

# The Interactive Effect of Selenium and Farmyard Manure on Soil Microbial Activities, Yield and Selenium Accumulation by Wheat (*Triticum aestivum* L.) Grains

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#### Abstract

This study assessed the interactive effect of selenium (Se) and farmyard manure (FYM) on soil microbial activities, growth, yield, and Se accumulation by wheat grains. Preliminarily, the effect of Se (0–250  $\mu$ g kg<sup>-1</sup> soil) and FYM (0–12.5 g kg<sup>-1</sup> soil) was assessed on soil microflora. Selenium exhibited an adverse impact on soil microflora; respiration was decreased at  $\geq 10 \ \mu$ g kg<sup>-1</sup> soil while dehydrogenase and urease activities were decreased at  $\geq 125 \ \mu$ g kg<sup>-1</sup> soil. At 250  $\mu$ g Se kg<sup>-1</sup> soil, respiration, dehydrogenase and urease activities were decreased by 81, 40 and 35%, respectively, on unamended soil, and by 9, 47 and 22%, respectively, on FYM-amended soil. The subsequent plant experiments were conducted with same Se and FYM rates; one was harvested 42 days after sowing and other at crop maturity. The application of 125  $\mu$ g Se kg<sup>-1</sup> and 12.5 g FYM kg<sup>-1</sup> soil improved seedling biomass by 12.6 and 22%, respectively, while their combined use lacked synergistic effect. Similarly, at maturity Se and FYM increased grain yield while their combined effect was not synergistic. The Se-induced suppression in microbial activities was not related to yield which was improved (11% at the highest rate in unamended soil) by Se application. Selenium application increased grain Se content in a rate-dependent manner, it increased from 0 to 1025  $\mu$ g kg<sup>-1</sup> by applying 250  $\mu$ g Se kg<sup>-1</sup> soil. Moreover, FYM application decreased Se accumulation in grains. It is concluded that FYM application increased soil microbial activities and yield but reduced grain Se accumulation in wheat on Se-applied soil.

Keywords Soil respiration · Dehydrogenase · Urease · Farmyard manure · Selenium · Wheat

## Introduction

Selenium (Se) is an essential trace element for humans, animals and some species of microorganisms (Rayman 2012; Hossain et al. 2021). In humans, Se plays an important role in antioxidation, detoxification and functioning of immune system. Its deficiency induces the risk of Keshan disease, Kashin–Beck disease, muscle syndrome, liver disease, cancer, etc. (Arinola and Charles-Davies 2008; Huang et al. 2011; Guo et al. 2020; Stolwijk et al. 2020). The protection against these lethal diseases requires daily intake of

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Muhammad Imran muhammad.imran@niab.org.pk 50–55  $\mu$ g Se person<sup>-1</sup> for adults (WHO 2009). However, these levels are rarely met in most of the countries including Pakistan. Selenium is considered to be the 4<sup>th</sup> essential trace element after iron, zinc and iodine which is deficient in a huge population. The origin of Se deficiency in humans is believed to be low dietary intake of this element as most of the edible crops have low Se content. The low inherent soil Se content and lack of Se fertilization are the most reported reasons of low Se content in edible crops. Worldwide, the background level of Se in soils ranges from 0.01 to 2.0 mg kg<sup>-1</sup> with average value of < 0.5 mg kg<sup>-1</sup> soil (Fordyce, 2007). In Pakistan, Se concentration in soils is very low and reported values range between 0.032 and  $0.372 \text{ mg kg}^{-1}$  soil (Ahmad et al. 2009; Khan et al. 2006; Zou et al. 2019). This implies that soil application of Se may help improve Se levels in food crops and hence could improve dietary intake of Se in Pakistan.

