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BIOCHAR BASED BACTERIAL BIOFERTILIZER PROMOTES GROWTH AND MITIGATES COPPER TOXICITY IN *BRASSICA OLERACEA*

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ABSTRACT

The interest to bacterial biofertilizers (BFs) is currently increasing since their application is environment friendly and cheaper in comparison with chemical fertilizers. BFs application reduces the metals availability in the soil through chelation, thereby reducing the toxicity of pollutants. The aim of the research was to study the effect of BF based on biochar and metal tolerant plant growth promoting rhizobacteria (PGPR) on growth parameters, copper accumulation and lipid peroxidation level in *Brassica oleracea* L. *Bacillus altitudinis* strain TF16a isolated from the rhizospheric soil of *Tussilago farfara* L. growing close to copper smelter was used to prepare the inoculum. To obtain BF, liquid inoculum of PGPR (10^8 CFU mL^{-1}) was mixed with sterile substrate. Plants were grown in pot for 30 days. In the control substrate and with the addition of BF, the copper content was two times lower than the maximum permissible concentration for Russia. It was found that combined action of BF with Cu had a positive effect on the growth parameters in *B. oleracea*. Copper application led to the significant increase in its accumulation in shoot and root which was accompanied by the increase in malondialdehyde content in cabbage leaves. The combined action of BF and Cu reduced Cu accumulation and mitigated peroxidation processes. Thus, it can be concluded that biochar based biofertilizer promoted growth and alleviated copper toxicity in *B. oleracea*.

Keywords: *Bacillus altitudinis, cabbage, biochar, plant growth, agrobiotechnology* .

INTRODUCTION

One of the global tasks of our time is to increase the productivity of cultivated plants, as well as to provide the population with high-quality and safe food. The uncontrolled use of chemical fertilizers and pesticides in the agricultural sector leads to the pollution of ecosystems with various pollutants, including heavy metals, and poses a threat to human health (Dubey and Nidhi Shukla, 2014; Yadav et al., 2017). In this regard, the search for safe biological products to stimulate plant growth without harming the environment is becoming increasingly important. One of the promising directions for improving agricultural technologies is the use of biofertilizers based on plant growth promoting bacteria (PGPB), which include both endophytic and rhizobacteria (Lugtenberg and Kamilova, 2009; Rana et al., 2011; Glick, 2012; Maleva et al., 2017; Kumar et al., 2021). In addition, the use of biochar (BC), a carbon-rich material produced by pyrolysis, gasification or hydrothermal carbonization from various organic wastes, is becoming increasingly popular in the agricultural sector (Gascó et al. 2019). The numerous studies have shown that the addition of BC leads to an improvement in the structure and physicochemical characteristics of the soil, promotes the development of soil microflora, and reduces the toxic effect of organic and inorganic pollutants (Lehmann, 2011; Wang et al., 2019). The use of BC allows not only the efficient use of industrial waste, but also a significant increase in crop yields, on an average by 10–30% (Sean 2020; Borisova et al., 2021). It has been shown that BC addition to the soil reduces the availability of metals including copper and their toxic effects on plants, thereby allowing their use for phytostabilization purposes (Kamran 2020). However, to date, data on the use of biochar as a carrier for PGPB are fragmentary.

Copper occurs in nature in different forms and it is one of the most used non-ferrous metals in industries. Wastes from metallurgical enterprises are the main sources of air, water and soil pollution with copper (Rehman et al., 2019; Kumar et al., 2020). Copper is an essential trace element for the growth and development of plants, since it is part of the active complex of enzymes that are involved in respiration, photosynthesis and many other key metabolic processes (Printz et al., 2016). It involved also in lignin synthesis and metabolism of carbohydrates and proteins (Printz et al., 2016; Tugbaeva et al., 2022). At the same time copper deficiency or excess can have a negative effect on agricultural plants (Mwamba et al., 2016; Tugbaeva et al., 2021; 2022).

The aim of the work was to study the effect of biofertilizer based on biochar and metal tolerant PGP rhizobacteria *Bacillus altitudinis* strain TF16a on growth parameters, copper accumulation and lipid peroxidation level in *Brassica oleracea* L.

