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Research Article

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Synergistic Effects of Biochar and Phosphorus Fertilization on Wheat Growth, Pollution Reduction, and Soil Enzymatic Behavior

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Abstract
This research, conducted in the wheat fields of West Mashhad, Iran, explores the synergistic effects of nano-technology and biochar on wheat cultivation and greenhouse gas emission. There is a growing global interest in recent years to decrease air pollution through augmentation of greenhouse gas emission. Moreover, this study investigated using a factorial experiment and various levels of phosphorus and biochar treatments, the study aimed to understand the dynamics of their combined influence on wheat's nutrient absorption and soil health. The results indicate that phosphorus fertilizer, particularly at full recommended doses, significantly enhances soil phosphorus content. This impact is further amplified when integrated with biochar, suggesting a collaborative relationship that is beneficial for both soil and crop health. The study also highlighted shifts in soil enzymatic activity: alkaline phosphatase enzyme levels are inversely related to phosphorus application but increase with biochar treatments. Given these findings, the research underscores the potential for refining wheat cultivation techniques. The paper suggests that more comprehensive studies and the integration of modern agricultural technologies could lead to more sustainable wheat cultivation strategies while optimizing yield and soil quality.

Keywords: Soil Health, Phosphorus Absorption, Biochar, Rhizospheric Soil, Sustainable Agriculture

1. Introduction
Situated in the western region of West Mashhad, Khorasan province, Iran, expansive stretches of wheat fields are distinguished by soils with high pH (8-9), notably low organic matter (less than 0.3%). Researchers have pointed out issues such as drainage, salinity, and alkalinity in many of these areas [1]. These conditions, particularly high lime content and pH range, present significant challenges for phosphorus mobility in these soils, which affects the wheat plant's capacity to absorb phosphorus. An extensive body of research supports these complexities. Given this backdrop and the long-standing tradition of wheat cultivation in this region—over 50 years in the northern areas and 30 years in the west of Mashhad—consistent application of phosphorus fertilizer remains a necessity, despite its long-term use.

At present, phosphorus fertilizer is applied only during the initial planting phase, with no additional fertilization during the regrowth stages, applied at a rate of 250-300 kilograms per hectare. There is a growing global interest in recent years to decrease air pollution through augmentation of greenhouse gas emission. This is achieved through methods such as compost application and the use of wheat industry by-products, notably wheat bran [2]. Studies suggest that organic compounds may increase the mobility of certain nutrients, especially phosphorus, aiding absorption by various plants, including wheat [3]. The results from these studies suggest an improvement in wheat growth and performance, as well as phosphorus absorption and efficiency of consumption [4-7]

However, the acquisition of necessary raw organic materials, such as farm by-products, involves significant costs. Also, a substantial amount of time may be needed to observe their impact on soil improvement. Given these constraints, alternative approaches have emerged, involving the use of extracts or low-volume compressed versions of these substances, including composts, biochars, and biochar-based nano fertilizers [8,9].

Biochar, renowned for its organic structure, has the ability to influence the mobility and availability of nutrients within the soil [10,11]. The most significant effects of biochar include improving nutrient absorption via root expansion and increasing the length and density of root hairs [12]. Numerous researchers have emphasized the role of biochar in increasing the soil microbial population, promoting the secretion of organic acids in the rhizosphere, and thereby facilitating nutrient mobility and absorption [13-16]

Soil enzymes play an integral role in the decomposition of organic materials and nutrient cycling [17,18]. Certain soil enzymes, found to accelerate reactions involved in plant residue decomposition and the subsequent release of nutrients, play a crucial role in the nutrient absorption process [19,20]. Phosphatase, a key soil enzyme, is responsible for the conversion of organic phosphorus into a form usable by plants, acting as a potential indicator of biological alterations within the soil [21,22]. Alkaline and acidic phosphatases, in particular, show increased activity in alkaline and acidic soils respectively, depending on the soil's pH level, with the presence of organic material sources and phosphorus influencing these enzymes' activity [23-25].

