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Enhancing the Vegetative Growth of Maize using Biochar from *Miscanthus x giganteus* Waste and Synthetic Nitrogen-Containing Heterocyclic Compounds

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Abstract

Objective: This study aimed to investigate the influence of Biochar derived from *Miscanthus x giganteus* waste when applied alone or in combination with synthetic nitrogen-containing heterocyclic compounds, derivatives of N-oxide-2,6-dimethylpyridine (Ivin), and sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methyur, Kamethur), on the vegetative growth of maize (*Zea mays* L.).

Materials and Methods: The experimental maize seeds were treated with a 10% solution of Biochar applied separately or in combination with synthetic nitrogen-containing heterocyclic compounds used in a 10^{-6} M solution, or auxin (Indole-3-acetic acid, IAA) used in a 10^{-6} M solution; control seeds were treated with distilled H_2O. After three weeks, a comparative analysis of morphological characteristics (shoot length (mm), root length (mm), root number (pcs), plant biomass (g)), concentration of chlorophylls and carotenoids (mg/g FW), and total soluble protein (g/100 g FW) of maize grown on an artificial substrate such as perlite was performed.

Results: Maize treated with Biochar, either separately or in combination with the synthetic nitrogen-containing heterocyclic compounds, exhibited significant improvements in both morphological and biochemical characteristics compared with the control plants. The application of Biochar, whether separately or in combination with the synthetic nitrogen-containing heterocyclic compounds, produced a synergistic effect that was comparable to or surpassed the effect of the IAA.

Conclusion: These findings underscore the potential of using Biochar in combination with synthetic nitrogen-containing heterocyclic compounds as a promising approach to enhance maize vegetative growth.

Keywords Maize · Biochar · Ivin · Methyur · Kamethur · IAA

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INTRODUCTION

Maize is one of the major grain and oilseed crops grown worldwide.[1] Maize grain is widely used for dietary consumption due to its high nutritional value; 100 g of grain contains: starch (66.2%), proteins (11.1%), oil (7.12%), minerals (1.5%), vitamins: carotene (90 mg), niacin (1.8 mg), thiamine (0.8 mg), and riboflavin (0.1 mg).[1] Maize grain is widely used for human consumption in a variety of forms, including meal, roasted kernels, and flour, as well as in the production of starch, syrups, drinks, juices, beer, sweets, flakes, chips, and chewing gum.[1] Maize grain and oil are also used as a source of nutrients and phytochemicals (alkaloids, flavonoids (anthocyanins), saponins, carotenoids, phenolic acids (coumaric acid, syringic acid, and ferulic acid), phytosterols, vitamins A, B1, E, and K and minerals potassium, phosphorous, magnesium and zinc), carbohydrates, and dietary fibre for the pharmaceutical and cosmetic industries.[1,2] The green part of the plant is used as fodder for livestock and grain for poultry, respectively.[3] Stove and dried leaves are used as raw materials for biofuel production.[3]

Modern agriculture faces a critical problem of creating new effective ecologically safe soil amendments and synthetic analogues of phytohormones to improve maize growth and productivity. Maize production in Ukraine is confronted with dual threats of climate change and active military operations. Therefore, new effective soil amendments and synthetic analogues of phytohormones are needed to improve maize cultivation. Annually, maize yields decrease due to global climate change, soil salinisation and heavy metal pollution, waterlogging or drought, diseases caused by phytopathogens that harm maize growth in the vegetative and reproductive phases and the quality of crop production.[4,5] The use of plant growth regulators and pesticides increases maize yields and improves resistance to abiotic and biotic stresses to be improved.[6,7]

