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Effects of Copper Compounds on Phenolic Composition of the Common and Tartary Buckwheat Seedlings

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Abstract: Food producers actively seek effective seed-coating agents to ensure optimal antimicrobial protection and/or nutritional support for young plants. In this context, our study aimed to investigate the impact of various copper compounds on the germination and early growth stages of two important crops, common and Tartary buckwheat. Microparticles (MPs) and nanoparticles (NPs) of copper oxide (CuO) were selected as potential seed treatment agents and compared to Cu salt in a comprehensive germination assay. The results indicated that seed germination remained unaffected by the tested copper compounds after eight days, while there was a significant reduction in seedlings fresh weight and root length. Treated common buckwheat seedlings exhibited extreme increases in all tested phenolic metabolites, even at low concentrations of Cu compounds. In contrast, in Tartary buckwheat seedlings, the already higher concentrations of flavonoids and tannins were mostly slightly decreased. Considering all the results, CuO NPs emerged as the most severe form of Cu, while CuO MPs may have the highest potential for applications in agriculture and food sciences. This finding has implications for producers seeking seedlings enriched in beneficial phenolic compounds for human health, as well as for farmers aiming to boost the antioxidative system of plants to mitigate stress.

Keywords: *Fagopyrum esculentum*; *Fagopyrum tataricum*; buckwheat; germination; phenols; flavonoids; tannins; copper; nanoparticles

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1. Introduction

Genus *Fagopyrum* contains nearly 30 species, predominantly wild and only two cultured—common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tataricum*). In the human diet, buckwheat is usually used as porridge, pasta, and for honey and tea production [1,2]. Recently, the international market promotes seedlings/sprouts or young plantlets, due to their rich content of various plant products, serving as valuable food supplements with high medical value [3–6]. In addition, buckwheat products are recognized as a source of trace elements and dietary fibers as well as safety for patients with celiac disease and gluten intolerance, which make a buckwheat crop on the rise.

The secondary metabolites in buckwheat, especially different phenolic compounds, play a key role in active defense mechanisms against radiation, pests, microorganisms, and particularly oxidative stress. Antioxidants prevent oxidative stress by trapping free radicals, chelation of metal ions, and/or their removal. Different non-enzymatic phenolic compounds are further divided into flavonoids, hydrolysable tannins, lignins, stilbenes, and tripolens. Besides many others, the role of non-structural phenols is also a chemical defense against the intrusion of microorganisms and resistance to biological degradation, which makes them commercially important in the pharmaceutical and food industry as well as in horticulture [7]. Tartary buckwheat is recognized as very rich in health-promoting substances such as rutine and tannins, and it contains additional flavonoids such as quercetin and quercitrin compared to common buckwheat [4,7,8].

Nowadays, growers are introduced to seed treatment techniques to enhance crop yield, protect seeds, and seedlings against microbes or/and to fertilize the crop with nutrients [9].

Various copper (Cu) compounds, with over 40 formulations, are one of the most natural substances to inhibit fungal and bacterial diseases of plants and are already in use for decades [10,11], particularly in organic farming. The mechanism involves free Cu^{2+} ions in water-soluble form entering the cell and binding to enzymes, impairing their functionality. On the other side, insoluble forms of Cu act externally on the cell walls and membranes of fungi and bacteria. The release of ions is impacted by the size of the particles; by reducing the size of particulate matter, the solubility and activity significantly increase, but on the other side also there is a toxicological and environmental risk [10]. Nanotechnology explores the use of nanoparticles (NPs) as nano-fertilizers to improve plant nutrition, as well as seed coatings for pests and microbes protection [12–16]. However, their exact mechanism of antimicrobial action remains unknown [17]. While Cu as cuprous oxide (Cu_2O) is already used as a fungicide for seed treatment [12], their low solubility suggests that their toxicity cannot be attributed only to metal ions, which applies to NPs of Ag and ZnO [18].

In plants, Cu in excess inhibits the growth and development of plants, causing discolouration of roots and chlorosis on the leaves as well as inhibits the development of sprouts [19]. Recent studies demonstrated that copper (II) oxide (CuO) NPs could be used as nano-fertilizers [20,21], but their impact on seed germination varies among studies, from no effect to its inhibition [21–23], with genotoxic effects reported that cause oxidative damage and DNA lesions [24,25]. Furthermore, these NPs have been shown to influence the antioxidant defense system in plants [13,15,26,27].

In our study, two closely related buckwheat species, common and Tartary buckwheat, were selected as an important functional food plant species. The main differences between them are the breeding system and preferred climate, with Tartary buckwheat more resistant to cold weather and drought, and as such it can grow in high-altitude regions [28–30]. Moreover, they exhibit significant variation in the content of secondary metabolites, and therefore we were interested in comparing the basic phenolic composition, including the total phenols, flavonoids, and tannins. To the best of our knowledge, buckwheat has been investigated in only three NPs' toxicity studies so far [25,31,32]. Due to high levels of a variety of organic acids and phenolic compounds chelating divalent metal ions of transition metals, buckwheat can accumulate Al and Pb in the shoots and survive high concentrations of Cu and Zn [33–36]. As such, it is a great candidate for investigating the impact of different Cu compounds, including Cu salt, CuO microparticles, and CuO nanoparticles, on seed germination and seedling performance with phenolic metabolites analyses. In light of the ongoing search for efficient forms of seed treatment agents, studies on important crop plants treated with potential antimicrobial agents, particularly those based on Cu—a widely accepted component in organic farming—are essential.