MATERIALS AND METHODS

As a carrier material for bacteria in bioformulation, a biochar was chosen, made by "DianAgro" (Novosibirsk, Russia) by the method of oxygen-free pyrolysis using high-density birch wood and representing 100% charcoal grade A (according to GOST 7657-84). Such wood produces good biochar due to higher percentage of carbon and minimum ash content. A liquid culture of *Bacillus altitudinis* strain TF16a (10^8 CFU mL^{-1}) was used as an inoculum for the preparation of bacterial biofertilizer (BF). This strain was previously isolated from the rhizosphere of *Tussilago farfara* L., growing closed to the copper smelter, and selected on the basis of the best Cu-tolerance and PGP attributes.

The pot-scale experiments were done on *Brassica oleracea* L. (variety Express F1) which were grown from seeds in 100 mL plastic pots in the growing chamber for 30 days with photoperiod 14:10 (day:night), light intensity – 130±20 µM m^{-2} s^{-1}, humidity – 55±5%, and temperature –23±2 °C. The experiment included four treatments: 1 – control (peat based soil substrate, S); 2 – substrate with the addition of 5% BF; 3 – substrate with addition of 100 mg kg^{-1} Cu (sulfate form); 4 – substrate with the addition of 5% BF and 100 mg/kg Cu (BF+Cu). Three seeds were planted in each pot, a total of 8 pots were used for each treatment, the experiment was repeated two times. The following growth parameters of *B. oleracea* were measured: number of seedlings, seed germination rate, shoot and root length, leaf area, fresh and dry biomass per plant.

To calculate the leaf area, a specialized program JMicroVision software (version 1.2.7) was used (Roduit, 2019). The level of lipid peroxidation was assessed by the amount of malonic dialdehyde (MDA) according to the generally accepted method (Heath, Packer, 1968). The copper content in shoot and root of *B. oleracea* was determined using the atomic absorption spectrometer AA240FS (Varian Australia Pty Ltd., Mulgrave, Victoria, Australia) after wet digestion in HNO_3 and calculated per dry weight (DW).

All data were analyzed statistically by analysis of variance using Statsoft Statistica 8.0. Data are presented as mean values ± standard errors (SE). ANOVA analysis of variance was used to determine the individual and joint effects of BF and Cu. The significance of differences between the treatments was assessed using Tukey's HSD test. Different alphabetical letters indicate significant difference between the treatments.

RESULTS AND DISCUSSION

A significant decrease in the rate of seed germination was observed in the treatment with copper. At the same time the maximum number of seeds germinated with the combined action of BF with copper (BF+Cu) (Fig 1a). Single application of BF and Cu led to a decrease in leaf area compared to the control by 15 and 28%, respectively. Combined action of BF and Cu (BF+Cu) increased leaf area of *B. oleracea* by 14% as compared to the control and by 50% compared to the single action of BF (Fig 1b). Moreover, the highest value of shoot length was also noted for this combined treatment, while the single application of BF or Cu had the inhibitory effect (Fig. 1c). The similar trend was also previously noted for *Brassica napus* by Dąbrowska et al. (2017), they reported that the combined action of biofertilizer with zinc and cadmium led to the increase in the length of its roots and stems.