Today, a very promising issue is the development of novel ecologically sound soil amendments based on the organic substance Biochar,[8,9] and new synthetic analogues of phytohormones, derivatives of nitrogen-containing heterocyclic compounds for stimulating the growth and development of maize.[10] Biochar is a charcoal-like organic carbon-rich substance produced through a controlled process called pyrolysis by heating organic material, usually derived from agricultural and wood bio-wastes, such as wood chip or crop straw, or animal waste under partial exclusion or limited oxygen conditions, within a temperature range of 300°C–1000°C.[9,11] Biochar, which is a porous carbon material, is a promising option for restoration of degraded land, increasing soil health carbon fixation, and decreasing soil greenhouse gas emissions, particularly nitrous oxide (N_2O) and methane (CH_4).[12,13] A global meta-analysis found that Biochar can reduce N_2O emissions by altering soil microbial activity and enhancing denitrification, the process of converting nitrate to N_2O. Biochar can also help reduce CH_4 emissions by influencing microbial communities that produce methane in anaerobic environments.[13] In particular, Biochar application significantly reduced N_2O and CH_4 emissions by 13.1% and 6.8%, respectively, while NH_3 emissions increased by 22.5%.[13] Biochar, containing biomass from agricultural and wood wastes, provides long-term carbon sequestration in amended soil due to its stable structure and improves soil fertility through renewable energy.[9,13] Three products obtained as a result of the conversion of agricultural waste into Biochar, bio-oil, and gas have received important practical interest due to the high demand for energy and concerns about greenhouse gas emissions and soil degradation worldwide.[9] UN Intergovernmental Panel on Climate Change recognised Biochar as a tool for negative emissions. The time frame for degradation and carbon loss to double in Biochar is approximately 100 million years, which supports Biochar carbon removals (BCR) as a permanent carbon dioxide removal (CDR).[14] BCR has been identified as the most relevant technology being capable of carbon removal at climate-relevant volumes within 15 years. Biochar has other added value, i.e. handling local residual materials, use in cement,[15] and enhancing the efficiency of anaerobic digestion.[16] Biochar is beneficial to soil and plant function by improving the accessibility and adsorption of water and soil nutrients; improving mineral nitrogen retention; reducing nitrogen losses; releasing a certain amount of macro- and micronutrients; improving nutrient use efficiency; reducing the demand for fertilisers; immobilising heavy metals; stimulating microbial activity and enzyme production; improving soil health and crop productivity; and protecting plants and soil from detrimental effects.[9,11,17] Biochar has an important impact on soil microorganisms by promoting the growth and abundance of most soil microbial communities; it enhances metabolic activity and reproduction of microorganisms. Immobilisation of soil pollutants by Biochar is beneficial for soil biodiversity by reversing degradation and enhancing ecosystem protection.[13,18]

Currently, new synthetic nitrogen-containing heterocyclic compounds, which are derivatives of pyridines and pyrimidines, are used in agriculture as plant growth regulators and pesticides.[19,20] The most common new environmentally friendly synthetic analogues of phytohormones, which are derivatives of nitrogen-containing heterocyclic compounds, are Ivin (*N*-oxide-2,6-dimethylpyridine), Methyur, and

Kamethur (sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine).[21-23] Numerous studies have shown that the new synthetic nitrogen-containing heterocyclic compounds, such as Ivin, Methyur, and Kamethur, have an effect similar to phytohormones, improving the growth of cereals, legumes, vegetables, and industrial crops, enhancing protein biosynthesis, photosynthesis, and delaying leaf senescence, increasing productivity, and improving the ability of plants to adapt to abiotic stresses.[21-23] The use of these synthetic nitrogen-containing heterocyclic compounds in nanomolar concentrations ranging from 10^{-4} M to 10^{-7} M for soaking or spraying plant seeds allows to decrease the consumption rates of pesticides, herbicides, and fungicides for plant protection and to eliminate their toxic effects on the environment and mammals.[24,25] It has important economic and environmental implications.

Existing literature and our previous research data analysis have shown growing interest in studying the effectiveness of soil amendments based on organic substances, such as Biochar produced from agricultural, food, and industrial waste and synthetic nitrogen-containing heterocyclic compounds like Ivin, Methyur, and Kamethur. Biochar derived from miscanthus waste plays a crucial role in enhancing soil structure, thereby improving soil fertility and water retention capacity. Furthermore, its production aids in mitigating carbon emissions by sequestering carbon in a stable and long-lasting form, contributing to sustainable environmental practices. However, no studies have explored the potential for the separate or combined application of these innovative environmentally friendly compounds, nor have they examined their possible interactions during maize cultivation. The objective of this study is to investigate the regulatory effects of Biochar produced from miscanthus waste, applied individually or in combination with nitrogen-containing heterocyclic compounds to accelerate maize growth during the vegetative phase.

MATERIALS AND METHODS

Characteristics of Biochar and Synthetic Nitrogen-Containing Heterocyclic Compounds

The Biochar studied in this work is produced from *Miscanthus x giganteus* waste by oxygen-limited pyrolysis at t=600°C.[12,26,27] The Biochar characteristics are shown in Table 1. Newel synthetic nitrogen-containing heterocyclic compounds such as Ivin (*N*-oxide-2,6-dimethylpyridine), Methyur and Kamethur (sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine) were developed at the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine

Table 1. Physicochemical properties of Biochar.

№	Property	Value
1.	Surface area, m^2/g	187
2.	Porosity, cm^3/g	0.55
3.	Mass fraction of the moisture, %.	24.9
4.	Mass fraction of volatile substances, %	8.2
5.	Mass fraction of fixed carbon, %.	82.5

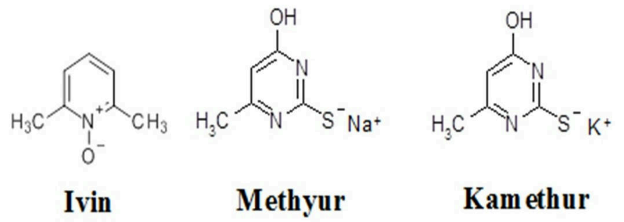

Figure 1. Chemical structures of synthetic nitrogen-containing heterocyclic compounds such as Ivin (N-oxide-2,6-dimethylpyridine), MW=125.17; Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine), MW=165.17; Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine), MW=181.28.

