Carbon nitride nano-biochar exhibit dose-dependent effect on rice growth

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Abstract. Biochar, a byproduct of biomass pyrolysis, has gained interest for its wide-ranging uses in agriculture and environmental remediation. Nano-biochar, in particular, holds promise for enhancing crop productivity and addressing environmental challenges faced by the plants. Its ability to improve soil properties and biological functions underscores its potential in sustainable agriculture. This study investigates the influence of a carbon-based nanobiochar- carbon nitride (C_3N_4, derived from melamine) on rice (*Oryza sativa*) growth. Despite the promise of nanostructured biochar materials in bolstering crop yields, their interaction with varying concentrations of C_3N_4 remains poorly understood. In this study, we studied the impact of five concentrations (0μM, 500μM, 1000μM, 1500μM, and 2000μM) of C_3N_4 on rice growth. Our results reveal a concentration-dependent response, with 1500μM and 2000μM concentrations exhibiting toxic effects on rice plants, while 500μM and 1000μM concentrations demonstrate positive effects on rice growth parameters (root-shoot length, fresh-dry biomass) with maximum values obtained in case of the latter. This research sheds light on the potential of C_3N_4 nanobiochars to influence the growth of crop plants, emphasizing the importance of optimizing concentrations for sustainable agricultural practices. Further exploration in this area could lead to finding of solutions for enhancing agricultural productivity in a sustainable manner.

Keywords: Root, Shoot, Rice, Carbon Nitride, Biochar, Nanobiochar, Biomass

1 Introduction

The escalating global population and finite arable land pose substantial challenges to global agricultural productivity and food security [1]. Scarce resources and the swiftly increasingly population of human, and probably reach 9.6 billion by 2050, propel the agricultural sector towards the urgent need for highly efficient practices to alleviate global poverty and hunger. Challenges such as limited nutrient efficiency and environmental constraints linked to chemical fertilizers persist as significant obstacles to achieving sustainable agriculture [2]. To address fertility of soil and crop growth enhancement, biochar has emerged as a promising soil amendment. Biochar, produced from various organic wastes, possesses properties such as high carbon content, alkalinity, and porous structure, contributing to improved soil pH, structure, and nutrient availability [3, 4]. Biochar's effectiveness depends on factors such as feedstock type, pyrolysis conditions, and post-treatment processes. Upon soil application, biochar influences soil solution composition, enhancing nutrient availability and reducing toxicity [5, 6]. Production methods for biochar include pyrolysis, gasification, and hydrothermal carbonization, each resulting in biochar with unique properties and benefits [7]. Incorporating biochar into agriculture enhances soil fertility and crop growth by adjusting soil conditions and nutrient availability [8]. Moreover, biochar can serve as an electron shuttle and substrate for photocatalysts, contributing to environmental remediation and sustainable agricultural practices [9, 10].

Recently, nanobiochar has garnered significant interest within the realm of engineered biochar varieties due to its advantageous chemical and physical characteristics. Various research endeavors have been undertaken to explore innovative techniques for the preparation and utilization of nanobiochar [11]. Nanobiochar has the potential for modification or engineering to yield "engineered nanobiochar" or biochar nanocomposites, which exhibit improved properties and expanded applications. Compared to bulk biochars, nanobiochar offers substantial enhancements in surface area (increasing by 0.4 to 97 times), pore size (increasing by 0.1 to 5.3 times), total pore volume (increasing by 0.5 to 48.5 times), and surface functionalities [12]. Nanobiochar offers a multitude of advantages, including enhancement in the growth of plant and properties of soil, efficient management of disease, remediation of pollutants and chemicals,

treatment of wastewater, and serving as a support material for immobilization of enzyme. Its cost-effective value, viable, and environmental affability marks it as a promising alternative to conventional approaches. Additionally, nanobiochar can contribute to mitigating climate change through its carbon sequestration function. Compared to biochar, nanobiochar exhibits superior capabilities in absorbing pollutants, nutrients, and contaminants, with increased mobility in soil, thereby presenting a potential substitute for waste management [13]. Graphitic carbon nitride (g- C_3N_4) stands out among carbon-based nanomaterials due to its distinctive layered structure, ease of fabrication, affordability, and eco-friendly nature [14, 15]. g-C_3N_4 exhibits potential in contaminant mitigation due to its high surface area and electrostatic forces, as evidenced by various studies [16]. Additionally, g-C_3N_4 finds application in nanoagriculture, such as ameliorating the toxic effects of arsenic (As) on rice plants [17].