2. Materials and Methods

In the germination test, the response of seeds and sprouts of common and Tartary buckwheat on treatment with various concentrations of Cu salt as a source of Cu^{2+} ions, copper (II) oxide (CuO), microparticles (MPs), and nanoparticles (NPs) was observed. Seeds of common (*Fagopyrum esculentum* Moench, cv. Trdinova) and Tartary (*Fagopyrum tataricum* Gaertn.) buckwheat used in our study were obtained from the Rangus mill (Otočec, Slovenia; 233 m a.s.l.), produced in the neighboring fields in 2012, on common buckwheat field (45.820681° N, 15.334260° E) and Tartary buckwheat field (45.819977° N, 15.334947° E). First, seeds were sterilized by 30% H_2O_2 and incubated for 2 h on a shaker in distilled water supplemented with different concentrations of CuO MPs (0.1; 1; 5; 10; 50; 100; 150 and 1000 mg L^{-1}), CuO NPs (0.1; 1; 5; 10; 50; 100; 150 and 1000 mg L^{-1}), copper salt $CuSO_4 \cdot 5H_2O$ (Sigma Aldrich, Taufkirchen, Germany; 0.01; 0.05; 0.1; 0.5; 1; 10 and 100 mg L^{-1}) and without supplement, which was used as a control. CuO MPs (Sigma Aldrich, Taufkirchen, Germany) and CuO NPs (Aldrich Chemistry, Milwaukee, WI, USA; particles size < 50 nm) are described in detail in our previous study [37]. Briefly, the size with TEM was measured to be 623 ± 45 nm for MPs CuO and 48 ± 3 nm for NPs CuO, and the impurities detected by XRF were for CuO MPs traces of Sn, Ca, Fe, and Ni, and for CuO NPs traces of Sn, Ca, Cr, Mn, Fe, and Ni. The experiment was repeated three times; each time it had five replicates with ten seeds. After imbibition, the seeds were transferred to Petri dishes with two filter papers and watered with 5 mL of appropriate treatment solution. In case of CuO, the suspensions were constantly stirred to assure the even distribution of the particles. During the first eight days, the plants were grown in the dark at 22 °C, and the percentage of germination was recorded daily. After eight days, the seedling's root length was measured, and the biomass was determined. For further analyses, seedlings were immediately frozen in liquid nitrogen, lyophilized, and powdered using liquid nitrogen and mortar with a pestle. The plant material from all experiments was combined based on treatments, due to the low amount of plant material, and it was further used for the determination of basic phenolic compounds. All experiments and analysis were done in Plant Physiology laboratories, Department of Biology (BF, UL).

2.1. Determination of Phenolic Metabolites in Seedlings

In our experiment, the basic phenolic compounds, namely the total phenols, total flavonoids, and tannins, were studied using spectrophotometric methods. For all three tests, the extraction procedure was the same. First, 200 mg of a powdered buckwheat seedlings sample was extracted with 10 mL of 60% ethanol overnight in a shaker. The mixture was centrifuged at 5000 rpm for 10 min, and the clear solution was used in further analyses.

Total phenols were determined by two aliquots, each of 20 µL of the sample extract [38]. To the first 160 µL of dH_2O and to the second 150 µL of dH_2O and 10 µL of Folin–Ciocalteau reagent was added. After 3 min, 20 µL of 20% Na_2CO_3 was added to both aliquots, and after 60 min the absorbance of both solutions was measured at 750 nm. The concentration was calculated from the differences between the measurements and by comparison to a standard curve of different ascorbic acid concentrations.

For the determination of flavonoids, two aliquots, each of 180 µL of the sample extract, were prepared [38]. To the first aliquot, 20 µL of 5% $AlCl_3$ in methanol was added, and 20 µL of pure methanol was added to the second aliquot. After 30 min, the absorbance of both solutions was measured at 425 nm. The concentration was calculated from the differences between the measurements and by comparison to a standard curve of different rutin concentrations.

The content of tannins in the seedlings was assessed by following the vanillin-HCl method [7]. Firstly, 25 µL of the extract was put into two aliquots. To the reagent-free aliquot, 125 µL of 4% HCl was added and to the other aliquot 125 µL 1% vanillin in 8% HCl in methanol was added. After 20 min, absorbance at 500 nm was recorded. The final tannins concentration was calculated from the differences between the measurements and by comparison to a standard curve of different catechin concentrations.