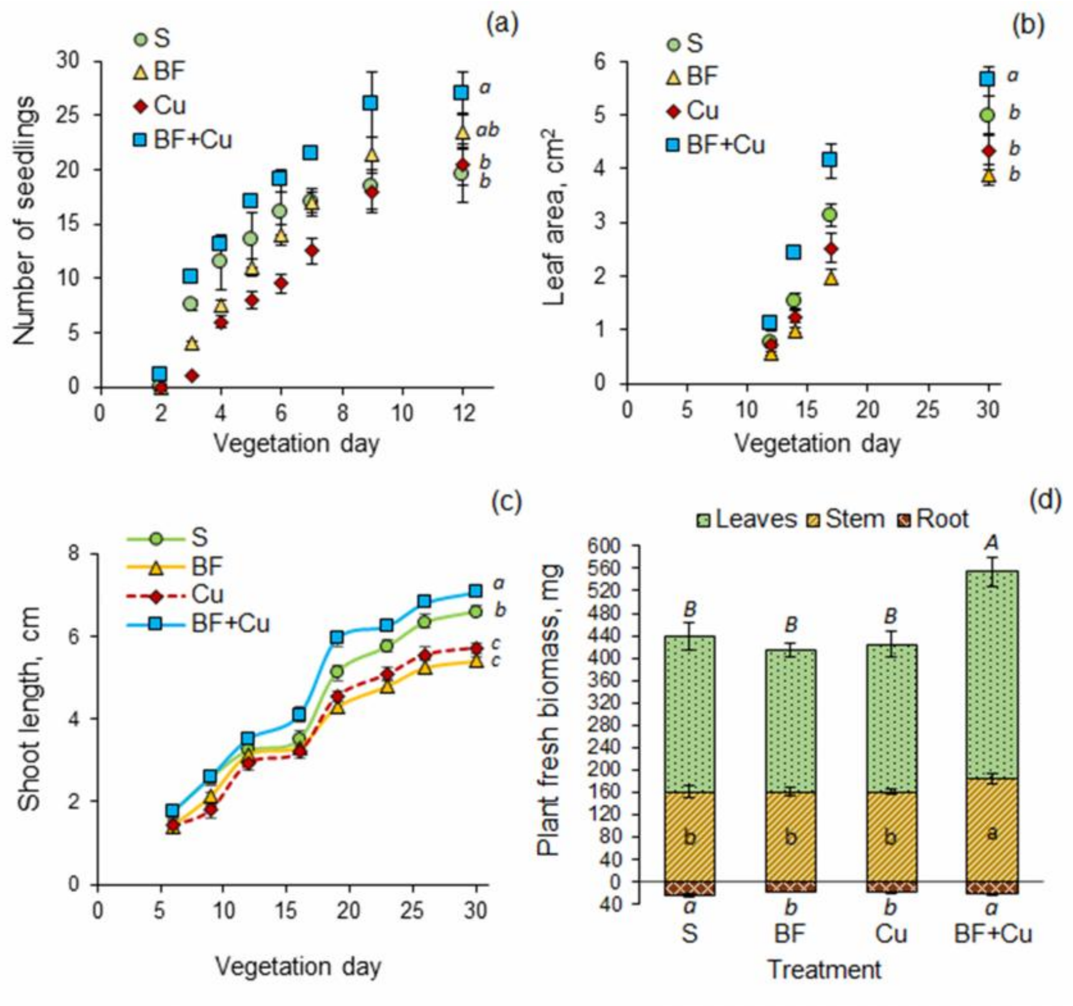

Figure 1. Number of seedlings (a), leaf area (b), shoot length (c) and fresh biomass (d) of *B. oleracea.*

The single action of BF as well as Cu did not affect fresh biomass of both *B. oleracea* aboveground and underground organs (Fig. 1d). However, the combined action of BF with Cu increased shoot biomass of *B. oleracea* by 26% compared to the control, mainly due to the increase in leaf weight. At the same time, the root weight practically did not change (Fig. 1d).

Similar trend was observed for dry biomass. In the control substrate and with the addition of BF, the copper content was 25 mg kg-1 which was 2.2 times lower than the maximum permissible concentration for Russia (Bakradze et al., 2008). After 30 days of *B. oleracea* vegetation at single copper treatment plants accumulated up to 24 and 27 mg Cu kg^{-1} DW in shoots and roots, respectively (Fig. 2a). The addition of BF did not affect the Cu accumulation by plants in comparison with the control. Combined effect of BF with copper (BF+Cu) significantly reduced its content in the roots and almost completely prevented the metal accumulation in the shoots which is of particular importance when growing this leaf vegetable on Cu-contaminated soils. The decrease in copper accumulation can be explained by metal sorption by rhizobacteria that was previously shown by other authors (Aust et al., 1985).

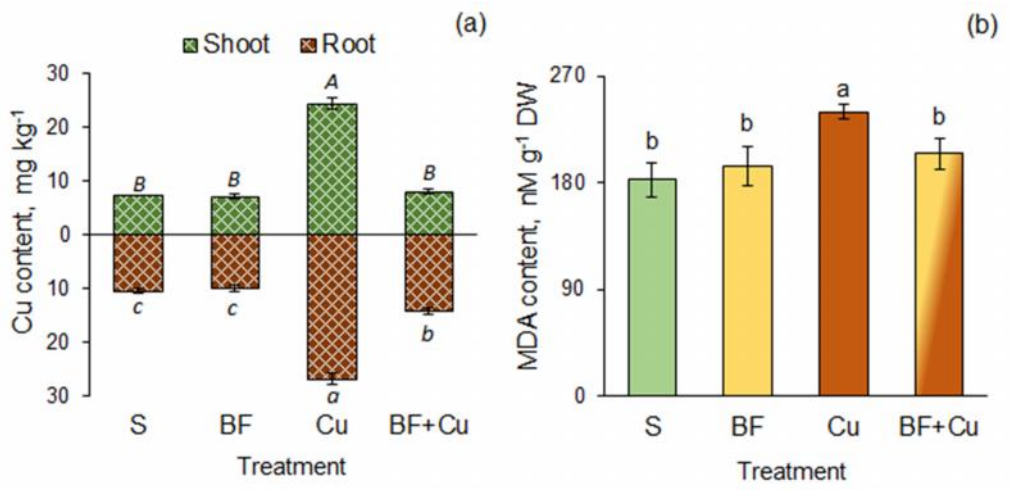

Figure 2. The copper accumulation in shoot and root of *B. oleracea* (a) and the malondialdehyde content in plant leaves (b).