In conclusion, the convergence of innovative materials such as nanobiochar such as C_3N_4 with traditional agricultural practices presents promising solutions to the multifaceted challenges of global food security, environmental sustainability, and human health. These materials offer a range of benefits, from enhancing soil fertility and crop productivity to mitigating environmental pollution and climate change impacts. Rice (*Oryza sativa*), a chief food for over part of the population of world, serves as a significant nutritive component rich in carbohydrates, protein, fat, and fibre. It presents opportunities for functional foods and pharmaceuticals leveraging its medicinal properties [18]. Being staple crop, rice continue to play pivotal roles in global nutrition and health, understanding the influence of C_3N_4 on growth of rice can unlock new opportunities for improving food quality, safety, and accessibility. Continued research and development efforts in these areas are essential to harnessing the full potential of these innovative materials and ensuring a resilient and sustainable future for agriculture and society.

2 Materials and methods

2.1 Growth Conditions of plants

O. sativa L. seeds were procured from a certified dealer located in the Prayagraj district. Seedlings were cultivated for 15 days following the protocols outlined by [19]. Prior to germination, seeds were sterilized for 10 minutes using 10% (v/v) sodium hypochlorite, followed by washing with distilled water and subsequent placement on moistened cloth of muslin in darkness for about 2 days for the promotion of germination rate. Seeds which were germinated then transferred onto Petri plates with Whatman Filter Paper No. 1 soaked in Hoagland nutrient medium [20]. These Petri plates were maintained in a growth chamber (Impact model IIC 129D, New Delhi, India) under

controlled conditions, providing photosynthetically active radiation (PAR) of 300 µmol photons m-2 s-1, relative humidity ranging between 65–70%, and a constant temperature of 26 ± 1 ºC with a 12-hour light/12-hour dark cycle. Throughout the growth period, seedlings were regularly supplied with nutrient medium. Later, seedlings of uniform size were selected and subjected to various chemical treatments.

2.2 Screening Treatment

The experimental treatments consisted of control (0 µM), 500µM, 1000µM, 1500µM, and 2000µM concentrations of C_3N_4 procured from Harcourt Butler Technical University, Kanpur, India. All solutions were prepared in Hoagland nutrient medium ($MgSO_4.7H_2O$, $Ca(NO_3)_2.2H_2O$, KH_2PO_4, KNO_3, Fe+EDTA($FeCl_3$), H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $Na_2MoO_4.2H_2O$) and were administered to seedlings for 7 days before subsequent analysis, as per the methodology outlined by [18]. Each treatment solution, total 40 ml, was allocated per pot. The pots were positioned within a plant growth chamber set to a photon flux density of 300 µmol photons $m^{-2} s^{-1}$, operating on a 12-hour light/12-hour dark cycle, with relative humidity maintained at 75–80% and a temperature of 26 ± 1 ºC for a duration of 7 days. To prevent root hypoxia, the solutions for all treatments were replaced every 3 days throughout the experimental period.

2.3 Analysis of Growth Parameters

2.3.1 Morphological Traits

Morphological characteristics, including root length, and shoot length of rice seedlings, were assessed using a centimeter scale.

2.3.2 Biomass Measurement

The fresh weight of distinct samples was determined by segregating roots and shoots and weighing them separately with a digital precision balance. Additionally, the dry weight of distinct samples was also determined by segregating roots and shoots and drying them in oven followed by weighing them separately with a digital precision balance.

2.4 Statistical Analysis

Statistical analyses were conducted by employing one-way analysis of variance (ANOVA) using SPSS 16.0 software, following verification of the normal distribution of the data. Duncan's multiple range test (DMRT) at a significance level of P<0.05 was utilized to discern variations among treatments. The reported

values represent the means derived from three independent biological replicates (n=3).

3 Results and Discussion

At the plant's harvest stage, assessments were conducted on various growth parameters. These encompassed measurements such as shoot- root length and both fresh and dry weights of shoots- roots. The morphological effects of the treatments are illustrated in Fig 1.

