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Original Research

Assessment of Wheat Biofortification for Iron (Fe) Using Vermicompst and Biochar along with Fe Application

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Abstract

Iron-biofortification is a sustainable method of addressing iron (Fe) deficiency in crops by increasing iron content and bioavailability under alkaline conditions. In this regard, the management of Fe-deficient soil is becoming a serious matter of concern for soil health and the food web. The current research was carried out to investigate the possible impact of vermicompost (VC) and biochar (BC) along with iron (Fe) for improving the Fe fortification in wheat (*Triticum aestivum L.)* grown in alkaline soil. The wheat genotype "Fakher-e-Bakkhr" was used as a test plant. The findings showed the highest variations among all the measured parameters. The use of Fe along with VC and BC significantly improved plant height, root length, root and shoot weight, number of leaves, and overall plant biomass of wheat crop. A greater increment was noticed in available Fe in soil when VC, BC, and VC+BC along with Fe were applied in alkaline soil by 31.4%, 36.4%, and 41.5%, respectively, over control. Additionally, Fe contents in plants were increased by 16.5%, 22.6%, and 25.1% when VC, BC, and VC+BC along with Fe were applied in alkaline soil. Similarly, maximum increases in soil OM, available P, and K by 53%, 21.9%, and 19% were recorded when VC+BC+Fe was added in combined form relative to control. This study indicated that using organic amendments like BC and VC along with iron can have the potential to improve the bioavailability of iron and enhance wheat nutritional quality, thereby enhancing the Fe fortification in wheat under alkaline conditions.

Keywords: iron, fortification, biochar, vermicompost, wheat

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Introduction

Wheat (*Triticum aestivum L.*) is widely recognized as a staple food across the globe due to its high nutritional value and need. In Pakistan, it has a great contribution of over 60% in the everyday diet of a common person. Pakistan is also one of the top 10 wheat-producing countries in the world. Pakistan grows wheat on an area of around 9.178 million hectares, producing 27.293 million tons annually. In Pakistan, wheat adds 9.2% to value and 1.8% to GDP (Pakistan Economic Survey, 2020–21). Macro- and micronutrients are essential for the growth, development, yield, and quality of different crop plants [1]. Micronutrients have a significant impact on the physiological growth and development of plants, particularly in wheat plants, where they increase yield and yield contributing parameters. Micronutrients are also required in trace amounts for proper plant growth and development [2].

Iron as an essential micronutrient plays a vital role in various physiological processes of plants, including oxidation-reduction, respiration, and photosynthesis [3]. However, the alkaline and calcareous nature of Pakistani soils, having low organic matter and micronutrient status and imbalanced application of NPK fertilizer, can hinder Fe availability [4]. Insufficient Fe content in soils can lead to Fe deficiency in the edible portion of the plant, ultimately causing Fe deficiency in humans. The alkaline and calcareous nature of Pakistani soils containing high calcium carbonate could lead to a prominent decrease in trace mineral element availability, which ultimately hinders plant growth [5].

Recently, malnutrition due to micronutrient deficiency in plant-based foods poses serious public health risks in developing countries. Particularly, hidden hunger due to iron deficiency is a prominent nutritional disorder in the world, which leads to anemia [6]. A recent study by Naveed [6] indicated that women and young children are anemic due to iron deficiency by 50% and 40%, respectively. Iron deficiency can be cured either through pharmacological iron supplementation or through agriculture-based iron biofortification.

The enhancement of Fe concentration through biofortification presents a sustainable solution to address Fe malnutrition in humans [7]. There are currently three common approaches utilized to increase micronutrient concentration in cereals: conventional breeding, genetic modification, and agronomic biofortification [8]. While breeding and genetic modification are effective and sustainable methods for combating Fe deficiency in grains, they are considered long-term solutions [2]. Conversely, agronomic biofortification is the most cost-effective and economical technique for augmenting micronutrient concentration in wheat and other crops [9]. In order to increase the availability of Fe, soil pH manipulation using acidifying materials such as microbial oxidation of elemental sulfur has been suggested [10]. Biochar and poultry manure have the potential to promote soil health and nutrient availability [11]. The use of biochar is particularly considered to be the most sustainable method for improving soil fertility and increasing crop productivity. Moreover, it has been observed to have a positive impact on Fe solubilization by reducing soil pH [12]. Vermicompost, which is a compost product created from organic matter degradation by earthworm digestive systems and associated environmental microorganisms, is an exceptional nutrient-rich organic fertilizer and soil conditioner that possesses high porosity, aeration, drainage, and water-holding capacity [13]. Furthermore, vermicompost regulates soil properties and the bacterial community in plastic shed soil, thereby improving plant growth and nutrient uptake [14]. In this current investigation, it was hypothesized that the addition of biochar and vermicompost along with iron sulfate could fortify wheat grains with iron and alter the chemical properties of alkaline soil. The precise objective of this investigation was to explain the influence of vermicompost on wheat yield and Fe concentration in wheat grains.