There are some studies on the soil application of Se on growth, physiology, yield, and its concentration in wheat grains, which is the  $2^{nd}$  most extensively cultivated crop in

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the world (Adams et al. 2002; Curtin et al. 2008; Xiaoqin et al. 2009; Xia et al. 2020). However, these studies lack consideration of the influence of Se on soil microorganisms which are a key component for soil fertility and crop production. Microorganisms facilitate plant growth through decomposition of crop residues, reduction in nutrient fixation in soil, phytohormones production, disease suppression, abiotic stress tolerance induction and biological nitrogen fixation (Khan and Joergensen 2009; Vanzolini et al. 2017; Fukami et al. 2019; Kalayu 2019; Frankenberger and Arshad 2020; Yadav et al., 2020; Basu et al. 2021) and thus play an important role in increasing the nutrient use efficiency of crop plants. The mechanisms by which Se application in soils improves growth and yield of plants are not well understood; apart from improving physiological functions, it may have some positive effects on the activities of soil microorganisms. On the other hand, Se application at high rates, for enriching grains with Se, may be toxic to soil microorganisms, thereby affecting their beneficial activities. Therefore, it is imperative to identify the critical levels of soil-applied Se that enhances grain Se content of wheat but non-toxic or beneficial for soil microorganisms.

The farmyard manure (FYM) is commonly applied to increase soil organic matter (SOM) in soils for higher crop yields all over the world including Pakistan. The SOM may affect Se uptake by crop plants through reduction of selenate to selenite (SeO<sub>3</sub><sup>2-</sup>), adsorption/complexation and volatilization of methylated Se from soil (Tokunaga et al. 1996; Dhillon et al. 2010; Li et al. 2017). The addition of FYM

Table 1Salientphysicochemical propertiesof the experimental soil andfarmyard manure

to soil is expected to enhance microbial activities, growth and yield of wheat. On the other hand, it may interfere with the Se uptake by wheat plants. However, in our soils, having very low indigenous Se and SOM, the efficiency of soil application of Se on FYM-amended soil has not been investigated. Thus, it was hypothesized that Se fertilization of soil adversely affects soil microflora, and FYM application to Se-fertilized soil ameliorates the adverse effects of Se on microflora, and reduces Se uptake and its accumulation in wheat grains. Therefore, the present study was conducted to investigate the interactive effect of Se and FYM application on soil microbial activities, growth, yield and Se accumulation by wheat grains in a pot experiment.

## **Materials and Methods**

# Collection and Preparation of Soil and Farmyard Manure

Surface soil (0–20 cm) was collected from the research field of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan (31° 23′ 58.92″ N, 73° 1′ 59.88″ E). Soil was air dried, sieved through a 2 mm sieve to remove stones and plant residues if any and analysed for various physicochemical characteristics (Table 1). Farmyard manure was collected from a nearby local farm and its composition was determined (Table 1). The FYM was dried, ground and

Parameter	Unit	Value	References
Soil			
Textural class		Loam	Gee and Bauder (1986)
pH <sub>s</sub>		$7.93 \pm 0.21$	McLean (1983)
EC <sub>e</sub>	$dS m^{-1}$	$2.21 \pm 0.23$	Allison and Richards (1954)
Organic matter	%	$0.65 \pm 0.31$	Walkley and Black (1934)
Total nitrogen	%	$0.07 \pm 0.002$	Bremner (1960)
Available phosphorous	mg kg <sup>-1</sup>	$10.06 \pm 0.38$	Olsen et al. (1954)
Extractable potassium	mg kg <sup>-1</sup>	$307 \pm 5.25$	Simard (1993)
Water-soluble potassium	mg kg <sup>-1</sup>	$93.3 \pm 3.60$	Richards (1954)
Water-soluble sodium	mg kg <sup>-1</sup>	$283 \pm 15.90$	Richards (1954)
Farmyard manure			
Total carbon	%	$19.21 \pm 0.91$	Walkley and Black (1934)
Total phosphorus	%	$0.260 \pm 0.004$	Chapman and Pratt (1961)
Total nitrogen	%	$0.793 \pm 0.016$	Bremner and Mulvaney (1982)
Total potassium	%	$1.931 \pm 0.046$	Estefan et al. (2013)
Moisture content	%	$14 \pm 0.5$	Niwa et al. (2007)
C:N ratio	_	$24.21 \pm 0.12$	-
pH (1:10 H <sub>2</sub> O)	_	$7.88 \pm 0.005$	_
EC (1:10 H <sub>2</sub> O)	$dS m^{-1}$	$5.83 \pm 0.18$	_

Values are means  $\pm$  SE of three technical replicates

passed through a 2 mm sieve. A portion of the soil required for the experiments was amended with 12.5 g kg<sup>-1</sup> FYM.