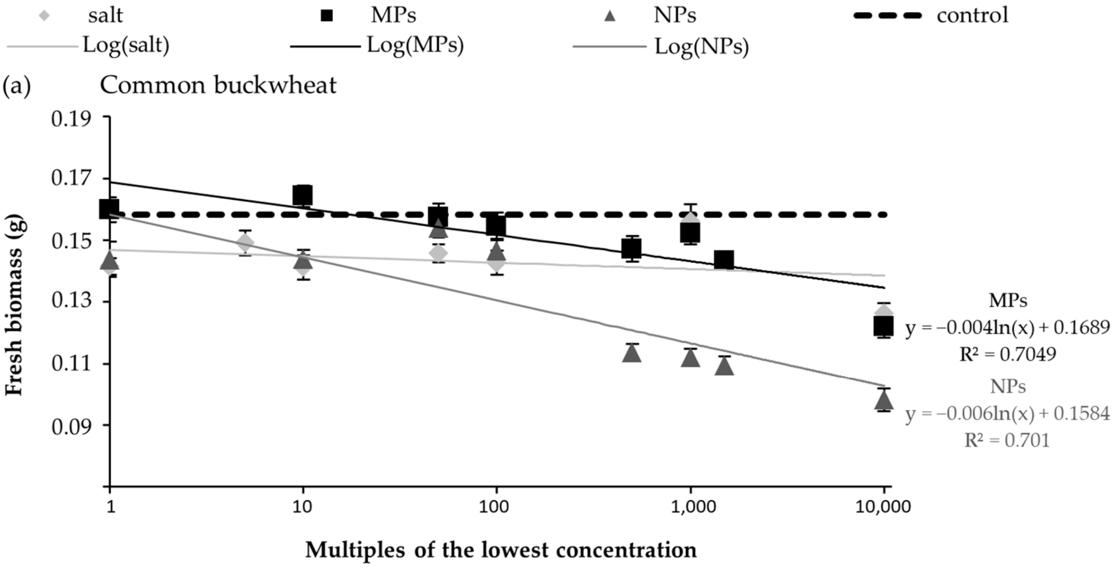
2.2. Statistical Analysis of Data

For basic statistic, a *t*-test and analysis of variance (ANOVA) with the accompanying Tukey post hoc test were used. To test a statistically significant effect of various factors and their interactions, factorial ANOVA was performed. Both ANOVAs and forward stepwise discriminant analyses were performed in Statistica 7 software (StatSoft; v7.0.61.0). The determination of the effective concentration (EC50) of copper compounds was performed with R software (v4.0.5) [39], using gplot and DRM packages, while the plots of the discriminant analysis were drawn with Microsoft office Excel's XLStat Add-in (v2014.5.03).

3. Results and Discussion

3.1. Seedlings Performance

First, the fresh biomass of common and Tartary buckwheat seedlings was evaluated. Both plant species exhibited comparable responses to treatment with different Cu compounds (Figure 1), with a decrease in biomass in correlation to the increasing Cu concentration. The treatments with CuO NPs proved to be the most detrimental for both species. Additionally, common buckwheat appeared to be more sensitive to CuO MPs than Cu salt. In line with our results, also Cu NPs reduced the growth of beans and wheat seedlings, with their toxicity attributed to the particles themselves, as the Cu^{2+} release from the particles' surface was minimal [40]. Similarly, the NPs CuO reduced the growth of roots and the shoots in soy [41], cucumber [42], and rice seedlings [43] as well as the roots of buckwheat due to their shown genotoxic effect [25]. In the case of Tartary buckwheat seedlings, biomass decrease was comparable between CuO MPs and Cu salt. However, the overall biomass of Tartary buckwheat seedlings was, in general, slightly smaller in comparison to common buckwheat seedlings (Figure 1). The fresh weight of seedlings was significantly ($p < 0.05$) influenced by plant species, Cu form, and Cu concentration, as well as their interactions (Table S1). This variance in seedling response could potentially be attributed to interspecific differences in morphology of seed hulls, impacting their permeability [44]. Furthermore, in the interpretation of the results, one should note that the response of plants to oxidative stress depends on the type of plants, as well as its variety [45].



(b) Tartary buckwheat

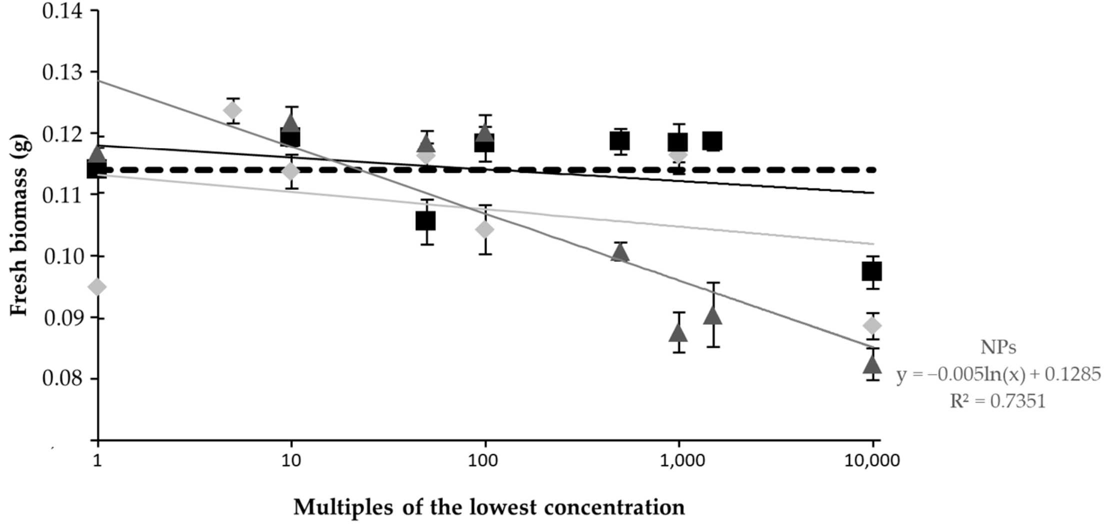


Figure 1. The effects of different concentrations and Cu compounds on fresh biomass of (**a**) common buckwheat and (**b**) Tartary buckwheat seedlings. Data are presented as mean ± SE ($n = 15$) and the lowest concentrations were for Cu salt 0.01 mg L^{-1}, for CuO MPs and NPs 0.1 mg L^{-1}. Trendline right of graph indicate biomass reduction with increasing Cu concentration. Legend: control—untreated seedlings, salt—$CuSO_4 \cdot 5H_2O$; MPs—CuO microparticles, NPs—CuO nanoparticles.

