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Preparation of copper glycinate and study of its effect on the germination of winter barley seeds

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Abstract. We analyzed the encrustation of winter barley seeds, which includes its preliminary treatment with cuprum glycinate, with the purpose of increasing yield and biochemical indicators of grain, and also improving the ecological condition of the soil. The objective of the research was to select methods for the synthesis of cuprum glycinate, study its chemical composition and the possibility of using this compound as a chelated microfertilizer for pre-sowing encrustation of winter barley seed as part of a tank mixture. Two well-known synthesis methods were used to obtain cuprum glycinate. The first method was the interaction between CuO with a solution of glycine during heating, during which pink metallic copper inclusions were noticed in the mixture of reaction products. It was found that it is expedient to synthesize the glycine complex of cuprum by the reaction of a suspension of $Cu_2CO_3(OH)_2$ with glycine during heating (the yield is 97%), since a complex compound $Cu(NH_2CH_2COO)_2 \cdot H_2O$ of sufficient purity is formed. The composition of the synthesized substance and the confirmation of the formula of the compound $Cu(NH_2CH_2COO)_2 \cdot H_2O$ were obtained by determining the infrared and the atomic absorption spectrum of the aqueous solution. Based on the obtained differences in the atomic absorption spectra of the synthesized copper sulfate and copper glycinate the formation of the latter was confirmed. The IR spectrum confirms the formula of the complex compound and the formation of strong covalent bonds between the metal cation and the ligands. The study of the effect of cuprum glycinate on the germination of winter barley Tutankhamun seeds was carried out in comparison with the similar effect of a complex compound of cuprum with ethylenediaminetetraacetate. The study of the influence of cuprum glycinate on the germination of winter barley revealed positive results in which the germination exceeded the control by 9–27%. Winter barley seeds treated with distilled water served as a control. Treatment of winter barley seeds with an aqueous solution of cuprum glycinate in the amount of 20 g of cuprum per 1 ton of grain led to better germination than pre-treatment of seeds with a twice concentrated corresponding solution. Treatment with a complex compound of copper with ethylenediaminetetraacetate had no significant effect on the germination and characteristics of sprouts. The results of laboratory studies confirmed the feasibility of using complex compounds of biometal copper with organic chelating ligands as microfertilizers for pre-sowing seed encrustation, as they have high stability and sufficient solubility in water, are non-toxic, are better absorbed by plants and are considered cost-effective and environmentally safe.

Keywords: atomic absorption spectrum; complex compound of cuprum; organic chelating ligand.

Introduction

Pre-sowing seed treatment makes it possible to increase the laboratory and field germination of seeds, to reduce the negative impact of pathogens, weeds, pests, to create favorable conditions for the proper growth of plants in the initial phases of ontogenesis, therefore producing higher yields of winter barley grain and increasing biochemical quality indicators of grown products, and also improving the ecological condition of the soil (Khalid et al., 2016; Szczepanek, 2018; Panakhyd et al., 2020). Pre-sowing seed encrustation of agricultural crops activates the immune system of the plants (Alqudah & Schnurbusch, 2015; Lyashenko et al., 2022), thus ensuring their increased resistance to droughts or excess moisture, and also helping them to increase the adaptation to adverse weather conditions (Popko at al., 2018). Some studies indicated shortening of the growing season and the enhancement of plant maturation, increase in number of grains in the ear, and accumulation of nutrients during grain filling in the plants' generative organs (Korotkova et al., 2021). An equally important problem that needs to be solved is the use of ecologically safe agricultural technologies that would not pollute the environment and allow obtaining safe food raw materials (Shubha et al., 2017; Yakhin et al., 2017).

The best way of introducing biometals into plants is in the form of chelated complex compounds, since they have high solubility in water, low toxicity, and better permeability through biological membranes. In addition, it is possible to use biologically active substances as ligands, as well as to vary the properties of complex compounds by changing the structure of their molecules. All this makes these chelate compounds promising microfertilizers (Kuznetsov et al., 2021).

The best ligands for such purposes are amines and their derivatives, since a strong, highly polar covalent bond is formed between the metal cation and the amino group. Therefore, compounds with ammonia and amines of some transition metals are so stable that they do not break down even in concentrated sulfuric acid (Brown et al., 2013). When comparing amino complexes of transition metal cations, compounds that are capable of forming bi- and multi-dentate chelate complexes have the greatest stability, as a result of the ligands forming a stable complex by closing their cycles around the metal center, which increases their stability by 30–70 times. Such compounds include non-aromatic di- and polyamines (ethylenediamine, diethylenetriamine, etc.), some heterocyclic diamines (dipyridyl, phenanthroline), etc. (Brown et al., 2013).