# Soil Incubation Experiment: Effect of Soil-Applied Se on Soil Microbial Activities in Unamended and FYM-Amended Soil

Soil incubation experiment was conducted to assess the alone and interactive effects of Se and FYM on soil microbial activities (soil respiration, dehydrogenase and urease activity). Fifty grams of both unamended and FYM-amended soils were taken in 100 mL glass beakers and spiked with Se to develop 0, 10, 25, 50, 125 and 250  $\mu$ g Se kg<sup>-1</sup> soil. The required quantities of Se were added to the soils as aqueous solution using sodium selenate salt so that the solution was sufficient to saturate the soil to field capacity. Treatments with three replicates were arranged according to complete randomized design (CRD) with factorial arrangements. For quantification of soil respiration, glass beakers containing different treatments were placed in mason jars (air tight) containing 30 mL NaOH (0.05 M) in the bottom, whereas empty mason jars were used for dehydrogenase and urease activities.

#### **Soil Respiration**

Soil respiration was determined following the method of Arshad et al. (2020). After 3 days of incubation at room temperature, NaOH was reacted with 10 mL BaCl<sub>2</sub> (0.5 M) and filtered. The filtrates were transferred to 100 mL conical flasks, added with 150  $\mu$ L phenolphthalein solution and titrated against 0.1 M HCl to the colourless end point. Carbon dioxide produced was calculated as follows:

$$R_{CO_2} = \frac{2.2(V_b - V_P) \times 1000}{m_s}$$
(1)

where  $R_{CO_2}$  is the rate of CO<sub>2</sub> evaluation (mg CO<sub>2</sub> kg<sup>-1</sup>),  $V_b$  is the volume (mL) of HCl consumed in the control,  $V_p$  is the volume (mL) of HCl consumed in the test sample,  $m_s$  is the mass (g) of dry soil sample and 2.2 is conversion factor (1 mL of 0.1 M HCl corresponds to 2.2 mg of CO<sub>2</sub>) (mg mL<sup>-1</sup>).

#### **Dehydrogenase Activity**

Dehydrogenase activity was measured following the methods of Singh and Kumar (2008). After 7 days of incubation, 5 g moist soil (14% moisture content) was taken in 50 mL Falcon tubes, added with 5 mL triphenyl tetrazolium chloride (1%) and incubated at 30 °C in the dark. After 24 h, 25 mL acetone was added to each tube. Resulting suspensions were filtered and optical densities of the filtrates were measured at 546 nm using a UV–visible spectrophotometer (Hitachi, U-2800). The calibration curve of triphenyl formazan (TPF) working standards was used to determine TPF concentration in Se- and FYM-added soils. Dehydrogenase activity is expressed as  $\mu g$  TPF g<sup>-1</sup> soil.

#### **Urease Activity**

Urease activity in the soil samples was determined according to the method of Tabatabai and Bremner (1972). Briefly, after 7 days of incubation, 5 g moist soil was taken in 50 mL volumetric flasks and added with 0.2 mL toluene and 9 mL tris(hydroxymethyl)aminomethane (THAM) buffer solution. The flasks were swirled to mix the contents, added with 1 mL of 0.2 M urea solution and swirled again. The flasks were tightly stoppered and incubated at 37 °C. After 2 h, about 30 mL of KCl (2.5 M)-Ag<sub>2</sub>SO<sub>4</sub> (100 ppm) was added into each flask and cooled to room temperature. The contents were diluted to 50 mL with KCl-Ag<sub>2</sub>SO<sub>4</sub> and ammonium (NH<sub>4</sub><sup>+</sup>)-N was analysed in the resulting soil suspensions. For this purpose, 20 mL aliquot from soil suspension was taken in distillation tube followed by the addition of 1 g MgO (pre-heated at 500 °C). The distillate was collected in boric acid-mixed indicator solution and titrated against 0.005 N sulfuric acid (Bremner and Keeney 1966). Urease activity is expressed as mg  $NH_4^+$ -N g<sup>-1</sup> soil.