The root growth of seedlings was strongly affected by the higher concentrations of tested Cu compounds (Figure 2), confirming the results of similar studies on buckwheat, wheat, beans, zucchini, and rice seedlings [32,46–48]. The research on the maize seedlings demonstrated that higher Cu concentrations inhibited cell division due to the toxic effects on the morphology of the chromosomes, but contrary to our findings, lower concentrations of Cu stimulated maize seedling growth [49].

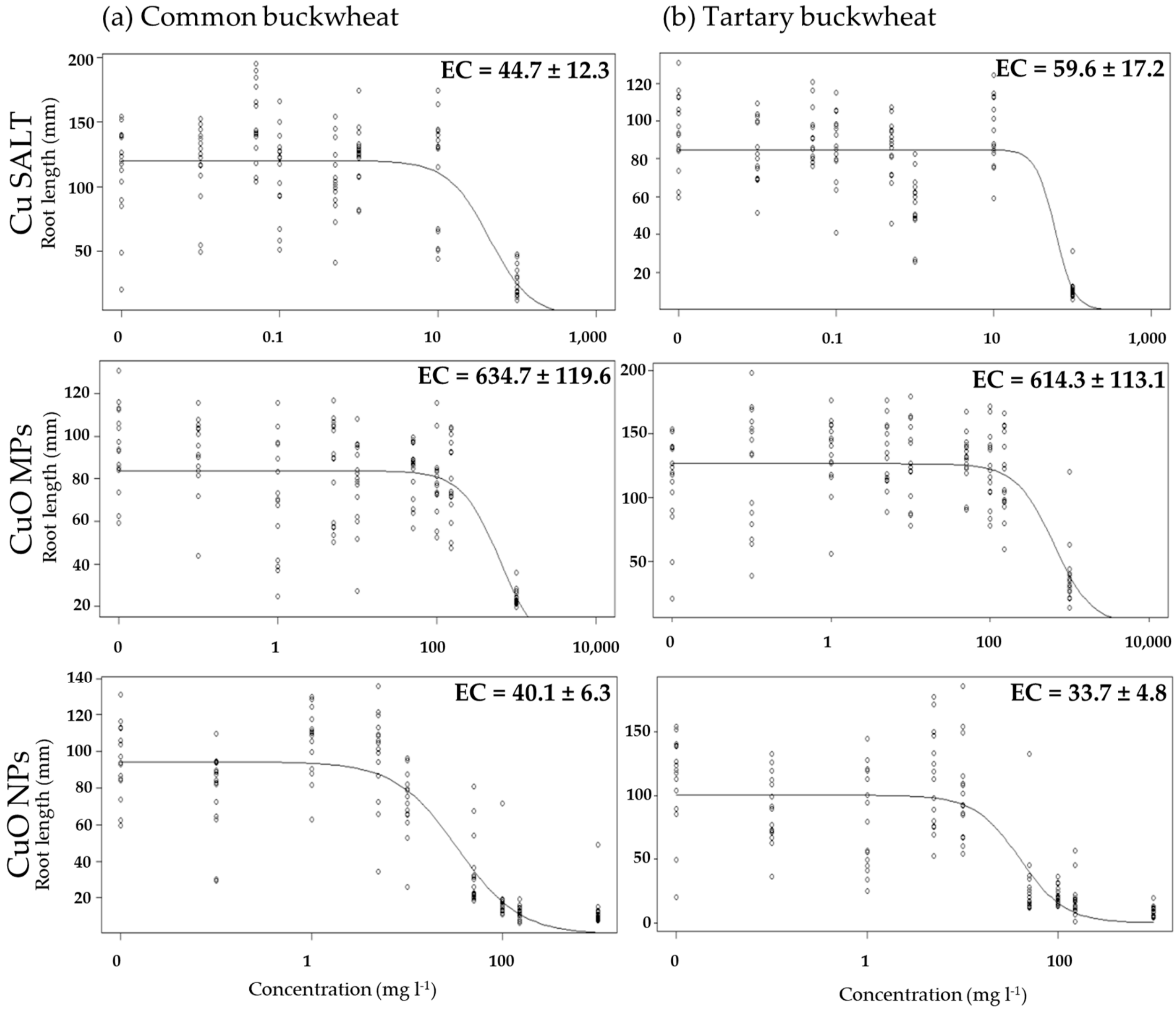


Figure 2. The effects of different Cu compounds on root length of treated buckwheat seedlings with defined effective concentration (EC) (mg L^{-1}) for (**a**) common buckwheat, (**b**) Tartary buckwheat, from top to bottom: Cu salt, CuO MPs and CuO NPs. Legend: Cu salt—$CuSO_4 \cdot 5H_2O$; CuO MPs—CuO microparticles, CuO NPs—CuO nanoparticles.