(Figure 1). Auxin (Indole-3-acetic acid, IAA) was obtained from Sigma-Aldrich, USA.

Soaking Seeds with Tested Compounds and Conditions for Plant Growth

The experiments were carried out using seeds of maize (*Zea mays* L.) of the Twist variety (hybrid FAO 270). The procedure for sterilising seeds involves treating them with a 1% solution of $KMnO_4$ for 15 min and a 96% solution of ethanol for 1-2 min and rinsing with sterile distilled water. Seeds were placed in plastic containers (20-25 seeds in each) filled with an artificial substrate such as perlite moistened with distilled water, an aqueous solution of IAA at a concentration of 10^{-6} M, or a 10% solution of Biochar, applied separately or in combination with synthetic nitrogen-containing heterocyclic compounds at a concentration of 10^{-6} M. Then, the plastic containers were placed in a thermostat set to 23°C in darkness for 48 h to allow seed germination. After sprout emergence, the seedlings were transferred to a climate box for growing with a 16-hour light and 8-hour dark cycle, maintaining a temperature of 21-23°C, humidity of 55-75%, and illumination of 3000 lux. After 3 weeks, a comparative analysis of the morphological characteristics of the maize was performed, including average indicators such as shoot length (mm), root length (mm), number of roots (pcs), and biomass (g) per 10 plants, following the methodology outlined in the relevant manual.[28] The ratio of morphological characteristics between the experimental and control plants was expressed as %.

Determination of Chlorophyll and Carotenoid Content

The chlorophyll and carotenoid contents in the plant leaves were determined following the methodology outlined in the relevant manuals.[29] A detailed description of the methodology and calculation formulas used in these studies is given in our previously published work.[30] The ratio of chlorophyll and carotenoid content between the experimental and control plants was expressed as %.

Measurement of the Total Soluble Protein Content

The content of total soluble protein (g/100 g FW) in maize leaves was determined using the Bradford technique.[31] A detailed description of the methodology and calculation formulas used in these studies is given in our previously published work.[32] The ratio of the total soluble protein concentration between the experimental and control plants was expressed as %.

Statistical Analysis

Each experiment was performed three times (n=3). Statistical processing of the experimental data was performed using Student's t-test with a significance level of $P<0.05$; mean values ± standard deviation (± SD).[33]

RESULTS

Impact of Biochar and Synthetic Nitrogen-Containing Heterocyclic Compounds on Maize Growth

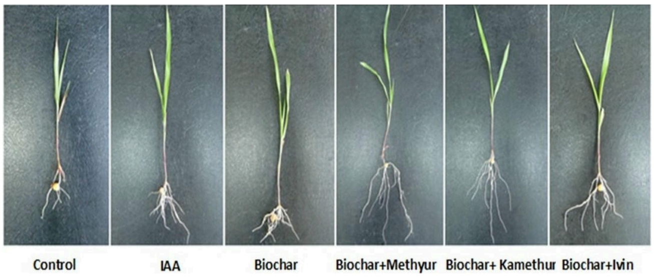

Figure 2. Impact of auxin IAA, Biochar, and its combination with synthetic nitrogen-containing heterocyclic compounds on maize growth compared to control plants.

Figure 2 illustrates the results of the impact of Biochar and its combination with synthetic nitrogen-containing heterocyclic compounds on the growth and development of maize during 3 weeks of vegetation. Under the influence of Biochar, used alone or in combination with synthetic nitrogen-containing heterocyclic compounds, an increase in the morphological characteristics of maize (shoot length (mm), root length (mm), number of roots (pcs), and biomass (g) per 10 plants) was observed. The growth-regulating activity of Biochar, applied separately or in combination with synthetic nitrogen-containing heterocyclic compounds, was equivalent to or superior to the activity of the auxin IAA (Figures 3-6).

Treatment of maize seeds with auxin IAA promoted the growth of shoots and roots, which increased the shoot length (mm) by 33.36%, root length (mm) by 73.83%, and number of roots (pcs) by 393.94%, which led to an increase in plant biomass by 48.15%, in relation to control plants (Figures 3-6). Treatment of maize seeds with Biochar and its combination with synthetic nitrogen-containing heterocyclic compounds, specifically Methyur and Kamethur, similarly increased the shoot length (mm): by 69.19%-with Biochar treatment, by 79.63%-with Biochar+Methyur treatment, and by 88.03% -with Biochar+Kamethur treatment; root length (mm) by 106.92% -withh Biochar treatment, by 370.09% - with Biochar+Methyur treatment, and by 361.68% -with Biochar+Kamethur treatment; the number of roots (pcs) by 339.39% -with Biochar treatment, by 421.21% -with Biochar+Methyur treatment, and by 345.45%-with Biochar+Kamethur treatment, respectively, which led to an increase in plant biomass by 54.56% -with Biochar treatment, by 68.10% -with Biochar+Methyur treatment, and by 78.48% -with Biochar+Kamethur treatment, respectively, in relation to control plants (Figures 3-6). The treatment of maize seeds with Biochar+Ivin similarly promoted the growth of shoots, with their length (mm) increasing by 55.90%. However, it was less effective in promoting the growth of maize roots, with their length (mm) increasing by 14.02% and the number of roots (pcs) increasing by 112.12%, which led to an increase in plant biomass by 39.07%, respectively, in relation to control plants (Figures 3-6).