# Plant Experiments: Effect of Se on Growth, Yield and Se Accumulation in Wheat Grains on Unamended and FYM-Amended Soils

#### Seedling Stage Experiment

A pot experiment was conducted to evaluate the effect of different levels of soil-applied Se on growth and Se accumulation by wheat grown on unamended and FYM-amended soils. 0.5 kg of both unamended and FYM-amended soils was taken in each plastic pot and spiked with different levels of Se viz. 0, 10, 25, 50, 125 and 250  $\mu$ g kg<sup>-1</sup> soil. The required quantities of Se were added to soil as aqueous solution which was sufficient to saturate the soil. Following treatment application, the pots were kept at room temperature for equilibration. Before sowing of seeds, N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were added to the soil at the rate of 60, 45 and 37.5 mg kg<sup>-1</sup> soil, respectively, as urea, di-ammonium phosphate and sulphate of potash. Six uniform-sized healthy seeds of wheat (cv. Faisalabad-2008), obtained from Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan), were sown in each pot. Before the sowing of seeds in pots, germination test was performed which showed that seeds had 100% germination rate (Arshad et al. 2020). At the three-leaf stage, seedlings were thinned to three plants  $pot^{-1}$ . Soil in pots was

maintained at about field capacity throughout the experimental period. The pots were placed in a wire-house having average day-time temperature 21 °C, night-time temperature 13 °C, humidity 64%, and photoperiod time of 14 h during the experimental period. Plants were harvested after 42 days of sowing. Roots and shoots were separated, well washed with distilled water, air dried and placed in an oven at 65 °C for 72 h to determine their dry weight (g plant<sup>-1</sup>).

#### **Maturity Stage Experiment**

In parallel to seedling stage experiment, a pot experiment was conducted to evaluate the impact of soil-applied Se and FYM, applied at the same levels as described above, on yield, Se content and uptake in wheat grains. All the procedures were the same as described for seedling stage experiment, except for the soil quantity which was 5 kg pot<sup>-1</sup> and two additional applications of 15 mg N kg<sup>-1</sup> soil at tillering and booting stages. At crop maturity, data regarding tillers (No. plant<sup>-1</sup>), straw yield (g plant<sup>-1</sup>), spike weight (g plant<sup>-1</sup>), grain yield (g plant<sup>-1</sup>), grain Se content ( $\mu$ g kg<sup>-1</sup>), and grain Se uptake (mg pot<sup>-1</sup>) were recorded.

Dry weights of straw, spike and grains of wheat were determined after oven drying of samples at 65 °C for 72 h. For determining total grain Se content, 3 g of oven-dried ground grain samples were digested by  $HNO_3:H_2O_2$  method (Costa et al. 2016). The digests were diluted to 50 mL with distilled water and filtered. Total Se content ( $\mu g k g^{-1}$ ) in the digest was determined by ICP-OES (2100 dv, Perkin-Elmer). Total Se uptake ( $\mu g pot^{-1}$ ) was calculated by multiplying Se content ( $\mu g g^{-1}$ ) of wheat grains with grain yield (g pot<sup>-1</sup>).

## **Statistical Analysis**

Statistical analyses were performed using Statistix-8.1 program. Data of all three experiments were analysed by twoway ANOVA. Least significance difference (LSD) test was used to compare the treatment means at 5% probability level (Steel and Torrie 1996). Before applying ANOVA, data were tested for normality distribution and homogeneity of variances using Shapiro–Wilk's test and Levene's test, respectively. The tests showed that all the data were normally distributed and showed homogeneity of variances. Descriptive statistics including means and standard deviation of means were calculated by Microsoft Excel 2013 (Microsoft Office).

