4301bc	10.84ef
	ZnO-NPs	4380bc	15.02a
	Si-NPs	4480b	10.88ef
	PS + ZS	4525ab	13.10a-d
	PS + ZnO-NPs	4490b	14.22ab
	ZS + Si-NPs	4402bc	13.63abc
	Si-NPs + ZnO-NPs	4784a	14.88a

Means in columns followed by the same letter(s) are not significantly different at $P \leq 0.05$.
AM: animal manure; ZnO-NPs: zinc oxide nanoparticles, Si-NPs: silicon dioxide nanoparticles.

Nutrients uptake

The results of combined analysis of variance showed that the individual impact of AM was highly significant (P<0.01) only on protein content in grain. Also, the Si concentration in grain, Zn concentration in grain and protein content in grain were significantly (P<0.01) affected by the main effects of Si + Zn application. The two-way interaction between AM and Si + Zn application (P<0.05) was significant for protein content in grain (Table 7).

Table 7. Multivariate analysis of variance for silicon and zinc concentration and protein content in grain of wheat

Source of variation	df	Si concentration in grain (%)	Zn concentration in grain (mg kg^{-1})	Protein content in grain (%)
Year (Y)	1	0.003^{ns}	50.37^{ns}	2.05^{ns}
Replication (Y)	4	0.091	4.30	4.00
Animal manure (AM)	1	0.001^{ns}	21.90^{ns}	217.40**
Y×AM	1	0.027^{ns}	5.02^{ns}	0.001^{ns}
Error	4	0.18	19.18	1.27
Si + Zn	8	0.58**	83.36**	37.66**
Y×Si + Zn	8	0.005^{ns}	6.20^{ns}	1.41^{ns}
AM×Si + Zn	8	0.043^{ns}	10.03^{ns}	3.43*
Y×AM×Si + Zn	8	0.10^{ns}	2.14^{ns}	0.15^{ns}
Error	64	0.10	16.93	1.62
CV (%)	-	17.05	10.23	10.82

^{ns}, *, and ** are non-significant and significant at the 5 and 1% probability levels, respectively.

Table 8. Mean comparison of main effects of Y, AM and Si+Zn on Si and Zn concentration and protein content in grain of wheat

Experimental treatments	Si concentration in grain (%)	Zn concentration in grain (mg kg^{-1})	Protein content in grain (%)
Year			
2018-2019	1.93a	39.51a	11.64a
2019-2020	1.92a	40.88a	11.91a
AM			
Control (non-application of AM)	1.93a	39.74a	10.36b
Application of AM	1.93a	40.65a	13.19a
Si+Zn			
Control	1.67c	36.30c	9.40c
Zinc sulfate (ZS)	1.68bc	40.19ab	12.40ab
Potassium silicate (PS)	1.94ab	37.40bc	9.49c
ZnO-NPs	1.66c	42.87a	13.44a
Si-NPs	2.18a	37.83bc	9.59c
PS + ZS	1.94ab	40.18ab	12.40ab
PS + ZnO-NPs	1.95ab	43.33a	13.44a
Si-NPs + ZS	2.18a	40.31ab	12.40b
Si-NPs + ZnO-NPs	2.18a	43.36a	13.44a

Means in columns followed by the same letter(s) are not significantly different at P≤0.05.

AM: animal manure; ZnO-NPs: zinc oxide nanoparticles, Si-NPs: silicon dioxide nanoparticles.

In this research, the application of AM failed to help improve the Si concentration in wheat grain. Our results showed that all Si and Zn treatments except for the individual application of Zn sources (zinc sulfate or ZnO-NPs) resulted in ameliorated grain Si concentration compared with control treatment. In fact, in all treatments where Si was applied either via nanoparticles or as soil, grain Si accumulation showed a significant increase. Previous studies showed that application of Si from any source was able to increase the Si content in wheat plants (Salem et al., 2022). Our results are in agreement with Kheyri et al. (2019a) who reported that application of Si from both soil application and nanoparticle sources resulted in a significant increase in Si concentration in rice grain. In this research, when Si-NPs was added to wheat plants individually or in combination with Zn sources, we observed higher Si accumulation in grain compared with soil application of Si. The highest grain Si concentration was observed in plants treated by Si-NPs, Si-NPs + zinc sulfate, and Si-NPs + ZnO-NPs with an average of 2.18%, whereas the lowest Si concentration in grain was recorded in treatments of ZnO-NPs (1.66%) and control (1.67%) (Table 8). This result shows the superiority of nanoparticle fertilizers over their conventional form. The reason for this superiority could be the small diameter of nanoparticles, which facilitates the speed of absorption, transport, and accumulation of these particles compared with conventional particles (Torabian and Zahedi, 2013). It has been reported that nanoparticles-plants treated had ultrahigh uptake capacity when compared with the conventional fertilizer due to the small diameter of the nanoparticles (Kheyri et al., 2018). Our findings are further strengthened by the findings of Saberiyan-Ranjbar et al. (2019), who reported that the Si concentration in wheat shoots was higher by the Si application as nanoparticles compared with different levels of potassium silicate. In another study, Kheyri et al. (2017) found that the simultaneous application of Si and Zn as nanoparticles had a similar impact as soil application in terms of nutrient uptake, although the positive effects of nanoparticles were higher than that of soil application.