Impact of Biochar and Synthetic Nitrogen-Containing Heterocyclic Compounds on Chlorophyll and Carotenoid Content in Maize Leaves

The impact of Biochar and its combination with synthetic nitrogen-containing heterocyclic compounds on the content of chlorophylls and carotenoids (mg/g FW) in maize leaves was studied. The results obtained showed that under the influence of Biochar, used alone or in combination with synthetic nitrogen-containing heterocyclic compounds, an increase in chlorophyll and carotenoid content (mg/g FW) was observed in maize leaves, as illustrated in Figure 7. The regulating activity of Biochar, applied separately or in combination with synthetic nitrogen-containing heterocyclic compounds, was equivalent to or superior to the activity of the auxin IAA.

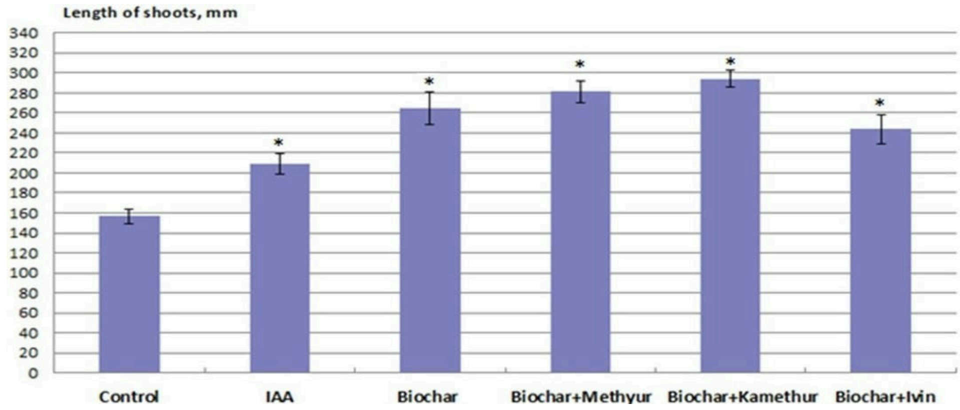

Figure 3. Impact of auxin IAA, Biochar, and its combination with synthetic nitrogen-containing heterocyclic compounds on the average length of maize shoots (mm) compared to control plants. Note. *Significant differences from control values, $p<0.05$, $n=3$, the values are mean±SD.

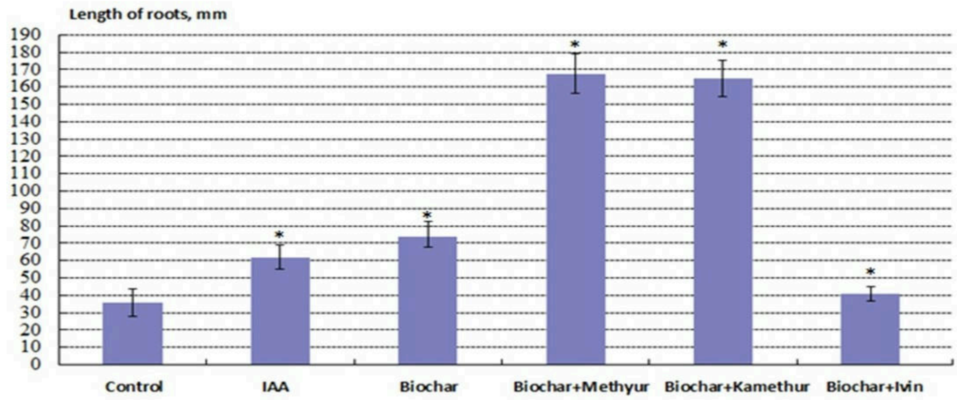

Figure 4. Impact of auxin IAA, Biochar, and its combination with synthetic nitrogen-containing heterocyclic compounds on the average length of maize roots (mm) compared to control plants. Note. *Significant differences from control values, $p<0.05$, $n=3$, the values are mean±SD.