The highest effective concentrations were found for CuO MPs, exceeding 600 mg L^{-1} in both buckwheat species, making them the least toxic among all the Cu forms tested (Cu salt, CuO MPs and CuO NPs). In contrast, Cu salt and CuO NPs had comparable effective concentrations, around 40 mg L^{-1}, although the values for CuO NPs were slightly lower compared to salt. The EC results revealed that common buckwheat seedlings demonstrated greater sensitivity to Cu salt in comparison to Tartary buckwheat seedlings (Figure 2, upper charts), while Tartary buckwheat seedlings demonstrated greater sensitivity to CuO NPs in comparison to common buckwheat seedlings (Figure 2, bottom charts). The root length was significantly affected by all factors, including plant species, Cu form, and Cu concentration (Table S1).

In contrast to our findings, research in wheat sprouts indicated that small amounts (0.5 mg/mL) of CuO NPs increased the radicle and plumule length, while higher concentrations (6 mg/mL) had inhibitory effects due to Cu accumulation and phytotoxicity in plant tissue [50]. NPs of CuO reduced the root length of salad and alfalfa [51] and rice [43], inducing backwardness of root growth, greater lignification, and cytotoxicity in root cells of soybean [41]. Proposed mechanisms behind these findings include NPs of Cu and Cu_2O blocking water channels due to adsorption on the surface of the plant or/and causing injuries on the membranes, enabling their penetration into the roots and, consequently, the malfunction of cell division [24,25,32]. Since the production of ROS and the oxidative-modified components of the cell after NP exposure can increase in plants, this may further lead to mutagenesis and modified growth [24]. Excess of Cu can alter gene expression involved in the metabolism of fatty acids and the biogenesis of cellular components in roots [52].

3.2. Phenolic Metabolites in Treated Seedlings

In addition to the quantitative traits related to the growth and development of seedlings, our study also investigated the fundamental metabolic response of plant defense by measuring the concentrations of phenolic compounds, specifically the total phenols, flavonoids, and tannins. The concentrations of the total phenols, flavonoids, and tannins (% dry weight) in common buckwheat were measured at 1.3 ± 0.1, 0.5 ± 0.03, and 0.3 ± 0.02, respectively. In Tartary buckwheat seedlings, the concentrations (% DW) were 2.6 ± 0.04 for phenols, 2.7 ± 0.1 for flavonoids, and 0.4 ± 0.02 for tannins. This is in line with other studies [4], demonstrating that Tartary buckwheat plants exhibit higher concentrations of phenols and flavonoids compared to common buckwheat. Surprisingly, distinct metabolic responses to Cu compounds were observed. In the case of common buckwheat, all tested phenolic metabolites, total phenols, flavonoids, and tannins increased on average by 32% compared to the control (considering all treatments). In contrast, the Tartary buckwheat response considering the total phenols, flavonoids, and tannins exhibited an opposite trend, with average differences to the control of 1%, −4%, and −10%, respectively (Table S2, considering all treatments).

Differences between treatments are listed in Table 1 (raw values in Table S2). Regarding the total phenols in Tartary buckwheat seedlings, a very diverse response to Cu was observed, with a slight increase in the case of salt and MPs, and a decrease in the case of NPs. Flavonoids increased in the middle-tested concentrations of Cu salt, CuO MPs, and, predominantly (49%) in all concentrations of CuO NPs, in common buckwheat seedlings. Surprisingly, only a few significant changes in concentrations of flavonoids in the treated Tartary buckwheat seedlings were observed, mostly with the negative impact of CuO NPs. The concentrations of tannins in the treated seedlings of common buckwheat content increased with the addition of Cu salt 0.1–1 mg L^{-1}, MPs CuO > 10 mg L^{-1}, NPs CuO > 5 mg L^{-1}, while in the treated Tartary buckwheat seedlings concentrations were lower in comparison to the control.

Tartary buckwheat exhibited much higher concentrations of total phenols and flavonoids, emphasizing interaction between buckwheat species and Cu form as a crucial factor influencing the content of phenolic metabolites in seedlings (Table S1). In general, NPs had a consistently negative impact on all measured metabolites in the case of Tartary buckwheat, indicating the lack of sensitivity of this species to such forms of Cu (Tables 1 and S2). Even though there is a noticeable decrease from the initially high concentrations, they obviously still offer effective protection. Nevertheless, when analyzing the outcomes of phenolic compound contents, it is essential to consider that these values could vary based on the chosen plant cultivar, the experimental design (whether conducted in a laboratory or a field), and environmental conditions such as temperature and light. The studies of the impact of NPs on the enzymatic part of the antioxidative system of plants are relevant and frequent [45], commonly showing the increased activity of antioxidant enzymes exposed to NPs [13,46,51]. However, our study focused on the phenols, flavonoids, and tannins, as a part of the non-enzymatic antioxidative system of plants because during germination, it plays a crucial role in enabling the embryo to survive in polluted environments and is important in mechanisms of Cu resistance [53,54].

Table 1. Total phenols, flavonoids, and tannins concentrations (% difference from the control) in common and Tartary buckwheat seedlings treated with Cu salt, MPs CuO, and NPs CuO in different concentrations (mean ± SE; *n* = 5). Asterisks next to the values represent statistically significant differences between the treatment and control ($p < 0.05$). Red color stands for negative value and green for positive, in comparison to the control. Legend: Cu salt—$CuSO_4 \cdot 5H_2O$; MPs—CuO microparticles, NPs—CuO nanoparticles.