The main objective of this research is to investigate the extent of phosphorus absorption by wheat plants to increase soil health and decrease greenhouse gas emissions. This involves exploring changes in the plant's rhizosphere, particularly focusing on phosphatase enzyme activity and accessible rhizospheric soil phosphorus, under the influence of phosphorus fertilizer and biochar treatments, across two distinct harvest periods.

2. Material and Methods

To compare the combined effects of phosphorus fertilizer and biochar on the activity of alkaline phosphatase enzymes, and soil phosphorus in the wheat rhizosphere (including total phosphorus and Olsen phosphorus), a pot experiment was carried out in a greenhouse in western Iran, at the Hakim Farabi Agro-Industry Company (48º36' E, 30º59' N). This experiment involved various levels of phosphorus (0, 50, and 100 percent of the recommended fertilizer equivalent to 250 kilograms per hectare), applied as soil amendments and placed beneath the wheat cuttings, treatments of biochar (three levels of soaking cuttings in 0, 0.3, and 0.5 percent biochar solutions, and soil application at the rate of 10 kilograms per hectare of biochar), and two harvest times (45 and 90 days after planting) were examined. In the soaking treatments, after removing the husk, wheat cuttings were soaked for 30 minutes in biochar solutions at the aforementioned concentrations.

2.1 Soil Analysis and Plant Preparing

The soil used for this research was obtained from the surface layer (0-15 centimeters) of a wheat farm situated within the premises of the Hakim Farabi Agro-Industry Company. The collected soil samples were air-dried, ground, passed through a 2-millimeter sieve, and then placed in pots with a diameter of 30 centimeters and a depth of 50 centimeters. To ensure the roots were not damaged during plant removal, plastic bags of suitable sizes were placed inside the pots. The soil sample's physical and chemical properties were assessed using standard laboratory procedures.

Soil texture was established using the hydrometer method, electrical conductivity was measured according to the, and the equivalent of calcium carbonate was evaluated by acid titration [26-28]. The soil's pH was measured in a 1:2 soil-to-water suspension. The soil's organic carbon content was identified using the wet oxidation method, and phosphorus extracted with sodium bicarbonate (Olsen phosphorus) was measured using the method outlined by Biochar extracted from wheat bran underwent an elemental analysis (Costech ECS 4010 CHNSO model) to establish the quantities of carbon, nitrogen, sulfur, and hydrogen. The determined quantities of these elements were 12.53%, 2.52%, 1.2%, and 0.2%, respectively [29-31].

The planting process commenced in the first week of October 2016, utilizing the 69CP-1062 wheat variety's single-bud cuttings. Prior to planting, the cuttings were prepared by immersing them in either 0.3% and 0.5% biochar solutions, or water as a control treatment, for a duration of 30 minutes. The soil, mixed with sieved and thoroughly washed sand in a 1:1 ratio, was placed in the pots. Phosphorus fertilizer was introduced at varying concentrations—zero, 50%, and 100%—based on the recommended amount for the region, and this was done before the planting stage. Nitrogen fertilizer, in the form of urea, was then applied in two stages throughout the growth period. This application was standardized across all treatments, with a rate equivalent to 50 kilograms per hectare. To ensure the soil moisture remained within 80% of field capacity, a regular irrigation schedule was established, taking place every two days. Prior to initiating each irrigation session, the soil's moisture content was measured in destructive pots to determine the precise moisture level. This meticulous process ensured the optimal conditions for plant growth were consistently maintained. Following a growth period of (45-90) days post-planting, the wheat plants were carefully harvested. The harvest involved cutting the plants at the intersection of the soil and the plant collar, and the harvested specimens were then secured in plastic bags for later analysis. Subsequently, the soil within the pot was delicately extracted, ensuring the internal plastic covering was also removed. After the wheat roots were isolated from the soil, the rhizosphere soil—defined as the soil attached to the roots—was collected with utmost care and precision.