## Results

#### **Soil Microbial Activities**

The application of FYM substantially increased the soil respiration both in - Se and + Se soils. Across the range of

soil-applied Se levels, the quantity of CO<sub>2</sub> evolved ranged from 43 to 223 mg kg<sup>-1</sup> soil in unamended and 621 to 681 mg kg<sup>-1</sup> soil in FYM-amended soils. The application of Se exhibited adverse influence on soil respiration, however, variable response was observed for unamended and FYM-amended soils. In unamended soil, CO<sub>2</sub> emission significantly decreased at or above the Se application rate of 10 µg kg<sup>-1</sup>. However, in FYM-amended soil, soil respiration was suppressed by the application of Se above 50 µg kg<sup>-1</sup> soil. In control soil (– Se, – FYM), the quantity of CO<sub>2</sub> evolved was 223 ± 16 mg kg<sup>-1</sup> soil and it decreased to only 43 ± 10 mg kg<sup>-1</sup> at 250 µg Se kg<sup>-1</sup> soil. In contrast, on FYMamended soil, the quantity of CO<sub>2</sub> evolved decreased from 681 ± 20 mg kg<sup>-1</sup> soil without Se to 621 ± 10 mg kg<sup>-1</sup> with 250 µg Se kg<sup>-1</sup> soil.

Dehydrogenase activity of soil was significantly changed by the addition of both Se and FYM after 7 days of incubation (Table 2). Across the range of soil-applied Se levels, dehydrogenase activity ranged from 3.19 to 5.31 and 6.10 to 11.58  $\mu$ g TPF g<sup>-1</sup> soil on unamended and FYM-amended soils, respectively. Similar to soil respiration, the application of Se exhibited adverse influence on dehydrogenase activity, however, the variable response was observed on unamended and FYM-amended soils. Soil spiking with Se beyond 50  $\mu$ g kg<sup>-1</sup> resulted in significant decrease in dehydrogenase activity on unamended soil. Dehydrogenase activity decreased up to 40% by applying 250  $\mu$ g Se kg<sup>-1</sup> soil. In FYM-amended soil, the addition of Se at or above  $10 \,\mu g \, \text{Se} \, \text{kg}^{-1}$  significantly reduced the dehydrogenase activity. Selenium application at 250 µg Se kg<sup>-1</sup> soil decreased the dehydrogenase activity by 47% compared to control  $(-Se_{1}+FYM)$ .

The application of FYM increased while Se decreased the urease activity of soils measured after 7 days of incubation (Table 2). Across the range of soil-applied Se levels, the urease activity ranged from 14.47 to 22.18 and 27.44 to  $35.21 \text{ mg N g}^{-1}$  soil in unamended and FYM-amended soils, respectively. Soil spiking with Se beyond 50 µg kg<sup>-1</sup> resulted in significant decrease in urease activity on both unamended and FYM-amended soils. Urease activity decreased up to 35% and 22% by applying 250 µg Se kg<sup>-1</sup> soil on unamended and FYM-amended soils, respectively, compared to respective controls (Table 2).

#### **Growth and Yield of Wheat**

The results of seedling stage experiment showed that Se application at 10 and 25  $\mu$ g kg<sup>-1</sup> soil did not significantly affect the seedling biomass. However, application at 50 and 250  $\mu$ g kg<sup>-1</sup> soil increased seedling biomass by 11.9 and 12.6%, respectively, compared to control (Figs. 1, 2). The application of FYM alone improved the seedling biomass by 22% compared to control. The combined applications of

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Table 2Effect of differentrates of soil-applied Se onsoil microbial activities inunamended and FYM-amendedsoils