Treatment (mg L^{-1})	**Phenols (% Difference from Control)**						**Flavonoids (% Difference from Control)**						**Tannins (% Difference from Control)**					
	Common			**Tartary**			**Common**			**Tartary**			**Common**			**Tartary**		
Cu salt 0.01	19.9	*	🟩	1.3		🟩	24.5	*	🟩	2.3		🟩	0.1		🟩	−8		🟥
Cu salt 0.05	27.9	*	🟩	−1.3		🟥	24.2	*	🟩	−3.1		🟥	19.8		🟩	−5		🟥
Cu salt 0.1	31	*	🟩	10.1	*	🟩	33	*	🟩	−2.1		🟥	24.6	*	🟩	3.7		🟩
Cu salt 0.5	35.3	*	🟩	10.4	*	🟩	30.3	*	🟩	2.4		🟩	31.4	*	🟩	−10.8		🟥
Cu salt 1	26.1	*	🟩	−1.1		🟥	33.5	*	🟩	0.1			19.5	*	🟩	−11.2	*	🟥
Cu salt 10	34.5	*	🟩	−0.7		🟥	21.4	*	🟩	−2.1		🟥	7.8		🟩	−14.7	*	🟥
Cu salt 100	3.1		🟩	−3.5		🟥	20.2	*	🟩	−3.6		🟥	2.4		🟩	−19.4	*	🟥
MPs 0.1	30.7	*	🟩	−3.6		🟥	28.6	*	🟩	−7	*	🟥	16.6		🟩	−11.7	*	🟥
MPs 1	27	*	🟩	0.7		🟩	38	*	🟩	−3.9		🟥	16.2		🟩	−6.7		🟥
MPs 5	48.3	*	🟩	3.3		🟩	38.1	*	🟩	−0.4		🟥	30.3		🟩	2.9		🟩
MPs 10	54.6	*	🟩	6.1	*	🟩	42.3	*	🟩	2.7		🟩	29.8	*	🟩	−2.7		🟥
MPs 50	50.9	*	🟩	2.9		🟩	37.3	*	🟩	−1.6		🟥	29.5	*	🟩	−0.1		
MPs 100	28.3	*	🟩	6	*	🟩	37.7	*	🟩	−5.1		🟥	32.2	*	🟩	−9.2		🟥
MPs 150	46.3	*	🟩	1.4		🟩	33.6	*	🟩	−7.1	*	🟥	28.6	*	🟩	−4.6		🟥
MPs 1000	22.4	*	🟩	−0.7		🟥	27.6	*	🟩	−4.3		🟥	18.9	*	🟩	−12		🟥
NPs 0.1	13.8		🟩	3.9		🟩	42.7	*	🟩	−7.4	*	🟥	32.5		🟩	−6.2		🟥
NPs 1	32	*	🟩	1.5		🟩	44.5	*	🟩	−6	*	🟥	19.3		🟩	−21.1	*	🟥
NPs 5	45.7	*	🟩	8.2		🟩	49.5	*	🟩	−3.7		🟥	32.3	*	🟩	−12.3		🟥
NPs 10	48.1	*	🟩	0.9		🟩	47.1	*	🟩	−6.5	*	🟥	45.9	*	🟩	−14.1	*	🟥
NPs 50	36.4	*	🟩	2.2		🟩	60.5	*	🟩	−6.2	*	🟥	58.9	*	🟩	−9		🟥
NPs 100	14.3	*	🟩	−1.5	*	🟥	53.2	*	🟩	−5.9	*	🟥	43.2	*	🟩	−24	*	🟥
NPs 150	15	*	🟩	−6.1	*	🟥	43.4	*	🟩	−7	*	🟥	33	*	🟩	−17.8	*	🟥
NPs 1000	22.2	*	🟩	−18	*	🟥	48.3	*	🟩	−4.1		🟥	50.9	*	🟩	−6.3		🟥

The results point to a positive effect of Cu on the antioxidant response (phenols, flavonoids and tannins) of common buckwheat plants, which is in line with the study of CuO NPs' influence on *Arabidopsis thaliana*, where the flavonoid content was enhanced [55], and the study on Cu^{2+} impact on Tartary buckwheat seedlings [54]. Increased concentrations of total phenols were found also in buckwheat exposed to metals, such as Zn and Al [54,56], and seeds treated with 2% chlorcholine chloride [57]. Furthermore, the seed coating of *Brassica juncea* with CuO NPs had a 41% increase in proline content, which is also an indicator of antioxidant non-enzymatic activity [15]. Altogether, our results confirm the role of phenylpropanoid metabolic pathways in the response of plants to metal or/and metallic NPs [15,58]. This suggests that CuO NPs and MPs at appropriate concentrations could protect plants from stress by boosting their antioxidative defense system and may also contribute additional nutritional value to human nutrition.

In contrast to common buckwheat, all tested treatments had a negative impact on the basic phenolic metabolism of the Tartary buckwheat seedlings, which is surprising since Horbowicz et al. (2013) [58] found that a buckwheat variety more resistant to heavy metal treatments contained higher flavonoid levels in cotyledons compared to a sensitive variety. Kovačik et al. (2009) [59] suggested that the synthesis of polymerized phenols plays a role in forming complexes with Cd ions, which might also be a case for Cu ions. However, as the concentrations of the total phenols, flavonoids, and tannins in the Tartary buckwheat are 1.95, 5.23, and 1.3 times higher than those in common buckwheat (Table S2), respectively, we hypothesize that Tartary buckwheat is genetically pre-adapted to diverse forms of stress. This important Tartary buckwheat trait originates from the growth conditions at high elevations in the Himalayas [30], where phenolic compounds and flavonoids serve as plant protection against UV radiation [60].