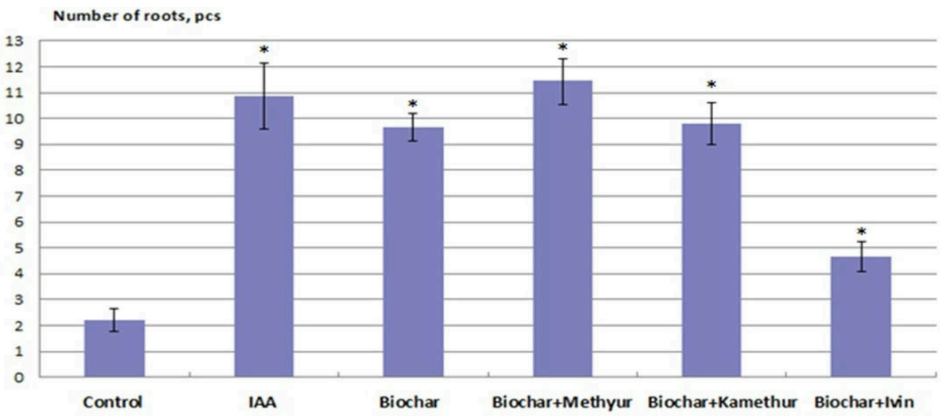

Figure 5. Impact of auxin IAA, Biochar, and its combination with synthetic nitrogen-containing heterocyclic compounds on the average number of maize roots (pcs) compared to control plants. Note. *Significant differences from control values, $p<0.05$, $n=3$, the values are mean±SD.

Treatment of maize seeds with auxin IAA increased the content of chlorophyll a in maize leaves by 46.62%, chlorophyll b by 46.2%, chlorophylls a+b by 46.52%, and carotenoids by 22.22%, respectively, in relation to control plants (Figure 7). Similar results regarding chlorophyll content in maize leaves were obtained when maize seeds were treated with Biochar and its combination with synthetic nitrogen-containing heterocyclic compounds. The treatment of maize seeds with Biochar increased the content of chlorophyll a in maize leaves by 29.30%, chlorophyll b by 43.39%, and chlorophylls a+b by 32.47%, respectively, in relation to control plants (Figure 7). Treatment of maize seeds with Biochar+Methyur increased the

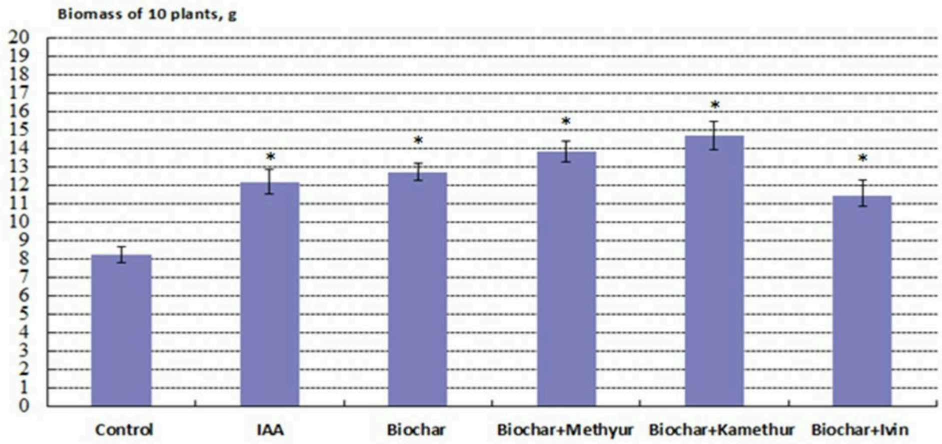

Figure 6. Impact of auxin IAA, Biochar, and its combination with synthetic nitrogen-containing heterocyclic compounds on the average biomass (g) of 10 maize plants compared to control plants. Note. *Significant differences from control values, $p<0.05$, n=3, the values are mean±SD.