Fig. 1. Morphological impact of various concentrations of C_3N_4 (0µM, 500µM, 1000µM, 1500µM, 2000µM) on root and shoot of rice seedlings.

3.1 Shoot length

In this study, at a concentration of 500µM, C_3N_4 concluded a 4.4% increase in shoot length with respect to the control, while at 1000µM concentration, there was a significant 8.9% enhancement in shoot length relative to the control (Fig 2). Conversely, concentrations of 1500µM and 2000µM led to reductions in shoot length by 1.7% and 9.8%, respectively, related to the control. Notably, concentrations up to 1000µM of C_3N_4 exhibited a positive impact on rice shoot length, while higher concentrations proved to be toxic (Fig 2). These findings suggest that while C_3N_4 can positively impact plant growth at optimum concentrations, as reported previously by Hao et al [21] in rice against cadmium (Cd) stress. However, excessive application can lead to negative effects, marking the significance of optimal dosing in agricultural practices. Overall, these studies broadly emphasize the practicability of C_3N_4 as a beneficial means in enhancing plant growth and stress tolerance, while also emphasizing the importance of dosage optimization for maximizing its benefits in agriculture.
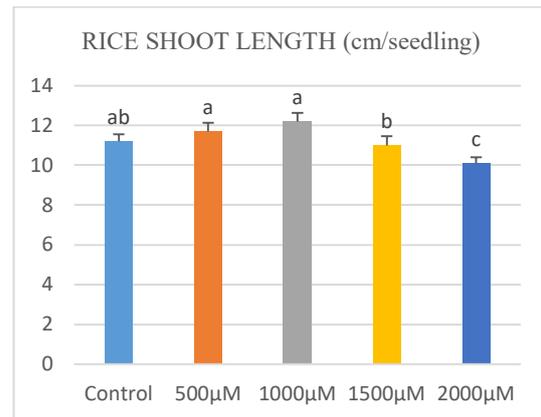

Fig. 2. Impact of varying concentrations of C_3N_4 (0µM, 500µM, 1000µM, 1500µM, 2000µM) on the shoot length of rice seedlings.

3.2 Root length

In the research conducted, at concentration of 500µM, C_3N_4 led to a 5.4% increase in length of the roots as compared to the control, while at 1000µM concentration, a significant 9% enhancement in root length was observed relative to the control (Fig 3). Conversely, concentrations of 1500µM and 2000µM resulted in reductions of length of root by 1.8% and 10%, as related to the control. Notably, concentrations up to 1000µM of C_3N_4 demonstrated a positive impact on rice root length, while higher concentrations were found to be detrimental (Fig 3). These findings suggest that while C_3N_4 can positively impact plant growth at optimum concentrations, as reported previously by Ma et al [17] in rice against Cd and arsenic (As) stress. However, excessive application can cause toxic effects, emphasizing the potential of optimal dosing in agricultural practices. These findings collectively suggest that C_3N_4 holds promise as a versatile agent for enhancing plant growth and stress tolerance, particularly in the context of heavy metal contamination in agricultural soils.
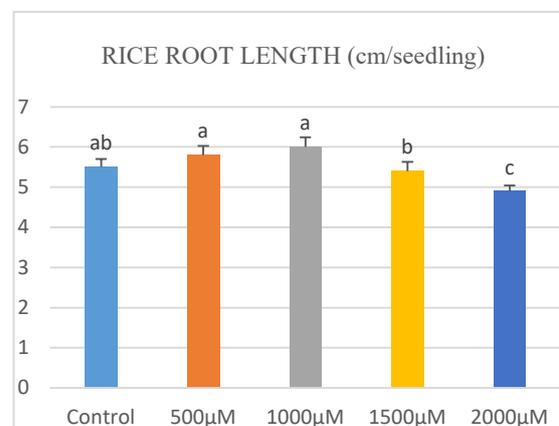
Fig. 3. Impact of varying concentrations of C_3N_4 (0μM, 500μM, 1000μM, 1500μM, 2000μM) on the root length of rice seedlings.