The calculation of the copper bioconcentration factor (BCF) in shoots and roots of *B. oleracea* showed that cabbage does not belong to copper accumulators, since in all treatments BCF was significantly less than 1. When adding biofertilizer, the BCF value did not change considerably.

In all treatments, Cu was predominantly accumulated in the roots, as evidenced by the low translocation coefficient (<1). The addition of BF prevented the translocation of the metal into *B. oleracea* shoots to an even greater extent.

It is known that an excess of heavy metals can cause oxidative stress in plants due to the generation of reactive oxygen species (ROS) and disruption of the native conformation of enzymes. Since copper is a redox active metal, it is involved in the direct generation of ROS, such as superoxide radical, hydrogen peroxide, and hydroxyl radicals (Aust et al., 1985). The intensity of lipid peroxidation processes is usually judged by the content of malonic dialdehyde. It was noted that the addition of copper led to a significant increase in the content of MDA in the leaves of *B. oleracea* by 32 % (Fig. 2b). At the same time, when copper was added together with BF, the MDA content did not significantly differ from the control. This is in good agreement with a decrease in the accumulation of copper, which led to a decrease in the process of peroxidation.

Thus, the greatest positive influence on the *B. oleracea* growth parameters was observed in the BF+Cu treatment. This fact is confirmed by the results of a two-way ANOVA, which showed that the most significant effect on studied growth parameters was exerted by the combined action of copper with biofertilizer ($p < 0.01$). The absence of the noticeable effect at single BF addition on the cabbage growth allows us to suggest that the PGP activity of used bacterial strain depended on copper concentration in the soil.

CONCLUSIONS

The study of the effect of biofertilizer based on metal tolerant PGP rhizobacteria *Bacillus altitudinis* strain TF16a and biochar on growth parameters and copper accumulation in *Brassica oleracea* L. are presented. The combined application of BF and copper had a positive effect on the germination rate, leaf area, shoots length, biomass value of cabbage. The addition of copper led to a significant increase in its accumulation in shoot and root and in the content of MDA in the leaves of *B. oleracea*. However, when copper was added together with BF, the Cu content and lipid peroxidation level did not significantly differ from the control. Thus, it can be concluded that biochar based biofertilizer promoted growth and alleviated copper toxicity in *Brassica oleracea*. Biofertilizer usage suggest an environmentally sustainable approach to increase crop production contributing substantially in development of agrobiotechnology.

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REFERENCES

Aust S. D., Morehouse L. A., Thomas C. E. (1985) Role of metals in oxygen radical reactions. Journal of Free Radicals in Biology & Medicine, vol. 1, No 1, pp. 3-25.

Bakradze E., Vodyanitskii Yu., Urushadze T., Chankseliani Z., Arabidze M. (2018) About rationing of the heavy metals in soils of Georgia. Annals of Agrarian Science, vol. 16, No 1, pp. 1-6.

Borisova G., Maleva M., Atambire A., Davydova D., Tripti (2021) Wood biochar as an amendment for enhanced growth of *Phacelia tanacetifolia*. AIP Conference Proceedings, vol. 2388, 040007.

Dąbrowska G., Hrynkiewicz K., Trejgell A., Baum C. (2017) The effect of plant growth-promoting rhizobacteria on the phytoextraction of Cd and Zn by *Brassica napus* L. International Journal of Phytoremediation, vol. 19, No 7, pp. 597-604.

Dubey R. K., NidhiShukla (2014) Organic farming: an eco-friendly technology and its importance and opportunities in the sustainable development. International Journal of Innovative Research in Science, Engineering and Technology, vol. 3, No 3, pp. 10726-10734.

Gascó G., Álvarez M. L., Paz-Ferreiro J., Méndez A. (2019) Combining phytoextraction by Brassica napus and biochar amendment for the remediation of a mining soil in Riotinto (Spain). Chemosphere, 2019, vol. 231, pp. 562-570.