FYM (g kg <sup>-1</sup> soil)	Selenium (µg kg <sup>-1</sup> soil)	Soil respiration (mg $CO_2 kg^{-1}$ soil)	Dehydrogenase (µg TPF g <sup>-1</sup> soil)	Urease (mg $NH_4^+$ -N g <sup>-1</sup> soil)
0	0	$223 \pm 16 \text{ d}$	5.31±1.12 c	$22.18 \pm 0.84$ f
	10	131±23 e	$5.64 \pm 0.82$ c	$24.79 \pm 2.41 \text{ df}$
	25	$71 \pm 10$ f	5.66±2.39 c	23.87 ± 1.33 ef
	50	$61 \pm 12 \text{ fg}$	$5.40 \pm 0.54$ c	$20.68 \pm 0.94 \text{ f}$
	125	$49 \pm 10 \text{ fg}$	$3.18 \pm 0.72 \text{ d}$	15.83±2.14 g
	250	$43 \pm 10$ g	$3.19 \pm 0.23 \text{ d}$	14.47 ± 1.69 g
12.5	0	$681 \pm 20$ ab	11.58±1.38 a	$35.21 \pm 4.23$ ab
	10	699±6 a	8.16±0.63 b	35.31 ± 1.86 a
	25	$684 \pm 4$ ab	7.96±0.68 b	35.29 ± 1.44 a
	50	665±13 b	6.32±0.78 c	$30.94 \pm 3.54$ bc
	125	640±8 c	$6.60 \pm 0.32$ bc	$28.95 \pm 4.29$ cd
	250	$621 \pm 10 c$	$6.10 \pm 0.63$ c	27.44 <u>+</u> 1.87 с–е
LSD		23	1.59	4.31
CV		3.58	14.95	9.70

Values are means  $\pm$  SE of three independent pot replicates. Values followed by different alphabet(s) in a column are statistically different from each other at 5% probability

50  $\mu$ g Se kg<sup>-1</sup> soil and FYM produced the maximum seedling biomass, however, it was statistically at par with that obtained from alone FYM-amended soil (Table 3).

In crop maturity experiment, Se application tended to increase the straw biomass (5.8%), spike weight (8.8%) and productive tillers (7.6%), but the effect remained non-significant as compared to control. However, Se application significantly improved grain yield (by 11.4%) compared to control (– Se, – FYM). The grain yield in FYM-amended soil was recorded to be 17% higher than unamended control soil. The combined application of FYM and 25  $\mu$ g Se kg<sup>-1</sup> soil improved the grain yield by 24%, however, it was statistically at par with that obtained from alone FYM-amended soil.

## **Grain Se Content and Total Grain Se Uptake**

The soil application of Se significantly increased the Se content in wheat grains both on unamended and FYMamended soils (Fig. 3A). However, grain Se content on FYM-amended soils was significantly lower than unamended soil at each level of soil-applied Se. On unamended soil, Se was not detected in the grains of control plants while Se application increased grain Se content in a rate-dependent manner, the value reaching to  $1025 \ \mu g \ kg^{-1}$  at 250 g Se kg<sup>-1</sup> soil. On FYM-amended soil, the application of same rate of Se produced grains of 941  $\ \mu g$  Se kg<sup>-1</sup>, respectively. A similar decrease was recorded at other rates of Se on FYM-amended soils. The average Se content of wheat grains at all levels of soil-applied Se was 342 and 245  $\mu$ g kg<sup>-1</sup> on unamended and FYM-amended soils, respectively.

The soil-applied Se also resulted in a significant increase in total Se uptake in wheat grains (Fig. 3B). The average Se uptake in wheat grains at all levels of soil-applied Se was 8.3 and 6.8  $\mu$ g pot<sup>-1</sup> on unamended and FYM-amended soils, respectively.

# Discussion

Selenium has been considered as non-essential, but a beneficial element for plants as it promotes plant growth at low concentrations (Djanaguiraman et al. 2010; Jóźwiak and Politycka 2019). Our results showed that soil application of Se increased seedling growth and yield of wheat on unamended soil, whereas non-significant effect of Se was observed on FYM-amended soil. The maximum increase in biomass (12.6%) of 42-day-old seedlings was recorded at 125  $\mu$ g Se kg<sup>-1</sup> soil, while the highest increase in yield (11%) occurred at 250  $\mu$ g kg<sup>-1</sup> soil compared to control (-FYM, -Se). However, this increase in growth and yield of wheat was statistically at par observed with other levels of Se. Similar to our results, a significant increase in seedling growth of wheat through soil-applied Se has been reported (Xiaoqin et al. 2009). Xia et al. (2020) found that Se application at 37.5 g ha<sup>-1</sup> enhanced shoot dry weight of wheat by 9.6% and grain yield by 16.8% during two consecutive year experiments. However, there are reports that at lower application rate of 10 g ha<sup>-1</sup> Se did not significantly affect wheat yield (Broadley et al. 2010; Ramkissoon et al., 2019).