Materials and Methods

Collection of Soil and Preparation of Soil Amendments

Soil was gathered from a calcareous Fe-deficient zone of Dera Ghazi Khan (29°59’22”N 70°30’42”E) and was moved to the University Research site for further processing and experiment. Soil was taken from the ground and all the extra impurities were removed and passed through a 2 mm sieve for pot study. The physico-chemical properties of soil are: sandy loam texture, organic matter 0.57%, soil pH 7.78, EC 3.02 mS cm^{-1}, available-P 8.15 mg Kg^{-1}, available potassium 78 mg Kg^{-1}.

Vermicompost and Biochar Production

A plastic container of 100 liters was used for the production of vermicompost and biochar produced through the pyrolysis process as described in the [15, 16].

Pot Experiment

A pot experiment was conducted at the experimental site of the Department of Soil and Environmental Sciences Ghazi University, Dera Ghazi Khan (30°1′59″N 70°38′24″E), Pakistan to investigate the effects of vermicompost and biochar along with iron application for fortification during the Rabi season 2022-23. The wheat genotype “Fakher-e-Bakkhr” was used as a test plant. The experiment involved eight different treatments, each of them containing 7 kg of sandy loam soil. The recommended doses of treatments were applied at the time of sowing. There are the following treatments as applied: T0: Control (without fertilizer), T1 (VC@ 10 t ha^{-1}), T2 (Biochar@ 4 t ha^{-1}), T3 (Iron@ 20 kg h^{-1}), T4 (Iron@ 20 kg h^{-1} + VC@ 10 t ha^{-1}), T5 (Iron@ 20 kg h^{-1} + Biochar@ 4 t ha^{-1}). $FeSO_4 7H_2 0$ was used as an iron source. The treatment calculations were made for each pot. The recommended doses of NPK

