Carbon nitride nano biochar imparts concentration dependent impact on finger millet growth

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Abstract. Biochar, a sustainable green technology product, is essential for promoting plant development and growth. Nano biochar emerges as a promising solution for environmental sustainability. Despite its potential, the intricate dynamics between plants and nano-biochar, particularly in finger millet (*Eleusine coracana*), remain largely unexplored, necessitating a deeper understanding of their interaction. This study investigates the effects of carbon nitride (C_3N_4) nano-biochar, derived from melamine, on finger millet. A screening setup was employed with varied concentrations of C_3N_4: 0 µM (control), 500 µM, 1000 µM, 1500 µM, and 2000 µM. Morphological parameters of plants includes root-shoot length, fresh and dry weights, were analysed to determine the optimal dosage. Results reveal that the most effective concentration of C_3N_4 for enhancing finger millet growth was found to be 1000 µM. Beyond this threshold, higher doses proved to be toxic, impeding plant growth and development. This research sheds light on the unexplored relationship between nano-biochar and plant physiology, offering insights crucial for sustainable agricultural practices.

Keywords: biochar, finger millet, screening, soil fertility, plant nutrient, optimum dose.

1 Introduction

Plant encounters a variety of environmental stress, both biotic and abiotic, which might further obstruct their ability to grow and develop [1]. The increase in the population can simultaneously increase in the demand of food supply and therefore there is a need to fulfil the food demand and its production [2]. There are various methods from which the food production can be increased like the application of chemical fertilizers, using HYV seeds (High Yielding Variety), FYM (farm yard manure), compost, biofertilizers, etc [3]. Application of biochar is one such method from which the quality of food can be improved and food production can be increased. Biochar (carbon-based solid obtained by biomass pyrolysis) is gaining interest for researchers in agriculture field. Addition of biochar can remarkably improve the integrity of soil as the soil needs a specific number of aggregates, solids, and organic matter to function as the optimal growing medium for plants [4,5]

According to a recent study by Noreen and Elsalam [6], nano sized biochar has a higher surface area than a biochar and is unique and advantageous due to its numerous functional groups that provide improved mechanical and thermal stability [6]. The production of the process of carbonization and pyrolysis from which nano biochar is formed from the biomass that is the rich source of carbon. Due to its physical and chemical characteristics and the porous structure, it has emerged as a viable option for soil amendment it is also an excellent absorbent and an efficient enzyme support [7,8]. Plants can absorb nutrients that are bioavailable when they are supplied by biochar. There are numerous ways biochar can enhance the soil health that ultimately leads to improved crop productivity. For plants that cannot fix nitrogen on their own, biochar helps makes it available. Borchard et al [9] mentioned, when applied biochar in maize (*Zea mays*) plant @15g/kg, there is an increase in the yield of maize with increase in the nitrogen, calcium content [9]. Furthermore, nitrogen retention increased with the application of biochar, on applying 90g/kg biochar on legume crops, the rate of nitrogen fixation of common beans increased from 50% to 72% [5, 10]. Biochar also helps in increasing the water holding capacity (WHC) in soil through surface area and porosity. WHC increased by 100% with only 9% of biochar (yellow pine wood that has been pyrolyzed at 400℃) [5, 11]. In another study, it was discovered that the WHC was increased by 30% with the application of sunflower husk biochar [5, 12].

In this study, carbon nitride (C_3N_4) nano-biochar is used (extracted from thermal polycondensation of melamine). Cereals are the source of low-cost energy, contributes significantly to calorie intake (19%) and protein (20%) consumption [13, 14]. One of the important food crops that significantly affects developing countries' economies in finger millet. Millets offers numerous advantages over another crop such as they are resistant to pest and drought [15, 16]. In

India, finger millets are the staple food in different parts, contributes around 60% of the global producer [17, 18, 19]. One of the key features of finger millets is that, it adapts itself to various agroclimatic conditions which accounts for its best yield among other millets. Highlighting its nutritional value, the grain has a carbohydrate content of 81.5%, protein 9.8%, minerals 2.7% and crude fibre 4.3% [17, 18, 19]. To address this increasing food demand and population and to produce improved versions of crops (biotic and abiotic resistance varieties) to provide more nutrients and minerals, the application of nano biochar in crop can be done. The objective is to analyse the optimum dose of biochar by applying the various concentrations in finger millet crop.