3.3. The Pattern Recognition of Buckwheat Seeds and Seedlings Response to Different Cu Compounds

In this part of our study, a comprehensive analysis was conducted on seven measured variables (seed germination on first day, seed germination on eighth day, fresh weight, root length, phenols, flavonoids, and tannins) across 24 groups representing various Cu treatments. Interestingly, factorial ANOVA revealed that buckwheat species and Cu significantly affected five (Table S1: germination on first day, fresh weight, root length, phenols, tannins) out of seven measured variables, and Cu concentration is important in only three (Table S1: fresh weight, root length, phenols). This suggests the importance of individual testing for each plant–compound combination.

We also performed a forward stepwise discriminant analysis in order to reveal the discriminant values of each variable. The root length and concentrations of the total phenols in seedlings are the two most important discriminant variables in both species. In particular, the Tartary buckwheat fresh weight is the most important variable explaining Tartary buckwheat response to Cu treatments according to the model (Table 2).

Table 2. Discriminant variables of common buckwheat and Tartary buckwheat seeds and seedlings response to treatment with different Cu compounds with a forward stepwise discriminant analysis (7 variables, 24 groups).

Common Buckwheat	**F**	***p***	**Tartary Buckwheat**	**F**	***p***
Fresh weight	4.209329	0.000000	Root length	8.138269	0.000000
Phenols	4.145876	0.000001	Germination first day	4.816375	0.000000
Root length	3.773790	0.000003	Phenols	3.353833	0.000021
Flavonoids	2.689075	0.000457	Fresh weight	3.730093	0.000004
Tannins	1.851816	0.021093	Flavonoids	1.939112	0.014514
Germination first day	1.823449	0.023856	Tannins	1.775372	0.029548
Germination eighth day	0.944755	0.541639	Germination eighth day	1.186192	0.278248

The only irrelevant discriminant variable in our performed experiment was the seed germination on eighth day. Similar to our findings, studies on tomato and maize have reported that initially, the germination rates may differ due to treatments, but these differences disappear by the end of the experiment [14,61]. Germination of pumpkin seeds was not affected by MPs and NPs of Cu, Si, ZnO, or carbon nanotubes [47], as well as corn and rice seeds by NPs of CuO, TiO_2, SiO_2, CeO, Fe_3O_4, Al_2O_3, and ZnO [48]. Treated Tartary buckwheat seeds had greater germination on first day in comparison to the control, which is in line with the findings of others [62,63]. The proposed explanation is that treated seeds germinate faster due to the increased uptake of water and elements [64]. On the other side, some authors reported the negative impact of CuO NPs on seed germination of lettuce, radish and cucumber [22], and rice [43], Cu on wheat [65,66], and grapevine [67] germination, which can be attributed to the Cu sensitivity of these species and the destruction of stored biomolecules in the seed and altered membrane permeability [68]. In general, the effect of Cu on seed germination depends greatly on plant species, intraspecific differences in tolerance, and the thickness of the seed hulls [45,69,70].

With the first two functions in the linear discriminant analysis, we successfully explained 93.33% and 97.7% of the total variability for common and Tartary buckwheat, respectively (Figure 3). The charts exhibit distinct separation between the tested compounds, with CuO NPs being the most far from the control and with CuO MPs and Cu salt in the middle of Function 1 axis. The negative contribution of variables to Function 1 of common buckwheat response confirmed the observed results of higher concentrations of phenols, flavonoids, and tannins in the treated seedlings with lower biomass, root length, and seeds germination rate in comparison to the control (Figure 3a, Table S3). In the case of Tartary, buckwheat germination on first day played a distinctive role, contributing in the opposite directions compared to other variables distinguishing between the control and NPs CuO treatments (Figure 3b, Table S3).