Glick B. R. (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica, No 5, pp. 1-15.

Heath R. L., Packer L. (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, vol. 125, No 1, pp. 189–198.

Kamran M., Malik Z., Parveen A., Huang L., Riaz M., Bashir S., Mustafa A., Abbasi G. H., Xue B., Ali U. (2020) Ameliorative effects of biochar on rapeseed (*Brassica napus* L.) growth and heavy metal immobilization in soil irrigated with untreated wastewater. Journal of Plant Growth Regulation, vol. 39, pp. 266-281.

Kumar A., Tripti, Maleva M., Kiseleva I., Maiti S.K., Morozova M. (2020) Toxic metal(loid)s contamination and potential human health risk assessment in the vicinity of century-old coppersmelter, Karabash, Russia. Environmental Geochemistry and Health, vol. 42, pp. 4113-4124.

Kumar A., Tripti, Voropayeva O., Maleva M., Panikovskaya K., Borisova G., Rajkumar M. (2021) Bioaugmentation with copper tolerant endophyte *Pseudomonas lurida* strain EOO26 for improved plant growth and copper phytostabilization by *Helianthus annuus*, Chemosphere, vol. 266, 128983.

Lehmann J., Rillig M. C., Thies J., Masiello C. A., Hockaday W. C., Crowley D. (2011) Biochar effects on soil biota – A review. Soil Biology and Biochemistry, vol. 43, No 9, pp. 1812-1836.

Lugtenberg B., Kamilova F. (2009) Plant-growth-promoting rhizobacteria. Annual Reviews of Microbiology, vol. 63, pp. 541-546.

Maleva M., Borisova G., Koshcheeva O., Sinenko O. (2017) Biofertilizer based on silicate solubilizing bacteria improves photosynthetic function of *Brassica juncea*. AGROFOR International Journal, vol. 2, No 3, pp. 13-19.

Mwamba T. M., Ali S., Ali B., Lwalaba J. L., Liu H., Farooq M. A., Shou J., Zhou W. (2016) Interactive effects of cadmium and copper on metal accumulation, oxidative stress, and mineral composition in *Brassica napus*. International Journal of Environmental Science and Technology, vol. 13, No 9, pp 2163-2174.

Printz B., Lutts S., Hausman J., Sergeant K. (2016) Copper trafficking in plants and its implication on cell wall dynamics. Frontiers in Plant Science, vol. 5, 601.

Rana A., Saharan B., Joshi M., Prasanna R., Kumar K., Nain L. (2011) Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Annals of Microbiology, vol. 61, No 4, pp. 893-900.

Rehman M., Liu L., Wang Q., Saleem M. H., Bashir S., Ullah S., Peng D. (2019) Copper environmental toxicology, recent advances, and future outlook: a review. Environmental Science and Pollution Research, vol. 26, No 18, pp. 18003-18016.

Roduit N. JMicroVision: Image analysis toolbox for measuring and quantifying components of high-definition images. Version 1.2.7. https://jmicrovision.github.io (accessed 5 April 2019).

Sean C. T. (2020) Biochar effects on germination and radicle extension in temperate tree seedlings under field conditions. Canadian Journal of Forest Research, vol. 51, No 1, pp. 10-17.

Tripti, Kumar A., Darkazanli M., Maleva M., Rajkumar M., Bruno L.B. (2021) Metal and drought tolerant biochar based biofertilizer for enhanced growth of *Raphanus sativus.* AIP Conference Proceedings, vol. 2388, 020036.

Tugbaeva A. S., Ermoshin A. A., Plotnikov D. S., Kiseleva I. S. (2021) Activity of cell wall-bound and cytosolic peroxidases under the after effect of copper ions in *Nicotiana tabacum* plants. Journal of Siberian Federal University. Biology, vol. 14, No 3, pp. 318-327.

Tugbaeva A., Ermoshin A., Wuriyanghan H., Maleva M., Borisova G., Kiseleva I. (2022) Copper stress enhances the lignification of axial organs in *Zinnia elegans*. Horticulturae, vol. 8, 558.

Wang J. Wang S. (2019) Preparation, modification and environmental application of biochar: A review. Journal of Cleaner Production, vol. 227, pp. 1002-1022.

Yadav A. N., Verma P., Singh B., Chauahan V. S., Suman A., Saxena A. K. (2017) Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. Advances in Biotechnology & Microbiology, vol. 5(5), 555671.