**Fig. 1** Effect of different rates of soil-applied Se on biomass of 42-day-old wheat seedlings in unamended and FYM-amended soils. The data are means of three independent pot replicates. Bar showing different alphabet(s) are statistically different from each other at 5% probability (LSD 0.61, CV 12.58)

Selenium at high rates (2 mg kg<sup>-1</sup>) may also be toxic and reduce plant biomass (Zhang et al. 2017). In our study, Se may have improved the growth and yield of wheat as it has been reported to be involved in the regulation of photosynthesis and anti-oxygenation (Ulhassan et al. 2019; Alves et al. 2020).

The Se content and uptake in wheat grains increased with soil Se application in a rate-dependent manner on both unamended and FYM-amended soils. Curtin et al. (2008) observed that grain Se increased linearly with the application rate of Se. An application of 10 g Se ha<sup>-1</sup> increased the Se concentration of wheat grain by 10-fold from its low ambient levels (Adams et al. 2002). There are a few other reports that soil application of Se is helpful in increasing Se content and its uptake in wheat grains (Galinha et al. 2015; Ali et al. 2017; Ramkissoon et al. 2019; Manojlović et al. 2019). Agronomic biofortification of wheat with Se appears to be a feasible option for increasing dietary Se intake in Pakistan as Se content was not detected in grains of control plants (-Se), while it increased to 941–1025  $\mu$ g kg<sup>-1</sup> with soil Se application. Moreover, it was found that Se content in grains of wheat was lower on FYMamended soil than unamended soil. Wheat grains produced on FYM-amended soil had 29 and 18% low Se content and uptake, respectively, than unamended soil. Thus, it is clear that FYM amendment decreased both Se content and total uptake, but decline in the former attribute was more, probably due to growth dilution effect which aroused from the higher grain yield obtained on FYM-amended soil. On seleniferous soils, organic FYM decreased 23% Se accumulation in wheat grains (Sharma et al. 2011). In another study, the application of press mud and poultry manure reduced Se accumulation by 85–92% in wheat straw, 45-74% in wheat grains, 45-74% in rapeseed straw and 76-92% in rapeseed grains under field conditions (Dhillon et al. 2010). In root, shoot and leaves of canola and tall fescue plants. Se accumulation was also reduced by the addition of organic materials (Ajwa et al. 1998). Soil organic matter can immobilize Se and reduce its bioavailability in soil (Li et al. 2017). The addition of FYM to soil might had reduced Se uptake and its acquisition by three different ways. Firstly, the addition of organic matter could stimulate microbial activity in soil which may lead to bio-reduction of  $SeO_4^{2-}$  to  $SeO_3^{2-}$  and finally to elemental Se (Tokunaga et al. 1996). The reduced forms of Se are less water soluble and have low bioavailability. Secondly, the addition of organic matter reduces uptake by adsorption and complexation phenomena (Li et al. 2017). Some studies found that exogenous Se applied into the soil are quickly immobilized by soil organic matter (Johnsson 1991; Thavarajah et al. 2015; Di Tullo et al. 2016). Thirdly, organic matter favours the formation of methylated Se and its volatilization from soil reduces Se content in soil (Dhillon et al. 2010).