2.2 Biochar Preparation

Biochar was obtained from wheat bran and prepared through slow pyrolysis. This involved heating the wheat bran in an oxygen-free environment at temperatures between 300 and 600 degrees Celsius. This process yielded a stable form of carbon, biochar, which was then crushed and sieved to achieve a uniform particle size. The biochar underwent an elemental analysis to establish the quantities of carbon, nitrogen, sulfur, and hydrogen. Soil used for the experiment was collected from a wheat farm and mixed with sieved and washed sand at a 1:1 ratio. Biochar was then added to this soil-sand mixture, thoroughly incorporating it to ensure even distribution.

2.3 Statistical Analysis

In this study, a factorial experiment was conducted using two factors, phosphorus fertilizer and humic acid, with three replications in a completely randomized design. The statistical calculations were performed using SAS software, and the comparison of means was conducted using Tukey's test at the 5% level.

3. Results

The findings derived from the variance analysis of different treatments' mean squares are detailed in Tables 2 and 3. As suggested by the data within these tables, applying biochar and phosphorus fertilizer led to notable shifts (at both 1% and 5% levels) across all the parameters investigated, with the sole exception of acidic phosphatase during one or both of the harvest periods.

S. O. V	df	Total P (mg kg^{-1})	Olsen P (mg kg^{-1})	Alkaline Phosphatase (µmol h^{-1} g^{-1})	P absorption (mg P plant^{-1})
Phosphorus fertilizer (A)	2	24047**	136.69**	8.07*	26.72*
Biochar (B)	3	10426^{ns}	3.88^{ns}	1.84^{ns}	63.53**
(A)×(B)	6	4663^{ns}	2.88^{ns}	0.75^{ns}	2.42^{ns}
Error	24	4433	2.36	1.26	2.87

Non-significant differences (ns), significant difference at 5% (*), significant difference at 1% (**)

Table 1: Variance decomposition of the effect of phosphorus fertilizer and biochar on phosphorus, phosphatase enzymes in the rhizosphere soil, and absorption of phosphorus by sugarcane plant 45 days after planting

S. O. V	df	Total P (mg kg^{-1})	Olsen P (mg kg^{-1})	Alkaline Phosphatase (µmol h^{-1} g^{-1})	P absorption (mg P plant^{-1})
Phosphorus fertilizer (A)	2	15652.9**	50.11**	2.37*	120.85*
Biochar (B)	3	9.803^{ns}	1.36^{ns}	0.9853^{ns}	210.00**
(A)×(B)	6	832.1^{ns}	2.75^{ns}	0.5108^{ns}	12.76^{ns}
Error	24	510.4	1.26	0.8182	14.25

Non-significant differences (ns), significant difference at 5% (*), significant difference at 1% (**)

Table 2: Variance decomposition of the effect of phosphorus fertilizer and biochar on phosphorus, phosphatase enzymes in the rhizosphere soil, and absorption of phosphorus by sugarcane plant 90 days after planting

Soil Phosphorus

Total Phosphorus

The variance analysis table highlights a statistically significant increase in total phosphorus at a 1% confidence level, attributed to the application of phosphorus fertilizer at both harvest times. However, alterations triggered by the application of biochar treatments were not statistically significant during either of the harvest periods. Tables 3 presents the comparative mean total phosphorus data in the rhizosphere soil. This information suggests that the observed variations were primarily associated with the different levels of applied phosphorus fertilizer.