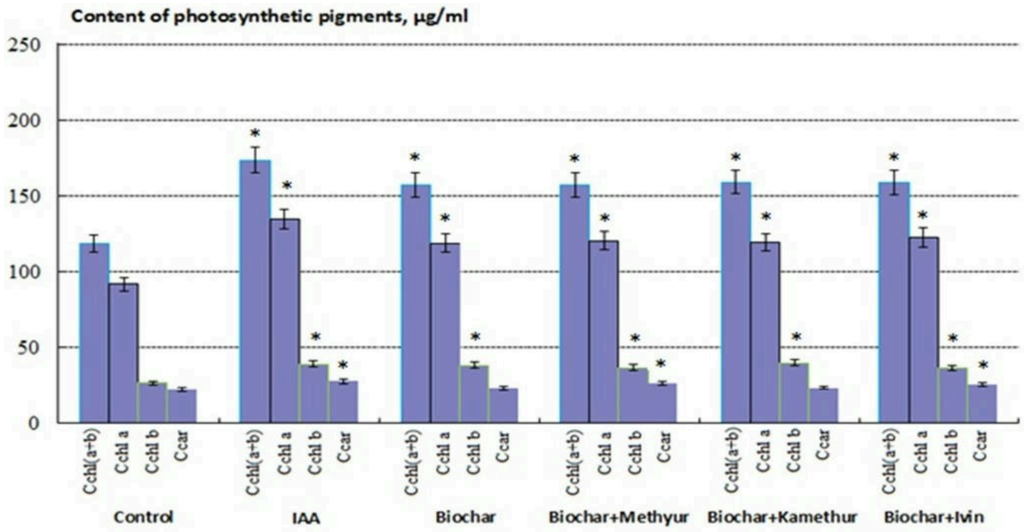

Figure 7. Impact of auxin IAA, Biochar, and its combination with synthetic nitrogen-containing heterocyclic compounds on the content of chlorophyll a, chlorophyll b, and carotenoids (µg/mL) in maize leaves compared to control plants. Note. *Significant differences from control values, $p<0.05$, n=3, the values are mean±SD.

content of chlorophyll a in maize leaves by 31.15%, chlorophyll b by 37.69%, chlorophylls a+b by 32.63%, respectively, in relation to control plants (Figure 7). Treatment of maize seeds with Biochar+Kamethur increased the content of chlorophyll a in maize leaves by 30%, chlorophyll b by 49.16%, chlorophylls a+b by 34.32%, respectively, in relation to control plants (Figure 7). Treatment of maize seeds with Biochar+Ivin increased the content of chlorophyll a in maize leaves by 33.54%, chlorophyll b by 36.33%, chlorophylls a+b by 34.17%, respectively, in relation to control plants (Figure 7).

The content of carotenoids after treatment of maize seeds with Biochar+Methyur and Biochar+Ivin increased by 15.56% and 12.02%, respectively, in relation to control plants (Figure 7). The content of carotenoids after treatment of maize seeds with Biochar and Biochar+Kamethur increased to a lesser extent: by 2.85% and 3.86%, respectively, but there was no statistically significant difference from the control plants (Figure 7).

Impact of Biochar and Heterocyclic Compounds Containing Synthetic Nitrogen on Total Soluble Protein Content in Maize Leaves

The impact of Biochar and its combination with synthetic nitrogen-containing heterocyclic compounds on the content of total soluble protein (g/100 g FW) in maize leaves was studied. The results showed that under the influence of Biochar, used alone or in combination with synthetic nitrogen-containing heterocyclic compounds, an increase in content of total soluble protein (g/100 g FW) was observed in maize leaves, as illustrated in Figure 8. The regulating activity of Biochar, applied separately or in combination with synthetic nitrogen-containing heterocyclic compounds, was

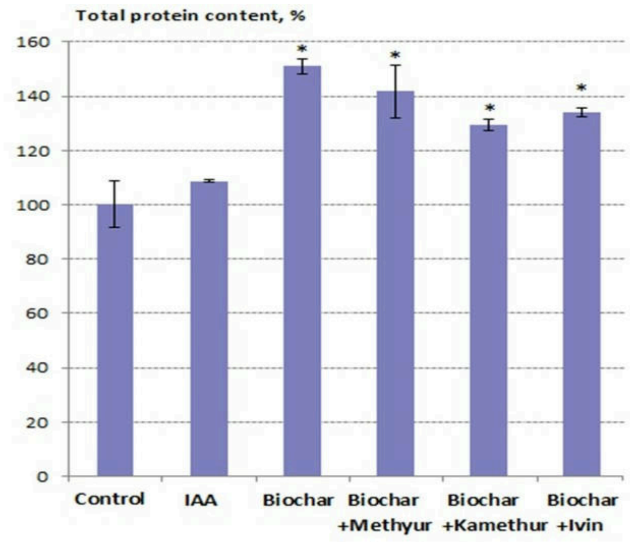

Figure 8. Impact of auxin IAA, Biochar, and its combination with synthetic nitrogen-containing heterocyclic compounds on the content of total soluble protein (%) in maize leaves compared to control plants. Note. *Significant differences from control values, $p<0.05$, n=3, the values are mean±SD.

superior to that of auxin IAA. Treatment of maize seeds with Biochar and its combination with synthetic nitrogen-containing heterocyclic compounds increased the content of total soluble protein: by 50.93% -with Biochar treatment, by 41.64% -with Biochar+Methyur treatment, by 29.45% -with Biochar+Kamethur treatment, and by 34% -with Biochar+Ivin treatment, respectively, in relation to control plants (Figure 8).

Treatment of maize seeds with auxin IAA increased the total soluble protein content to a lesser extent: by 8.66%, but there was no statistically significant difference from the control plants (Figure 8).