3.3 Root fresh weight

In the research conducted, 500μM and 1000μM concentration of C_3N_4 led to increase in the fresh weight of root by 8.8% and 14.9% as related to the control in the rice plants (Fig 4). Whereas 1500μM and 2000μM concentration of C_3N_4 proved to be toxic as these concentrations showed a decline in the root fresh weight by 6% and 6.6% respectively as compared to the control. These observations indicate that 1000μM C_3N_4 exhibited maximum positive influence on root fresh weight in comparison to the control, beyond this concentration the data for root fresh weight suggests of a toxic impact of C_3N_4 on rice plants (Fig 4). These findings indicate that C_3N_4 has the importance of positively influencing plant growth at optimal concentrations, consistent with previous observations by Hao et al [21] in rice under Cd stress conditions.
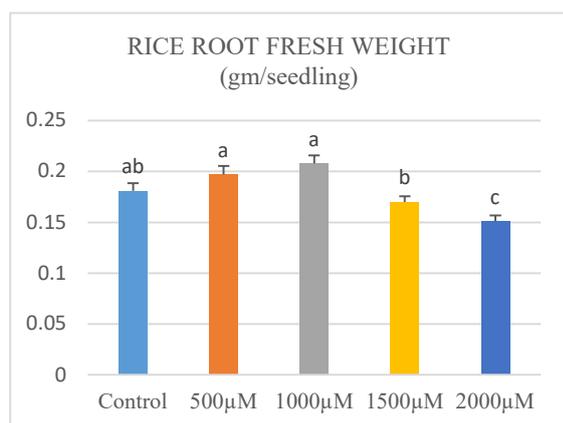

Fig. 4. Impact of varying concentrations of C_3N_4 (0μM, 500μM, 1000μM, 1500μM, 2000μM) on the root fresh weight of rice seedlings.

3.4 Root dry weight

In the experiments conducted, at different concentrations of 500μM and 1000μM, C_3N_4 resulted in an increase in root dry weight by 8.6% and 21.7%, respectively, in comparison of control in rice plants (Fig 5). Conversely, concentrations of 1500μM and 2000μM of C_3N_4 exhibited toxicity, with a decline in dry weight of root by 13% and 17.3%, respectively, compared to the control. Thus, concentrations up to 1000μM of C_3N_4 demonstrate a positive impact on the dry weight of rice plants. However, concentrations higher than 1000μM have been found to be toxic, indicating a detrimental effect on the fresh weight of rice plants (Fig 5). These results suggest that C_3N_4 possesses the capability to beneficially affect plant growth at suitable concentrations, aligning with earlier findings by Xu et al

[22] in soybean plants exposed to cadmium stress. These findings mark the importance of C_3N_4 as a promising strategy for removing heavy metal-created stress in plants and improving overall plant performance.
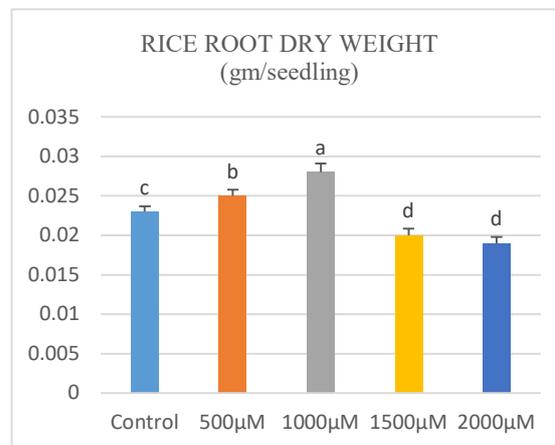

Fig. 5. Impact of varying concentrations of C_3N_4 (0μM, 500μM, 1000μM, 1500μM, 2000μM) on the root dry weight of rice seedlings.