Treatments	Total P (mg kg^{-1})	Olsen P (mg kg^{-1})	Alkaline Phosphatase (µmol h^{-1} g^{-1})	P absorption (mg P plant^{-1})
H_0P_0	404.33^{ab}	2.80 ^{d}	6.84 ^{ab}	5.41 ^{e}
H_0P_{50}	419.17 ^{ab}	5.67 ^{cd}	6.12 ^{ab}	5.73 ^{de}
H_0P_{100}	459.00 ^{ab}	7.50 ^{abc}	5.19 ^{b}	8.45 ^{be}
$H_{0.3}P_0$	394.5 ^{b}	2.53 ^{d}	8.51 ^{a}	10.41 ^{ad}
$H_{0.3}P_{50}$	446.50 ^{ab}	6.27 ^{bcd}	6.30 ^{ab}	10.29 ^{ae}
$H_{0.3}P_{100}$	467.56 ^{ab}	8.50 ^{abc}	5.94 ^{ab}	14.79 ^{a}

$H_{0.5}P_0$	392.5 [b]	2.53 [d]	6.40 [ab]	10.11 [ae]
$H_{0.5}P_{50}$	450.50 [ab]	5.47 [cd]	6.01 [ab]	11.11 [abc]
$H_{0.5}P_{100}$	556.42 [ab]	10.30 [ab]	5.46 [ab]	12.22 [ab]
$H_{SA}P_0$	387.50 [b]	2.67 [d]	7.09 [ab]	7.08 [cde]
$H_{SA}P_{50}$	529.67 [ab]	6.73 [ad]	6.95 [ab]	6.97 [cde]
$H_{SA}P_{100}$	590.92 [a]	11.23 [a]	5.69 [ab]	8.57 [be]

Table 3: Comparison of averages between phosphatase enzymes, rhizospheric soil phosphorus, and phosphorus absorption of sugarcane in the first harvest

H_0, $H_{0.3}$, $H_{0.5}$, refer to the solutions of zero, 0.3%, and 0.5% biochar, respectively. P_0, P_{50}, P_{100} refer to the solutions of zero, 50% and 100% biochar, respectively. HSA, indicates the soil application of biochar. (The average of the numbers with similar letters in each column is not significant according to Tukey's test at the 5% level).

The variance analysis table demonstrated a clear trend over both harvesting periods, establishing a strong correlation between increased application of phosphorus fertilizer and an upsurge in the total phosphorus content in the rhizosphere soil. For the initial harvest, the lowest measures of total phosphorus were linked to treatments where no phosphorus fertilizer was applied. In contrast, the highest measures were associated with treatments that involved application of phosphorus fertilizer, particularly at 100% of the recommended dose. Application of nano-fertilizer is so critical due to the particle size and their adhesion to the cell membrane they might have negative consequences and DNA degradations, it is recommended by scientist to pay great attention to the amount, time, and type of this fertilizers and also to the environmental conditions such as soil EC, which has great impact on effectiveness of the alternation and plant physiological responses [32]. Bio-fertilizer are recommended due to their less ecological issues. Most fertilizer treatments that included biochar showed elevated levels of total phosphorus compared to those that didn't include it. Now adays, different cropping practices such as using perennial covers instead of conventional cropping practices is also recommended by soil scientist. Perennials biofuel and cover crops increase carbon sequestration through their root biomass and decrease bulk density through the depth, which are two key soil indicators in advancing soil health [33]. This trend persisted in the second harvest, although the differences between treatments were more pronounced. In this phase, the peak total phosphorus content was recorded for treatments applying phosphorus fertilizer at 100% of the recommended dose, alongside biochar application. The smallest phosphorus content was found in treatments that omitted phosphorus fertilizer application, as illustrated in Tables 3. Numerically, the total phosphorus content from the first harvest exceeded that of the second. In treatments with biochar application, a more distinct disparity in total phosphorus content was noted. Despite a decrease in total phosphorus content during the second harvest, the phosphorus level consistently increased with rising levels of fertilization.