2 Material and method

2.1 Growth conditions of plant

Finger millet (*Eleusine coracana*) seeds were procured from Harcourt Butler Technical University, Kanpur, India and washed with distilled water (by removing all the contamination) and post surface sterilization take place using sodium hypochlorite solution (10% v/v) for the duration of 10 min and then were thoroughly washed again. For the duration of 24 hours, seeds were dipped in distilled water in dark conditions in 1:2 portion (1 part of seeds and 2 parts of water). After 24 hours the water was drained out and the seeds were transferred in muslin cloth into seed germinator for 2-3 days for better growth and establishment. Distilled water was supplied when needed, to keep the muslin cloth moistened containing finger millet seeds. Later, selected germinated seeds were grown in petri plates (150 mm, Riviera ™) using Whatman No. 1 filter paper poured with Hoagland's solution (half strength) ($MgSO_4.7H_2O$, $Ca(NO_3).2H_2O$, KH_2PO_4, KNO_3, Fe+EDTA, H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $Na_2MoO_4.2H_2O$, $CuSO_4.5H_2O$) grown in hydroponics system in a growth chamber having 65-70% relative humidity and photo-synthetically active radiation (PAR) of 300 µmol photons m-2 s-1 and 12/12 h day-night cycle at 25±2ºC for the duration of 7 days. During the growth of seedlings, when required, half strength Hoagland's nutrient solution was sprayed [24].

2.2 Screening of finger millet in different concentration of biochar

Different treatment were given to seedlings of uniform size in various cups. Treatments for C_3N_4 include-control 0 µM (Hoagland solution), 500 µM, 1000 µM, 1500 µM and 2000 µM. The solutions were changed after 3 days to protect the seedlings from hypoxia and the seedlings were harvested after 7 days.

2.3 Analysis of growth parameter

2.3.1 Root Length

After harvesting, to analyse the growth parameter, measurement of root length is done using centimetre scale in the finger millet at different concentration of biochar (0 µM, 500 µM, 1000 µM, 1500 µM and 2000 µM).

2.3.2 Shoot Length

With the same methodology, shoot length is also measured using centimetre scale at different concentration of biochar (0 µM, 500 µM, 1000 µM, 1500 µM and 2000 µM).

2.3.3 Fresh Weight

The fresh weight of finger millet roots and shoots is measured in grams [20].

2.3.4 Seedling Dry Weight

Using analytical weight balance, the dry seeds weight of finger millet was measure using an analytical weight balance [20].

2.4 Statistical Analysis

To analyze the data, we utilized one-way analysis of variance (ANOVA) with SPSS 16.0 software, ensuring the data followed a normal distribution. Duncan's multiple range test (DMRT) was employed at a significance level of $P<0.05$ to identify differences among treatments. The reported values represent the means obtained from three independent biological replicates (n=3).

3 Result and Discussion

Throughout the experiment, numerous parameters were tested that includes seedling dry weight, root-shoot fresh weight and root length. Morphological analysis indicates a significant decline in growth parameters as the concentration of C_3N_4 increased beyond1000 µM, as illustrated in Fig 1.

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Fig. 1. Morphological impact of C_3N_4 in root and shoot length of finger millet at different concentrations of biochar.