3.5 Shoot fresh weight

At a concentration of 500μM, C_3N_4 worked in a 2.3% increase in fresh weight of shoot as related to the control, however at 1000μM concentration, there was a significant 13.3% enhancement in shoot fresh weight relative to the control (Fig 6). Conversely, concentrations of 1500μM and 2000μM led to reductions in shoot fresh weight by 13.3% and 20.4%, respectively, compared to the control. Notably, concentrations up to 1000μM of C_3N_4 exhibited a positive impact on rice shoot fresh weight, while higher concentrations proved to be detrimental (Fig 6). These findings indicate that C_3N_4 has the potential to positively impact plant growth at appropriate concentrations, consistent with previous observations Xu et al [22] in soybean plants subjected to Cd stress.
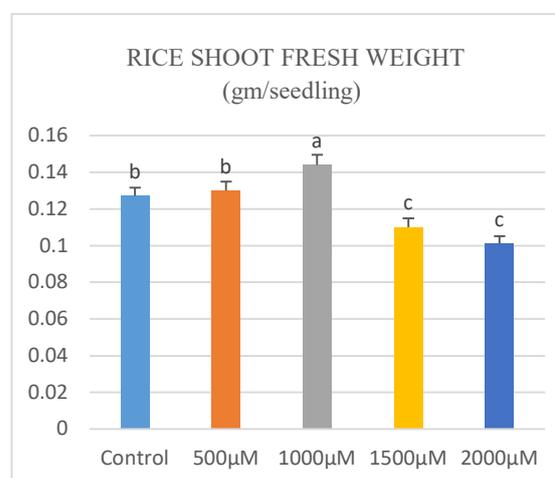
3.6 Shoot dry weight

At a concentration of 500μM, the application of C_3N_4 turned out a 5% increase in dry weight of shoot in comparison of the control, while at 1000μM concentration, a notable enhancement of 7.8% in shoot dry weight was observed relative to the control (Fig 7). On the contrary, concentrations of 1500μM and 2000μM led to decreases in shoot dry weight by 2.1% and 7.8%, respectively, in comparison to the control. Notably, concentrations up to 1000μM of C_3N_4 exhibited a favorable impact on rice shoot dry weight, whereas higher concentrations were observed to have detrimental effects (Fig 7). These results indicate that C_3N_4 can have a beneficial effect on plant growth at optimal concentrations, consistent with earlier observations by Ma et al [17] in rice plants under Cd and As stress. These findings underscore the importance of optimizing C_3N_4 dosage to maximize its beneficial effects on plant growth while avoiding potential toxicity.
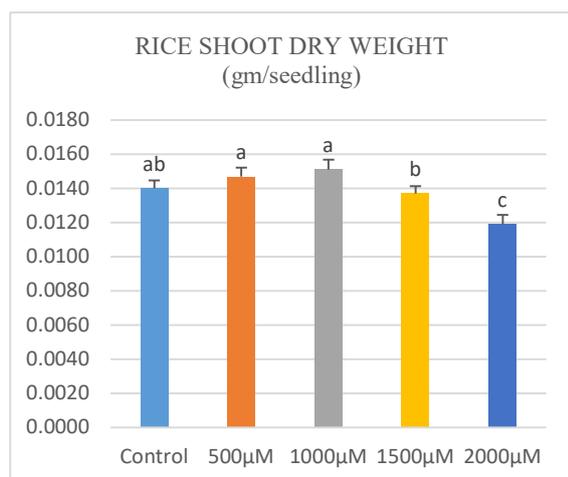

Fig. 7. Impact of varying concentrations of C_3N_4 (0μM, 500μM, 1000μM, 1500μM, 2000μM) on the shoot dry weight of rice seedlings.

4 Conclusion

Biochar application in agriculture and environmental rehabilitation offers a range of advantages, despite encountering obstacles. Nano-biochar emerges as a promising solution, particularly in addressing harmful contaminants and enhancing crop yields, while also positively impacting soil and biological factors, rendering it a suitable option for agricultural endeavors. This research delves into the influence of various nanobiochars on rice cultivation, specifically examining the concentrations of C_3N_4 derived from melamine. While nanostructured biochar has demonstrated efficacy

in bolstering crop output, its interaction with different concentrations of C_3N_4 remains ambiguous. Our findings reveal a concentration-dependent reaction, with 1500μM and 2000μM concentrations displaying toxicity, whereas 500μM and 1000μM exhibit favorable effects on rice growth. This study illuminates the potential of nanobiochar- C_3N_4 to influence the growth of agricultural crops, underscoring the necessity of concentration optimization for sustainable farming practices. Exploring the long-term effects of these interactions on soil microbial communities, nutrient cycling, and overall ecosystem resilience would be invaluable in shaping holistic approaches to sustainable agriculture. Additionally, investigating the scalability and feasibility of implementing nanobiochar-C_3N_4 technologies on a larger scale, including field trials across diverse agroecological settings, could provide practical insights into their real-world applicability and potential impact on global food security.

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