DISCUSSION

The results indicate a positive interaction between Biochar and synthetic nitrogen-containing heterocyclic compounds, on the growth on an artificial subtract such as perlite during the vegetative stage of maize, with notable improvements in root and shoot development, photosynthetic pigment content, and protein content in maize leaves. These findings are largely consistent with the existing literature, which highlights the individual benefits of Biochar and synthetic nitrogen-containing heterocyclic compounds on the growth and productivity of agricultural crops.[8,9,10,21-23,32,34-37] However, our study also provides new insights into the mechanisms underlying the observed synergistic effects, which have been less explored in prior research.

Biochar is an organic substance containing a certain amount of macro- and micronutrients that improve soil health and increase crop productivity.[8,9,34] Biochar quality depends on the chemical components of the feedstock, the pyrolysis system, and the production conditions, including temperature and residence time.[11] Adding Biochar to soils is an effective way to achieve agronomic benefits, such as increasing crop growth and yield, reducing plant diseases, stimulating the activity of antioxidant defense systems of plants under environmental stresses, reducing CH_4 emissions, and improving remediation and sequestration of soils contaminated with trace elements, heavy metals, and pesticides.[8,9,34-38] Biochar has long been recognised for its ability to improve soil physical and chemical properties and health, including enhancing the absorption of nutrients by plants, increasing water retention capacity, and stimulating beneficial soil microbial activity.[9,34-38] These characteristics support plant growth by providing a more favorable environment for root development and nutrient uptake. Our findings are consistent with those of earlier studies, demonstrating that Biochar significantly improves the conditions for maize root growth. Furthermore, our results support the view that Biochar can enhance soil fertility by increasing nutrient availability, particularly phosphorus, and improving microbial populations that are crucial for nutrient cycling.[38]

One of the key aspects of our study is the exploration of how Biochar works in conjunction with synthetic nitrogen-containing heterocyclic compounds. Previous research has primarily focused on Biochar's individual effects on soil and plant growth, with less emphasis on its interaction with other growth-regulating substances.[12,26,27] Our current findings suggest that Biochar's positive effects on water retention create an optimal growth environment for the application of synthetic nitrogen-containing heterocyclic compounds, which, in turn, may enhance the plant's response to Biochar.

The positive effect of Biochar on maize development during the vegetative stage can be attributed to the high quality of the Biochar used in the present study, as determined by its physicochemical characteristics. Biochar produced from miscanthus biomass demonstrated a significant potential for agricultural applications due to its favorable properties. Key attributes, such as a specific area of the surface (187 m^2/g), total pore size (0.55 cm^3/g), significant fixed carbon content (82.5%), relatively low volatile matter content (8.2%), and moderate ash content (9.3%), ensure its stability and contribute essential minerals to the artificial substrate for plant growth without causing excessive pH shifts.[12,26,27] These qualities make Biochar an effective amendment for improving soil water-holding capacity, especially in drought-prone regions, and enhancing nutrient retention for better root uptake. The high fixed carbon content also supports long-term carbon sequestration, making Biochar both agronomically and environmentally beneficial. The Biochar's physical and chemical characteristics synergistically support improved maize growth and development during the vegetative stage by fostering a more favorable growing environment, which enhances root growth, stress tolerance, and overall plant vigor. This opens up prospects for using high-quality Biochar to achieve optimal results in agricultural systems.

While Biochar's role as a soil amendment is well-established, the synergistic effects observed in this study underscore its potential to complement synthetic nitrogen-containing heterocyclic compounds, thereby magnifying their impact on maize growth even in the absence of soil on an artificial substrate such as perlite. In line with previous studies, our results indicate that synthetic nitrogen-containing heterocyclic compounds such as Ivin, Methyur, and Kamethur, as well as other pyrimidine derivatives, can regulate plant growth by modulating phytohormonal pathways, including those of auxins and cytokinins, which are crucial for processes like root and shoot development, photosynthesis, and stress response.[21–23,30,32,39–45] In our study, these synthetic nitrogen-containing heterocyclic compounds significantly enhanced maize growth, leading to increased root and shoot biomass, improved photosynthetic efficiency, and higher protein content in maize leaves. This finding supports prior research that demonstrated the regulatory role of synthetic nitrogen-containing heterocyclic compounds in promoting plant growth and increasing crop yields.[21–23,30,32,39-45] However, the key contribution of this study lies in the demonstration that the combination of Biochar and synthetic nitrogen-containing heterocyclic compounds resulted in even greater improvements in plant growth compared to either treatment alone. The observed synergistic effects between Biochar and synthetic nitrogen-containing heterocyclic compounds can be explained through several mechanisms. This synergistic effect suggests that Biochar enhances the efficiency of synthetic nitrogen-containing heterocyclic compounds by improving water uptake under growing conditions and introducing essential minerals into the artificial substrate for plant growth. Biochar's ability to increase water retention creates a more favorable environment for plant roots, thereby enhancing the plant's response to synthetic nitrogen-containing heterocyclic compounds. These findings are consistent with the concept that Biochar can act as a co-adjuvant, improving the effectiveness of other agricultural inputs, including synthetic nitrogen-containing heterocyclic compounds, by optimizing the plant growing conditions.[12,34] Moreover, literature data suggest that Biochar may play an additional role in improving nutrient cycling and enhancing soil fertility, which supports better plant growth by promoting nutrient and phosphorus availability by promoting the activity of phosphate-solubilizing bacteria (PSB), which are crucial for mobilizing phosphorus from soil organic matter, making it more accessible to plants.[34] Phosphorus is a key nutrient for plant growth, and its availability can significantly impact plant productivity when grown in soil conditions. This is consistent with previous research suggesting that Biochar may indirectly support plant growth by enhancing nutrient availability, especially in nutrient-limited soils.[38] The increased nutrient and phosphorus availability facilitated by the addition of Biochar to soils is likely to enhance the effects of synthetic nitrogen-containing heterocyclic compounds on maize growth under soil conditions, as the availability of this essential nutrient supports the growth-promoting functions of synthetic nitrogen-containing heterocyclic compounds. These important aspects will be explored in our further research.