Alkaline Phosphatase Enzyme

Applying different biochar treatments did not cause any substantial changes in the activity of the alkaline phosphatase enzyme. Intriguingly, however, the introduction of phosphorus fertilizer resulted in a significant decrease in this enzyme in the rhizosphere soil at a 5% level during both harvest times, as presented in Tables 2 and 3. These findings indicate a negative correlation between phosphorus fertilizer application and the activity of alkaline phosphatase throughout both harvest stages. A comparative analysis of mean values across various treatments demonstrated that, unlike phosphorus fertilizer, treatments without fertilization and those applying lower fertilizer levels (50% of the recommended dose) exhibited increased phosphatase levels during both harvest times when biochar was applied. Most treatments saw a decline in alkaline phosphatase enzyme activity over the two harvest periods, as shown in Tables 3. During both harvests, the highest alkaline phosphatase levels were found in the treatments involving biochar application. In contrast, the lowest levels were observed in the treatment with the highest phosphate fertilizer application (8.51 and 7.09 micromoles per hour per gram for the first and second harvests, respectively), as well as in the treatment that excluded phosphate fertilizer application regardless of biochar use (5.19 micromoles per hour per gram).

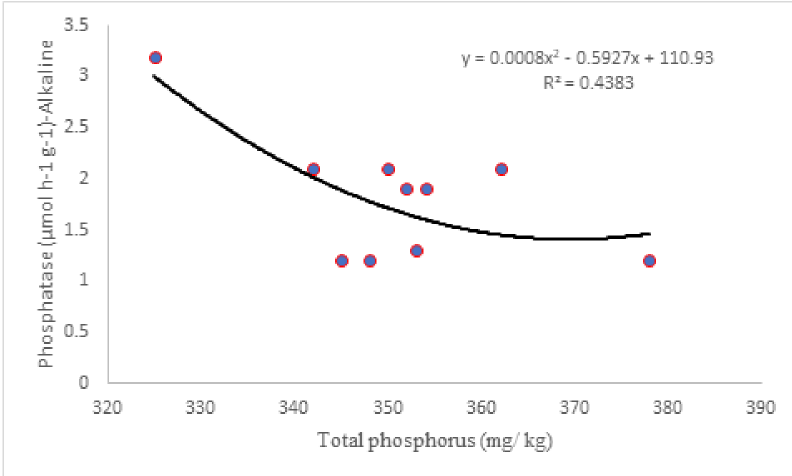

Figure 1: The relationship between total phosphorus and phosphatase enzymes in the first harvest

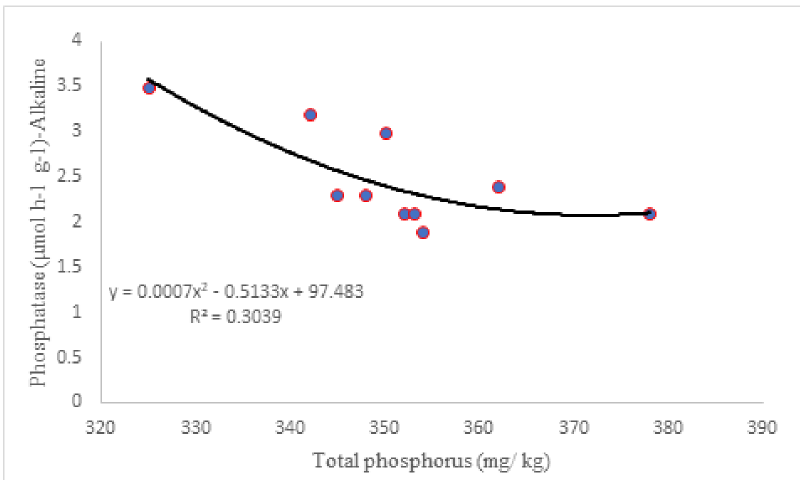

Figure 2: The relationship between total phosphorus and phosphatase enzymes in the second harvest. (a) Acidic phosphatase, (b) Alkaline phosphatase.