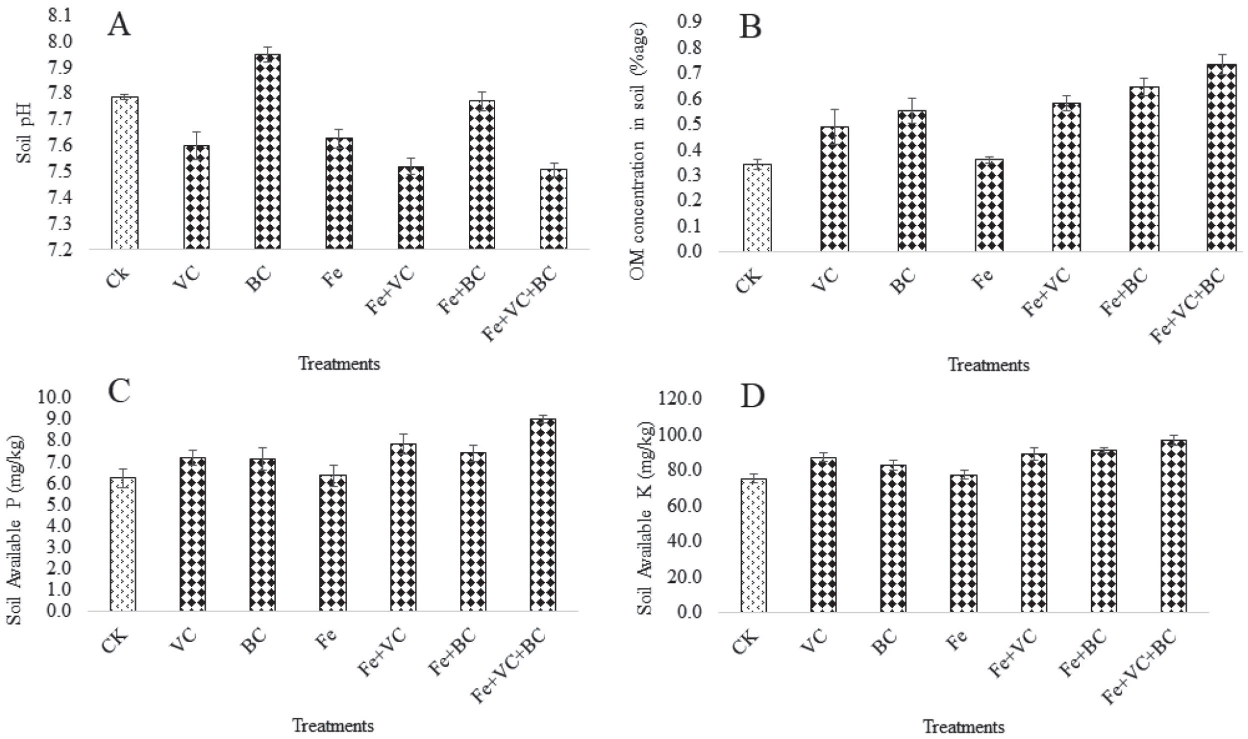

Fig. 1. Effect of vermicompost and biochar with and without iron application on soil pH (A), Soil organic matter (B), Soil available Phosphorus (C), and Soil available potassium (D). Treatments are as follows: Control (CK); Vermicompost (VC); Biochar (BC) and Iron (Fe). Error bars are the SD of the means (n=3) and different letters indicated that values are significantly different at $p<0.05$.

(120:60:60 kg ha^{-1}) to each pot were applied at the time of sowing. The irrigation was done by hand sprayer. All agronomic and physiological parameters were measured at the harvesting stage, while chlorophyll was measured at the vegetative stage.

Soil Analysis

After wheat harvesting, soil samples were taken from each experimental unit and analyzed for soil pH and EC [15]. The Walkley-Black method was used to estimate SOM after soil amelioration, as proposed by [15, 16]. Fe contents were estimated using the DTPA method [16, 17]. Soil available-P was calculated by the calorimetric method and K was determined as described in our previous studies [17, 18]. Iron contents in soil were determined using the DTPA method and analyzed by using an atomic absorption spectrophotometer (AA-240FS Varian, USA).

Plant Analysis

The chlorophyll levels in wheat leaves were calculated from each experimental pot as described in [8]. Afterward, the dried plant tissues were finely ground and digested using a combined mixture of HNO_3 and $HClO_4$ with (9:4 v/v) ratio to measure the Fe contents, as detailed in our previous study [15, 18]. The agronomic parameters like Plant height, shoot and root length, leaf length, spike length, number of spikelets/spike, number of leaves, and number of grains/spike were also estimated.

Statistical Analysis

The statistical analysis of the current study data was performed using the software Statistics 8.1. One-way ANOVA was observed for analysis and Duncan's multiple range test with p=0.05 was adopted. All the graphical figures were prepared using Microsoft Excel.

Results

Changes in Soil pH and EC with Soil Additives

The results illustrated in the Fig.1a) confirmed the significant alteration in soil pH. Soil amelioration using vermicompost (VC), biochar (BC), and Fe application significantly reduced soil pH. The prominent reduction was noticed by 0.2 and 0.3 units when the soil was amended with VC and VC, respectively, along with Fe relative to control. Similarly, soil pH was also decreased after mixing soil with combined soil additives (VC+BC+Fe) compared to control (Fig. 1).