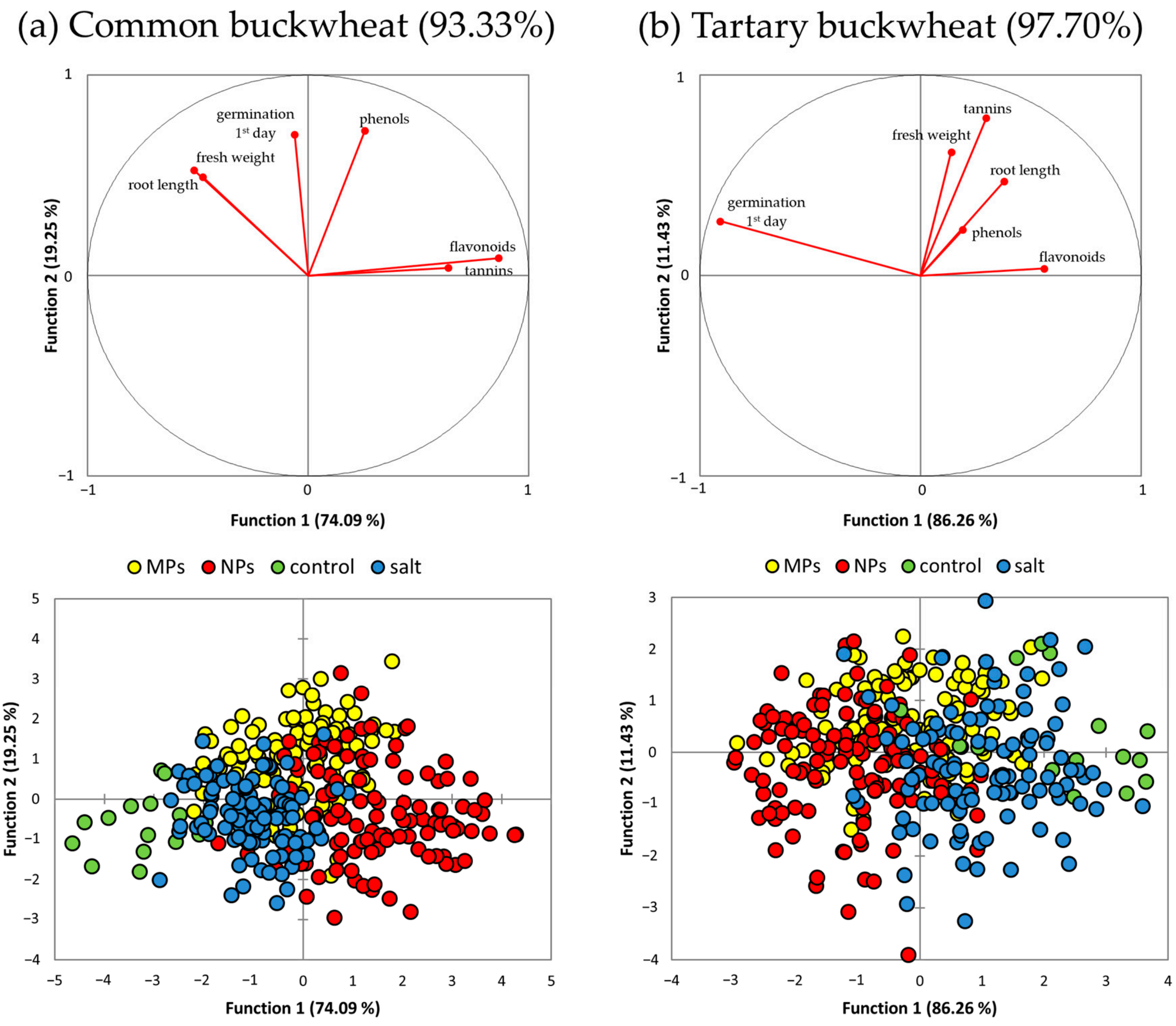


Figure 3. Linear discriminant analysis plot for measured parameters (germination first day, fresh weight, root length, phenols, flavonoids, tannins) of (**a**) common and (**b**) Tartary buckwheat seeds and seedlings in response to different Cu compounds. Legend: control—untreated seedlings, salt—$CuSO_4 \cdot 5H_2O$; MPs—CuO microparticles, NPs—CuO nanoparticles.

With the help of advanced statistical analysis, we can conclude that common buckwheat seedlings are more sensitive to this kind of treatment, because the groups are clearly separated, from the control, followed by salt, MPs, and NPs on other side. This is not so clearly visible in the case of Tartary buckwheat seedlings, which indicates that the data are less distinct and the effects of the tested compounds are smaller. This could be further connected to its secondary metabolism, since the most notable differences in the response patterns between the tested buckwheat species are the concentrations of phenolic metabolites, with a significant increase in the treated common buckwheat seedlings and a decrease or no changes in the treated Tartary buckwheat seedlings (Table 1). We assume that these differences occur because of the genetic and phenotypic plasticity of Tartary buckwheat compared to common buckwheat, since it has been cultivated in higher altitudes and is well adapted to environmental stress, e.g., UV radiation [71]. All in all, our results suggest that Cu treatment of seeds can affect the common buckwheat phenolic metabolism in a positive way, which is of interest to both farmers and consumers.

4. Conclusions

The application of copper oxide (CuO) may be an appropriate strategy for the pre-sowing treatment of common buckwheat seeds to achieve differences in the basic secondary metabolism with a significant increase in phenols, flavonoids, and tannins in seedlings. The results of the present study revealed that seed germination is not affected after eight days, while the seedlings' performance, including fresh biomass and root length, is negatively impacted. Notably, CuO nanoparticles emerged as the most severe form of Cu, indicating that CuO microparticles may have the highest potential for applications in agriculture and food sciences. In addition, this study emphasizes the importance of careful consideration and evaluation when employing seed dressing agents by recognizing their potential benefits with adverse effects on different aspects of plant performance.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture14020269/s1, Figure S1: Photos of untreated seedlings at the end of experiments for (a) common buckwheat and (b) Tartary buckwheat; Figure S2: Photos of roots exposed to different copper treatments for (a) common buckwheat and (b) Tartary buckwheat; Table S1: Impact of different factors, buckwheat species, Cu form, Cu concentration, and their interactions on measured parameters of common and Tartary buckwheat seeds and seedlings responses; Table S2: Average (means ± ster) of raw values (% dry weight) and cumulative differences of all treatments of same Cu compound in comparison to the control (%) for measurements of the total phenols, flavonoids, and tannins in common and Tartary buckwheat; Table S3: Barlett's test for eigenvalue significance and variables/factor correlations for (a) common buckwheat and (b) Tartary buckwheat in discriminant analyses.

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