The combined action of Biochar and synthetic nitrogen-containing heterocyclic compounds appears to work in synergy, not only by improving growing conditions but also by modulating plant metabolic processes. Synthetic nitrogen-containing heterocyclic compounds, such as pyridine and pyrimidine derivatives, have auxin- and cytokinin-like effects, enhancing plant growth, photosynthesis, protein biosynthesis, and stress tolerance.[21–23,30,32,39-45] In this context, Biochar helps optimize the growth environment, creating conditions that allow the synthetic nitrogen-containing heterocyclic compounds to exert their full regulatory potential. Together, these components promote improved root and shoot growth, higher photosynthetic efficiency, and increased biomass production. Our study supports the findings of earlier research that Biochar enhances soil properties and plant growth, particularly by improving nutrient availability and water retention.[9,34,38] Similarly, the effects of synthetic nitrogen-containing heterocyclic compounds—pyridine and pyrimidine derivatives—on plant growth have been well documented, with several studies reporting their positive impact on plant growth and stress tolerance.[21–23,30,32,39-45] However, our study shows that the combined application of Biochar and synthetic nitrogen-containing heterocyclic compounds may result in a synergistic effect on maize growth. Although individual treatments have been extensively studied, the interaction between Biochar and synthetic nitrogen-containing heterocyclic compounds has received less attention. Our findings provide valuable insight into the potential benefits of combining these two components, offering a promising strategy for improving crop productivity. However, one area where our findings diverge from the literature is the effect of Biochar and synthetic nitrogen-containing heterocyclic compounds on soil health, and there are significantly fewer studies examining the cumulative effects of Biochar and synthetic nitrogen-containing heterocyclic compounds on soil microbial communities and ecosystem resilience. Considering the aspect of environmental safety of the synthetic nitrogen-containing heterocyclic compounds we are studying, it is necessary to note their important advantage over other chemical means of plant growth control. These synthetic nitrogen-containing heterocyclic compounds can be used in nanomolar concentrations from 10^{-4} M to 10^{-7} M only at the stage of seed processing by spraying and subsequent drying of seeds before planting in the soil. Therefore, they do not accumulate in the soil and do not enter the grain, which is used as food for people and animals. Moreover, our earlier studies on rats indicate the ability of synthetic nitrogen-containing heterocyclic compounds, such as *N*-oxide-2,6-dimethylpyridine (Ivin), to reduce the toxic effects of pesticides on animals, which may be a useful tool for their combined use with pesticides in agriculture.[25] Further research is needed for assessment of cumulative effects of Biochar and synthetic nitrogen-containing heterocyclic compounds on soil sustainability and microbial diversity.

CONCLUSION

The results of this study confirm the benefits of adding Biochar as an amendment to the artificial substrate perlite and the effectiveness of using synthetic nitrogen-containing heterocyclic compounds to stimulate plant growth. In particular, our findings highlight the synergistic effects of combining Biochar with synthetic nitrogen-containing heterocyclic compounds on vegetative growth in maize. This synergy enhances maize growth by improving growth conditions and the physiological responses of plants to synthetic growth-regulating compounds. Although our results are consistent with existing studies on the individual effects of Biochar and synthetic nitrogen-containing heterocyclic compounds, they also require further research towards a new understanding of the driving mechanisms of their interactions on plant growth under soil conditions, especially in terms of phosphorus availability and nutrient cycling. These results suggest that the combined use of Biochar and synthetic nitrogen-containing heterocyclic compounds could offer a sustainable strategy for improving maize productivity, particularly in nutrient-limited or stressed environments. However, further research is needed to examine the effects of these treatments on soil health, microbial communities and the overall sustainability of agricultural systems. Understanding the full scope of the interactions between Biochar and synthetic nitrogen-containing heterocyclic compounds is crucial for optimizing their use in sustainable agricultural practices and enhancing crop resilience to environmental stresses.

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