3.1 Root Length

500 µM concentration of biochar showed 8.6 % increment in root-length as compare to the control whereas at 1000 µM a notable increase of 13% in root-length was observed in comparison to the control (Fig 2). However, at concentrations 1500 µM and 2000 µM, there was a drastic decrease of 6.5% and 10.8% respectively in root length as compared to the control. Therefore, at concentration up to 1000 µM showed a positive impact whereas the concentration higher than this showed slightly toxic effect. Overall, C_3N_4, when provided at optimum doses, can have positive impact on plant growth as reported in case of soybean plant (*Glycine max* L.). Xu et al. [21] mentioned in this study that the addition of C_3N_4 at 250 mg/L concentration helps in promoting the root length of the plant [21]. Another finding in rice plant, Hao et al. [22] mentioned that the application of C_3N_4 at the concentration 200 mg/L in rice results into the positive growth of root length [22]. Ma et al. [23] on the study in rice plant, when applied C_3N_4 it was observed that C_3N_4 gives a positive impact on root length growth [23].

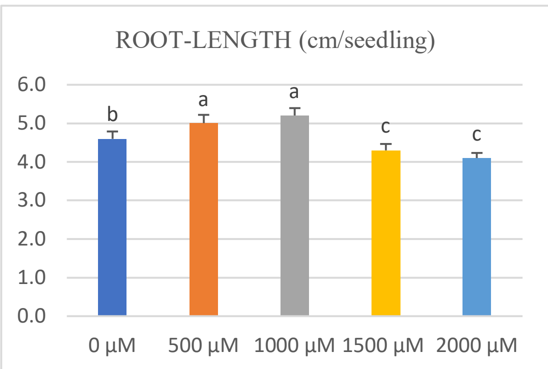

Fig. 2. Graphical analysis of finger millet root length (cm/seedling) at different concentrations of biochar.

3.2 Shoot Length

At the concentration 500 µM, the biochar showed 7.4 % increase in root-length as compared to control whereas at 1000 µM a significant increase of 14.8 % in root-length as compared to control (Fig 3). However, biochar at 1500 µM and 2000 µM induced reduction of 3.7% and 18.5% in shoot length as compared to the control. Therefore, concentration more than 1000 µM was observed to be slightly toxic. Overall, C_3N_4, when provided at optimum doses, can have positive impact on shoot length as reported by Xu et al. [21]. In his study, the biochar, C_3N_4 at the concentration 250 mg/L is given in soybean plant which results into the positive impact in shoot length [21]. Another finding by Hao et al. [22] in rice plant when applied C_3N_4, it was observed that the shoot length increased and gives the positive impact in yield [22]. Ma et al. [23] in their study in rice plant, C_3N_4 was observed to be beneficial for the development of the shoot [23]

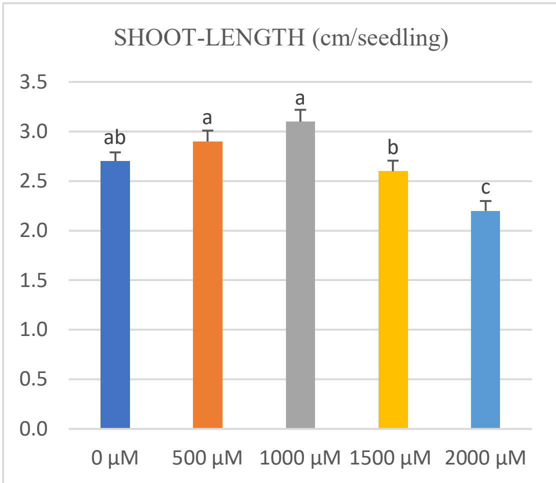

Fig. 3. Graphical analysis of finger millet shoot length (cm/seedling) at different concentrations of biochar.

3.3 Root Fresh Weight

The concentration at 500 µM showed 6.8% increase in root fresh weight as compared to control whereas at 1000 µM, 17.2% increase in the fresh weight of root was observed as compare to control (Fig 3). The biochar concentration at 1500 µM and 2000 µM induced 6.8 % and 13.7% decrease respectively in root fresh weight as compared to control. Overall, C_3N_4, when provided at optimum doses, can have positive result on plant growth as reported in case of rice plant, Hao et al [22] mentioned that the application of C_3N_4 at the concentration 200 mg/L helps in increasing the fresh weight of roots [22].