Phosphorus Absorption

The absorption of phosphorus by wheat was significantly increased by the application of both biochar and phosphorus fertilizer at both harvest times, with these changes significant at the 1% level for biochar application and at the 5% level for phosphorus fertilizer application (Tables 2 and 3). The comparison of average phosphorus absorption in different treatments and at both harvest times shows that phosphorus absorption was significantly higher in the treatments with biochar at both harvest times (Tables 3). Phosphorus absorption in various treatments increased significantly with the addition of phosphorus and biochar (either individually or in combination with phosphorus application) compared to the control treatment, and in most cases, this was significant at the 5% level. The highest and lowest phosphorus absorption at the first and second harvest were respectively related to the treatment of dipping cuttings in a 0.3% solution along with phosphorus fertilizer application (100% fertilizer recommendation) and the control treatment. Although the treatments with biochar application to the soil showed lower absorption compared to other biochar treatments, they still had significantly higher absorption compared to the control treatment. The amount of absorption in the second harvest increased significantly compared to the first harvest, such that in different treatments, the absorption in the second harvest was at least three times higher than the first harvest (Table 3). Among the factors under study (phosphatase enzymes and soil phosphorus), none showed a significant relationship with phosphorus absorption.

Discussion

Addressing the escalating need for food is paramount, and the agricultural sector plays a pivotal role in this endeavor. To meet this demand, the sector has leaned heavily on the use of chemical fertilizers and the cultivation of genetically modified crops. These methods, while effective in enhancing crop yields, have also ushered in a slew of environmental issues. Contamination of soil and water, coupled with air pollution, are some of the significant repercussions. The challenges are further compounded by factors such as rapid population growth, an increase in vehicular traffic, and swift urbanization, intensifying the pollution dilemma.

50 million metric tons of CO2 through traffic is annually produces just in Iran, making up a significant 5.5%-6% of the country's total greenhouse gas emissions, it is not limited to middle east part, for example in the United States, the environmental and economic toll is evident as well. In 2017, wasted fuel amounted to a staggering 3.3 billion gallons, leading to an economic setback of 179 billion USD [34]. it indicates the urgent need for taking a step to resolve this issue as soon as possible. In recent years, interdisciplinary collaboration has become increasingly prevalent as various fields endeavor to develop innovative solutions to mitigate greenhouse gas emissions and address the ramifications of global warming. This trend is evident across a multitude of disciplines, ranging from nanotechnology to chemistry and from transportation to geology [35]. For instance, the pioneering application of nanotechnology in areas such as fertilization and the advent of Cooperative Automated Vehicle (CAV)-based traffic control or spring–mass–damper (SMD) model systems underscore the concerted efforts of the scientific community in addressing these pressing environmental challenges [36].

In this regard agriculture sector play the most important role by applying cost effective alternative to sequester more carbon, increase soil health and decrease greenhouse gas emissions.

The obtained results related to the harvest time and bio-fertilizer treatments and humic acid highlight the distinctly significant role of fertilization in increasing total and available phosphorus in the rhizosphere soil with increasing root length in C3 plants such as sugarcane, and decreased greenhouse gas emission, and enhance soil health, which could be a great alternative in the time of global air pollution. The increase in phosphorus with increased phosphorus fertilizer is an obvious result that has been emphasized by many researchers. Along with the type of fertilizers, different cropping practices also have great impact on soil health indicators, The most significant indicators for soil health related to different cropping practices were organic carbon, total carbon, followed by total nitrogen, bulk density, and moisture, which are among the most important and effective indices [37].

However, other points have also been clarified in this research. Among them is the significant correlation between total phosphorus and available phosphorus in both harvests, albeit with a considerable difference in the first harvest, as well as a decrease in the amount of phosphorus (both available and total) over time. The reason for these changes can be attributed to changes in phosphorus absorption and its increased amount in the second harvest. With the increased use of phosphorus fertilizer in the 50% and 100% fertilizer recommendation treatments, under the combined effect of biochar with phosphorus fertilizer compared to treatments without biochar, the amount of total phosphorus and available phosphorus experiences a larger increase. These results are consistent with the findings of [38].