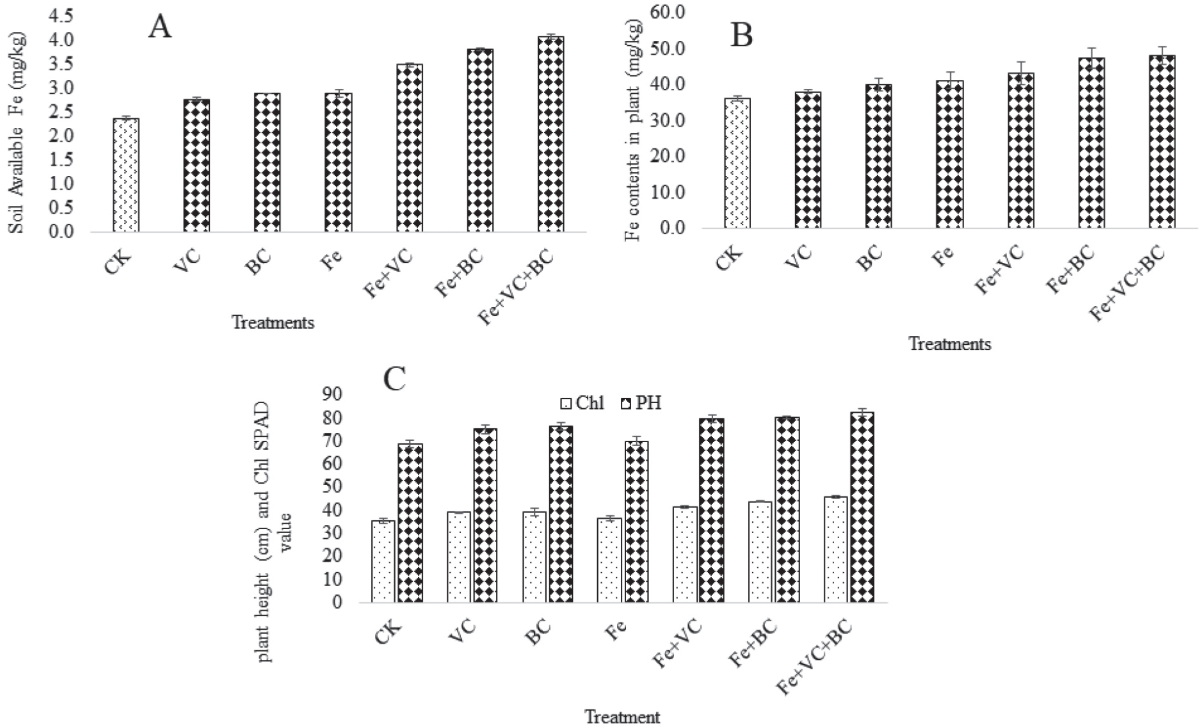

Fig. 2: Effect of soil additives on (A) soil available iron contents (B), iron uptake by wheat shoot (C) and plant height (PH), and chlorophyll contents (Chl). Treatments are as follows: Control (CK); Vermicompost (VC); Biochar (BC), and Iron (Fe). Error bars are the SD of the means (n=3) and different letters indicated that values are significantly different at $p<0.05$.

Changes in Soil Nutrient Status and Organic Matter with soil Additives

The findings of this study express that soil organic matter percentage was effectively boosted when alkaline soil was ameliorated with BC and VC along with Fe (Fig. 1) The highest percentage was estimated by 53% when the combined dose of VC and BC along with Fe was applied relative to control. While, the addition of VC and BC along with Fe showed also effective improvement in OM by 47% and 41% respectively against control. This study confirmed that the addition of organic materials in alkaline soil showed a significant improvement in soil nutrient status. The maximum improvement in soil available phosphorus (Fig. 1) was recorded by 21.9% over control. The alone incorporation of VC and BC along with Fe exhibited a prominent increment in soil P by 20.5% and 15.8%, respectively, over control. The maximum improvement in soil-available potassium (Fig. 1) was estimated at 19% when the mixture of VC and BC was added against the control treatment. However, when VC was mixed along with Fe enhanced soil-K by 17.5% and BC along with Fe increased by 15.4% over control.

Changes in Fe Contents with Soil Additives

The current findings express that the addition of VC and BC efficiently ameliorates soil pH, which leads to increased Fe availability in alkaline soil. The greatest increase in available Fe concentration was measured by 41.5% when VC and BC were applied mutually along with Fe relative to the control. Similarly, Fe availability was improved when Fe was applied without the combination of BC and VC by 36.8% and 31.4%, respectively, against control (Fig. 2).

Fe Fortification and Growth Parameters with Soil Additives

According to the findings, prominent increments in Fe contents were measured in wheat shoots and seeds after the amelioration of alkaline soil with soil additives. The maximum increment in Fe contents in the shoot was observed by 25.1% when the mixture of VC and BC was applied along with Fe relative to simple Fe addition in alkaline soil and control soil. Similarly, the Fe content in wheat grain was significantly improved by 22.6% and 16.5% after the addition of BC and VC along with Fe

Table 1. Effect of soil additives on plant growth parameters. Shoot weight (SW); Root weight (RW); Root Length (RL); Spike length (SPL); Number of spikelets (NSP); Grain per plant (G plant) and 1000-grain weight (1000-G wt). Treatments are as follows: Control (CK); Vermicompost (VC); Biochar (BC) and Iron (Fe). The SD of the means (n=3) and different letters indicated that values are significantly different at $p<0.05$.

Treatments	SW	RW	RL	SPL	NSP	G plant-1	1000-G wt
CK	3.1d	2.1d	11.3e	10.3d	15.7d	31.3d	38.7d
VC	4.4c	2.9c	12.9cd	11.0d	16.3d	35.0c	40.5cd
BC	4.7bc	3.3bc	13.2bc	11.3d	17.0cd	36.0bc	43.6ab
Fe	3.3d	2.9c	12.2de	11.6cd	17.0cd	33.3cd	42.7bc
Fe+VC	5.1bc	3.4b	14.3ab	12.7bc	18.0bc	38.6ab	44.2ab
Fe+BC	5.4ab	3.7b	15.0ab	13.8ab	19.5ab	40.0a	45.9ab
Fe+VC+BC	6.0a	4.2a	16.0a	14.6a	20.0a	41.7a	46.7a

dose relative to control. Similarly, all the growth parameters were promoted after the incorporation of soil additives (Table 1). The result showed that all parameters concerning wheat growth were significantly affected by the integrated application of VC and BC with and without Fe application over control. According to the data, all treatments showed a positive impact on plant height, biomass, and 1000-grain weight, as presented in (Table 1).