In our study, although the application of AM (40.65 mg kg^{-1}) slightly ameliorated the grain Zn concentration compared with the control treatment (39.74 mg kg^{-1}), there was no significant change (Table 8). We observed that the application of Zn in both zinc sulfate and ZnO-NPs forms improved Zn concentration in wheat grain compared with the control treatment. However, higher Zn concentrations were observed in treatments where ZnO-NPs were sprayed on wheat plants, such that application of ZnO-NPs alone, ZnO-NPs + potassium silicate, and ZnO-NPs + Si-NPs led to a 15.3, 16.2, and 16.3% increase in Zn content compared with control plants, respectively. Application of potassium silicate or Si-NPs alone was unable to increase grain Zn concentration, whereas application of Zn alone, either as soil application or nanoparticles, significantly improved grain Zn content. However, foliar application of ZnO-NPs had better effects in increasing grain Zn compared with soil application of zinc sulfate (Table 8). Our results are in agreement with Farooq et al. (2018) who found that the using different Zn application methods (foliar application, soil application, seed priming, and seed coating) enhanced grain Zn concentration. In another study, Toulabi et al. (2021) reported that the Zn foliar application resulted in improved Zn content in wheat grain. Kheyri and Abbasalipour (2021) reported that foliar application of ZnO nanoparticles at doses of 50 and 75 mg L^{-1} increased Zn concentration in rice grain by 44.2 and 51.8%, respectively, when compared with the control treatment. Other studies have shown that foliar application of ZnO-NPs and soil application of zinc sulfate increased Zn accumulation in wheat grain by 14.7 and 4%, respectively (Abdollahi et al., 2018), and in rice grain by 33.2 and 26.6%, respectively (Kheyri et al., 2019a), when compared with the treatment of no Zn application.

The results presented in Table 8 showed that the addition of AM to the wheat plants produced significantly higher protein content (13.19%) when compared with control plants

(10.36%). We found that the ZnO-NPs-treated plants had significantly higher protein content when compared with other experimental treatments. In this study, except for the individual Si treatments, all other experimental treatments resulted in a significant increase in grain protein content. However, the highest protein values (13.44%) were obtained when ZnO-NPs alone, ZnO-NPs + potassium silicate, and ZnO-NPs + Si-NPs treatments were applied. Also, the lowest protein content (9.40%) was observed in the control treatment or the lack of Zn and Si. We found that the role of Zn fertilizer in improving grain protein content was more prominent than Si, and also among Zn sources, foliar application of ZnO-NPs was superior to soil application of zinc sulfate.

The assay of interaction between AM × Si+Zn for protein content in grain revealed that highest grain protein content was obtained with averages of 14.78, 15.02 and 14.88% by co-application of AM + zinc sulfate, AM + ZnO-NPs and AM + Si-NPs + ZnO-NPs, respectively, although there was no significant difference by the simultaneous application of potassium silicate + zinc sulfate (13.10%), potassium silicate + ZnO-NPs (14.22%) and zinc sulfate + Si-NPs (13.63%). Our findings showed that adding AM to each of the Si and Zn application levels resulted in a significant increase in grain protein content compared with Si and Zn treatments without AM. When AM was combined with each of the Zn sources, it had more positive impacts on improving grain protein than when AM was used simultaneously with Si sources. There was no significant difference in grain protein content between the application of ZnO-NPs and zinc sulfate. However, ZnO-NPs foliar application had a slight advantage in increasing grain protein content compared zinc sulfate soil application. Other researchers studying the application of Zn to rice plants documented that the application of Zn in both the form of nanoparticles foliar application and soil application similarly improved protein content in rice plant tissue, although nanoparticles had slightly better effects (Kheyri et al., 2019a). By increasing the availability of soil absorbable N, AM led to a greater supply of absorbable N in the wheat root environment and subsequently improve the N concentration and protein content of wheat grain compared with the control treatment (Majidi and Shahbazi, 2020). Zn plays a vital role in the synthesis of protein and amino acids and is also an important factor in facilitating protein transport into wheat grain (Kheirizadeh Arough et al., 2016). In a study, Toulabi et al. (2021) reported that foliar Zn spraying and organic fertilizer application alone or in combination were able to increase the protein content of wheat grain. An earlier report by Mousavian et al. (2023) showed that soil application of zinc sulfate enhanced the grain protein content in wheat plants when compared with untreated plants.

CONCLUSIONS

Our findings illustrated that adding animal manure to the soil significantly increased yield components, yield, and grain protein content of wheat, except for 1000-grain weight and grain Si and Zn concentrations, when compared with plots that did not receive animal manure. The findings indicated that co-application of Si and Zn, either through foliar application of nanoparticles or soil application, produced the highest yields, which were significantly higher than the application of these elements alone. However, when Si and Zn in the form of nanoparticles were foliar sprayed on wheat plants, somewhat higher yields were achieved than when Si and Zn were applied as soil. Higher grain yield was observed when animal manure was applied along with ZnO-NPs and Si-NPs. In the comparison between Zn and Si, the application of both fertilizers produced similar grain yield, whereas the application of Si sources resulted in higher Si accumulation in grain and the application of Zn sources resulted in higher Zn accumulation and protein content in wheat grain. In general, higher Si concentrations were observed in treatments where Si-NPs were applied alone or in combination with Zn sources, or higher Zn concentrations and protein content were observed when ZnO-NPs were used alone or in combination with Si sources, indicating that foliar application of nanoparticles has better impacts in improving Si and Zn accumulation in wheat grain than soil application. Overall, the results of the present study revealed that by the co-application of animal manure + ZnO-NPs + Si-NPs can be improved both yield and quality of wheat plants by improved of nutrients uptake like Si and Zn and also enhancing the grain protein content.

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