Changes in soil phosphorus caused changes in enzyme levels, particularly alkaline phosphatase. In accordance with the findings of the amount of alkaline phosphatase was higher than acid phosphatase in both harvest times, due to favorable conditions in soils with a high pH [39]. With the increased use of phosphorus and consequently the increase in total phosphorus and available phosphorus in the rhizosphere soil, the activity of phosphatase enzymes, particularly alkaline phosphatase, decreased. This can be due to the availability of phosphorus in the plant root zone and the lack of need for phosphatase activity to provide the plant's required phosphorus from soil's organic phosphorus sources. This aligns with research.

The role of biochar in this regard, due to the much stronger role of phosphorus, is less observed. However, it seems that the use of biochar in fertilizer treatments has been able to increase the activity of the phosphatase enzyme. This could be related to the increased soil microbial population and improved root growth of the plant, as well as an increase in the secretion of this enzyme.

The overall role of biochar as an organic compound, alongside phosphorus fertilizer in escalating plant biomass production, could potentially explain the heightened phosphorus absorption. This hypothesis aligns with the findings of [40,41].

The absorption of phosphorus demonstrated a comparable pattern across both harvesting periods, with an observed escalation in absorption commensurate with the increase in phosphorus fertilizer application. This observation aligns with the research findings presented by A temporal absorption comparison between the first and second harvest cycles revealed that despite a reduction in phosphorus concentration, total absorption experienced an increase, which can be attributed to the rise in dry matter.

Besides phosphorus consumption, the application of biochar significantly elevated phosphorus absorption. Interestingly, improvements in phosphorus absorption were observed even in treatments devoid of phosphorus application, due to the employment of biochar. Analogous results have been reported for alternative crops, such as wheat [42,43]. Increased phosphorus absorption in treatments involving biochar may be ascribed to enhancements in the root system, as evidenced by an increase in root length and volume, as well as an augmentation in the length and density of root hairs. Consequently, the application of biochar appears to bolster the capacity for phosphorus absorption from soil and fertilizer sources by facilitating increases in root length and volume. These findings are congruent with the hypothesis that biochar and organic compounds can augment the utilization of phosphorus by plants [44-47].

Conclusion

Our research provides illuminating insights into the nuanced dynamics of phosphorus fertilizer and biochar in wheat cultivation. Specifically, phosphorus fertilizer has been found to significantly elevate the total phosphorus content in the soil, achieving its maximum efficacy when applied at full recommended doses. While biochar alone demonstrates modest effects, its combination with phosphorus fertilizer amplifies the latter's impact, suggesting a productive and synergistic relationship that benefits soil and crop health. This harmonized approach substantially bolsters phosphorus absorption in wheat across different harvest times, ensuring more consistent nutrient uptake. Furthermore, the study points to interesting shifts in the soil's enzymatic behavior, particularly in the activity of the alkaline phosphatase enzyme, which shows an inverse relationship with phosphorus application [48-63]. This is contrasted by treatments that incorporated biochar, which often displayed increased enzyme activity, indicating a complex interplay between the organic compound and soil enzymes. In essence, the data suggest that a carefully calibrated combination of biochar and phosphorus fertilizer not only promotes better nutrient absorption and soil enzyme activity but also paves the way for a more sustainable and efficient wheat cultivation strategy. Future investigations should delve deeper into the long-term effects of combined biochar and phosphorus fertilizer application, examining how they influence crop resistance to diseases, pests, and extreme climatic conditions. Additionally, research could focus on tailoring biochar's physicochemical properties to further enhance its synergistic effects with various fertilizer compositions.

Conflict of Interest

The authors declare that there is no conflict of interest for our research titled: "Enhancing Phosphorus Uptake in Sugarcane: A Critical Evaluation of Humic Acid and Phosphorus Fertilizers' Effectiveness.", and authors take full responsibility of it.

Ethical Approval

This study was approved by administrative committee of Research Farm (Islamic Azad University, Azerbayjan), Iran.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and/or AI-assisted technologies and authors take full responsibility for the content of the publication.

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