Discussion

Iron is a crucial mineral element and is essential for all living organisms [19]. The lack of iron is an emerging concern that poses some serious implications for the health of human beings [20]. According to the studies, the insufficiency of iron (Fe) in crops might have occurred due to the soil's high pH and calcareous characteristics. Studies have shown that Fe rapidly shifts from soluble Fe^{+2} to relatively insoluble Fe^{+3} oxides and hydroxides, ultimately resulting in the inadequacy of Fe in the soil [13, 19] Iron shortage is not only a serious concern for living organisms in alkaline soils but its restricted accessibility is also questionable because of greater amounts of carbonate and hydrogen carbonates [21]. The agronomic and genetic methods of Fe fortification are the most successful methods for coping with the problem of Fe deficiency. The availability of Fe in soil and plants is mainly influenced by OM and the pH of the soil. Pakistani soils are mostly calcareous in nature, which could reduce the solubility of micronutrients in the soil solution [22]. In the current research, the addition of organic byproducts (vermicompost and biochar) as organic soil passivators showed profound results in modifying the soil pH and organic matter as well as improving the nutrient status (N, P, and K). These findings are in line with the previous research performed by Saleem et al. [21] confirmed that the introduction of Fe in alkaline soil significantly improved the Fe status in soils and in plant tissues.

The soil pH gradually decreased in all units after the addition of Vermicompost (VC) through the inside mechanism after the decomposition of organic material of VC which could lead to the release of organic acids [23]. It can be described that the release of acids (humic and fulvic acids) has the potential to react with carbonates and bicarbonates of alkaline soils, which might promote neutralization and subsequent reduced soil pH [24]. However, biochar can increase soil pH because of its carbonaceous nature. In the current study, it has been observed that soil additives along with Fe prominently improved soil available nutrient status. Because they provide substrate and carbon to beneficial microbial communities that can further break down organic matter, acidic byproducts are liberated, which boosts the release and exchange of essential elements in the rhizosphere [25].

The results of the present study suggested that BC and VC with and without Fe exhibited a significant improvement in wheat growth and yield. They have the potential to improve soil quality and solve the alkalinity and nutrient deficiency of alkaline soils due to sufficient amounts of nutrients [14]. Additionally, BC as a soil additive can stimulate microbes activity as well as soil microbiological structure, which might have a great contribution to the promotion of plant root development [26, 27]. Similarly, VC has the potential to boost enzymatic activity which could promote chlorophyll synthesis, plant growth, and development, as well as enhance the accessibility of nutrients in the soil due to the presence of cytokinins, growth hormones, and humic acids in it [28, 29]. The previous study reported by Ramzan et al. [30] confirmed that the application of Fe effectively enhances plant height, number of tillers, number of spikes, and grain yield of wheat.

The incorporation of Fe along with VC and BC efficiently improved wheat growth parameters like plant height, root length, and yield parameters like spike length, no. of grains per plant, and 1000 grain weight. These findings are in line with Estefan et al. [31]. The study conducted by Nauš et al. [32] confirmed that iron (Fe) addition along with organic amendments, such as BC and PM, exhibited significant enhancement in various growth parameters as well as Fe accumulation in plants' edible portions. The findings of the research conducted by Ali [33] also demonstrated that the foliar application of Zn and Fe can have a constructive effect on the characteristics of the wheat crop. The study proposed by Hafeez et al. [1] also suggested the same conclusion.

Conclusions

The application of Fe with organic (vermicompost and biochar) significantly influenced the growth and yield attributes of the wheat as well as improved the bioavailability of Fe in wheat grains and soil. The results indicated that the fortification of Fe via the agronomic method is the best approach to overcoming iron deficiency in soils and humans. The application of Fe with organic amendments is the best approach to improve the growth and yield of wheat crops as well as Fe bioavailability, rather than Fe with inorganic fertilizers. The addition of Iron@ 20 kg/ha significantly facilitated the 1000-grain weight and the grain and biological yields of wheat when applied in combination with vermicompost and biochar. All the treatments except control have great contributions to improving soil organic matter, soil mineral Fe, and available phosphorus and potassium concentrations of the soil.

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Conflict of interest

The authors declare no conflict of interest.

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