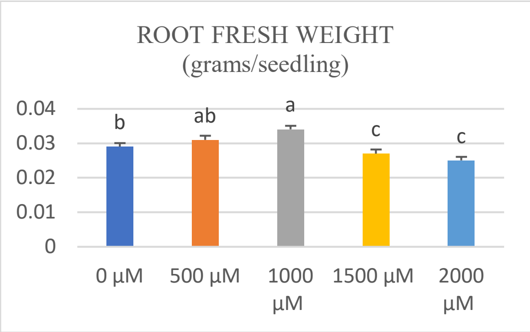

Fig. 4. Graphical analysis of finger millet root fresh weight (grams/seedling) at different concentrations of biochar.

3.4 Shoot Fresh Weight

The concentration at 500 µM showed 21.8% increment in shoot fresh weight in comparison to control. At 1000 µM, 28.1% increase in the shoot fresh weight as compare to control. Whereas at the concentration, 1500 µM and 2000 µM, reduction in shoot fresh weight was observed to be 3.1% and 12.5 % respectively as in comparison to control (Fig 5). Overall, C_3N_4, when provided at optimum doses, can have positive influence on plant growth as reported in case of rice plant, Hao et al. [22] mentioned that the application of C_3N_4 at the concentration 200 mg/L helps in increasing the fresh weight of shoot [22].

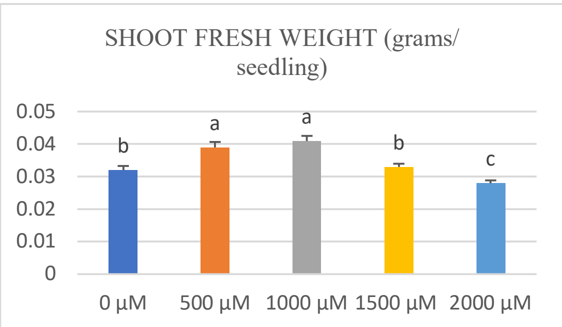

Fig. 5. Graphical analysis of finger millet shoot fresh weight (grams/seedling) at different concentrations of biochar.

3.5 Seedling Dry Weight

At the concentration 500 µM, the seedling dry weight was found to increase by 30% as compare to control. At 1000 µM, 46.1% increase in the seedling dry weight was observed as compared to control. Whereas at the concentration, 1500 µM and 2000 µM, the decrease in seedling dry weight was observed to be 0.7% and 6.9% as compared to control.

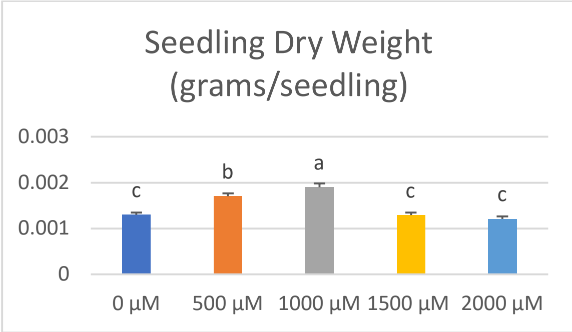

Fig. 6. Graphical analysis of seedling dry weight (grams/seedling) at different concentrations of biochar.

4 Conclusion

According to the data collected, biochar at the concentration up to 1000 µM was observed to be beneficial for finger millet. However, the concentrations above 1000 µM proved to be toxic for finger millet, as it inhibits the plant development and growth. From this study it was concluded that the application of biochar was dose dependent. Hence, finding underscores the potential of biochar application in agriculture by highlighting its part in promoting the plant productivity and growth. It also emphasises that application of biochar at right concentration could potentially promote the growth and development and thereby benefiting the crop production by making it resistance from biotic and abiotic stress, nutrient uptake, nourishment of soil, etc. This data emphasises that lower concentration of biochar is more affective, hence optimizing the concentration of C_3N_4 is of pivotal importance in agricultural practice as well as ensuring a sustainable crop yield.

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