Fig. 2 Effect of soil-applied Se  $(250 \ \mu g \ kg^{-1} \ soil)$  on growth of wheat in unamended and FYM-amended soils

Table 3Effect of differentrates of soil-applied Se ongrowth and yield of wheat inunamended and FYM-amendedsoil

FYM (g kg <sup>-1</sup> soil)	Selenium (µg kg <sup>-1</sup> soil)	Straw biomass (g plant <sup>-1</sup> )	Spike weight (g plant <sup>-1</sup> )	Productive tillers (No. plant <sup>-1</sup> )	Grain yield (g plant <sup>-1</sup> )
0	0	13.86 c	10.23 e	4.11 ab	7.54 d
	10	14.09 c	10.71 de	4.00 b	8.39 bc
	25	14.62 a–c	11.14 b–d	4.33 ab	8.34 b-d
	50	14.67 a–c	11.03 с-е	4.11 ab	8.27 cd
	125	14.56 a–c	10.95 с-е	4.33 ab	8.35 b-d
	250	14.22 bc	11.00 с-е	4.00 b	8.40 bc
12.5	0	15.31 ab	11.59 a–d	4.44 ab	8.82 a–c
	10	15.52 a	11.61 a–c	4.56 a	8.62 a–c
	25	15.58 a	11.70 a–c	4.44 ab	9.37 a
	50	15.39 ab	12.01 ab	4.56 a	9.28 a
	125	15.69 a	12.07 a	4.56 a	9.10 ab
	250	15.64 a	11.92 ab	4.44 ab	9.33 a
LSD		1.19	0.88	0.46	0.82
CV		4.69	4.60	6.26	5.57

Values are means  $\pm$  SE of three independent pot replicates. Values followed by different alphabet(s) in a column are statistically different from each other at 5% probability



**Fig. 3** Effect of different rates of soil-applied Se on grain Se concentration (**A**) and Se uptake by wheat grains (**B**) in unamended and FYM-amended soils. Bar showing different alphabet(s) are statistically different from each other at 5% probability (LSD<sub>A</sub> 16.41, LSD<sub>B</sub> 1.09)

Soil microorganisms are a key component for soil fertility, as they decompose crop residues and release nutrients to plants (Jenkinson 1988; Vanzolini et al. 2017; Chinta et al. 2020). Moreover, they protect nutrient against fixation by inorganic soil components (Khan and Joergensen 2009; Kalayu 2019; Yadav et al., 2020), thus play an important role in increasing nutrient use efficiency of crop plants. The application of Se decreased soil respiration, dehydrogenase and urease activities on both unamended and FYM-amended soils (Table 2). Moreover, soil microbial activities were higher on FYM-amended soil than unamended soil. It is known that Se has antimicrobial effect (Vasić et al. 2011) and alters bacterial community structure (Acuña et al. 2013). However, previously soil application of Se has not been scrutinized for its impact on soil respiration and enzyme activities. We found that Se-induced decrease in soil microbial activities was not related to yield reduction under different Se application levels. The soil respiration and enzyme activities were measured between 3 and 7 days of spiking. It is proposed that soil microbes might have recovered over the times after exposure to Se, and yield reduction did not occur. However, it is suggested to determine time-dependent changes in soil microbial activities in Se-applied soils.

## Conclusions

The application of Se on a loamy soil improved the seedling growth, yield and Se content of wheat grains in favour of human consumption. By applying Se at 250  $\mu$ g Se kg<sup>-1</sup> soil, grain Se content increased to more than 1 mg kg<sup>-1</sup> without compromise on the grain yield. Although, the application of FYM suppressed Se absorption from soil and its content in wheat grains, grain Se content was still substantially higher in + Se soil than – Se soil. Moreover, Se toxicity to soil microflora did not result in growth and yield reduction of wheat. However, more focussed studies are suggested to be carried out to determine the timedependent changes in microbial activities in Se-fertilized soils and their influence on wheat production.

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Data Availability Not applicable.

**Code Availability** Not applicable.

#### Declarations

**Conflict of interest** The authors declare that they have no conflict of interest/competing interests.

**Consent to Publish** All authors agree to publish the work in Journal of Plant growth regulation.

**Ethical Approval** Not applicable (this study does not report on or involve any animal or human data or tissue).

**Informed Consent** Not applicable (the manuscript does not contain data from any individual person).

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