Amino acids are also good complexing agents. Natural amino acids that are part of proteins usually have one amino and one carboxyl group, for example, aminoacetic acid (glycine HGl) NH_2CH_2COOH, α-aminopropionic acid (alanine) $CH_3CH(NH_2)COOH$, and others. The number of amino groups determines the high stability of the complexes, while the number of carboxyl groups – one or two – has little effect on their binding properties (Brown et al., 2013).

Alkaline and alkaline earth metals have a low affinity to the amino group, so they do not form stable complexes with amino acids. However, M^{2+} ions of d-metals form very strong chelated amino complexes. At the same time, the strength of the complexes depends little on the length and structure of the α-amino acid carbon chain. This indicates the same structure of the CuN_2O_2 coordination node and the nature of the chemical bond, which has a clearly expressed covalent character (Fig. 1).

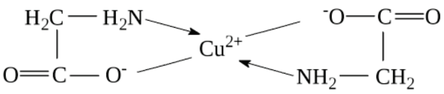

Fig. 1. Structural formula of cuprum glycinate

Cuprum is an important bioelement necessary for the normal development and growth of plants because it is included as a coenzyme in the composition of enzymes, in particular, it is a part of some oxidizing enzymes and increases the intensity of plant respiration and fosters the process of assimilation of mineral forms of nitrogen by them (Mustafa et al., 2024).

The purpose of our study was to choose a method for the synthesis of cuprum glycinate, to analyze its chemical composition and the possibility of using this compound as a chelated microfertilizer for pre-sowing encrustation of winter barley seeds as part of a tank mixture.

Materials and methods

Synthesis of the copper glycinate and study of its composition. Two well-known synthesis methods were used to produce copper glycinate:

1. Interaction between CuO and a solution of glycine during heating according to the reaction:

$$CuO + 2NH_2CH_2COOH = Cu(NH_2CH_2COO)_2 + H_2O.$$

We mixed 0.8 g of copper oxide, 1.5 g of glycine and 50 mL of distilled water, and stirred it using a magnetic stirrer while heating to 60–65 °C for one hour. At the same time, the black powder of copper oxide dissolves and a bright blue solution is formed, from which the complex compound of copper glycinate quickly crystallizes in the form of light blue mother-of-pearl flakes. The solution was left for a day to settle and crystallize the substances. After a day, the precipitate was filtered through a pleated "blue tape" filter and air-dried at room temperature.

2. The suspension of basic copper (II) carbonate $Cu_2CO_3(OH)_2$ and glycine interacted during heating according to the reaction:

$$Cu_2CO_3(OH)_2 + 4NH_2CH_2COOH = 2Cu(NH_2CH_2COO)_2 + 3H_2O + CO_2.$$

To the suspension consisting of 2 g of basic copper (II) carbonate and 30 mL of distilled water, we added 6 g of glycine, then heated it to 60–65 ℃ with constant stirring using the magnetic stirrer. The resulting mixture was left to stand for one day, and then filtered through a pleated "blue tape" filter. The synthesized substance was air dried at room temperature.

Absorption spectra of the analyzed aqueous solutions were measured on a UNICO UV2100 spectrophotometer (United Products and Instruments, USA) in glass cuvettes with an absorbing layer thickness of 1 cm. Infrared spectra (IR spectra) were recorded on a NICOLET Impact-400 IR spectrometer.

The amount of crystallization water was determined for the internal mass after drying in an oven at 120 °C to a constant mass. To determine the yield of the substance, the weight of copper glycinate was decomposed with an excess amount of sodium hydroxide during heating. The formed black precipitate of copper oxide was filtered and dried in oven at 200 °C to a constant weight.

Research of content and biometric parameters of winter barley Tutankhamun (root length and shoot height) was conducted in a thermostat at the Polymer Composite Materials laboratory of the Dnipro State Agrarian and Economic University. The selected winter barley (50 pieces each) was soaked in solutions of cuprum glycinate, cuprum ethylenediaminetetraacetate and in distilled water (control) at the temperature of 20–22 °C for 30 minutes. The concentration of coordination compounds in solutions used to treat winter barley was equivalent to 20 and 40 g of copper per 1 ton of grain. Then, they were placed in Petri dishes on circles of filter paper previously moistened with distilled water.

Laboratory germination (in percent) was measured two days later (n = 3). The experiment was repeated three times and the average value of the studied indicators was identified.

Analysis of results was expressed as mean (x) ± standard deviation (SD). A one-way analysis of variance (ANOVA) test was applied, considering $P < 0.05$ as statistically significant.

Results

To confirm the compatibility of the synthesized compounds, we obtained the IR spectrum (Fig. 2), the atomic absorption spectrum of the aqueous solution (Fig. 3), and determined the product yield (Table 1).

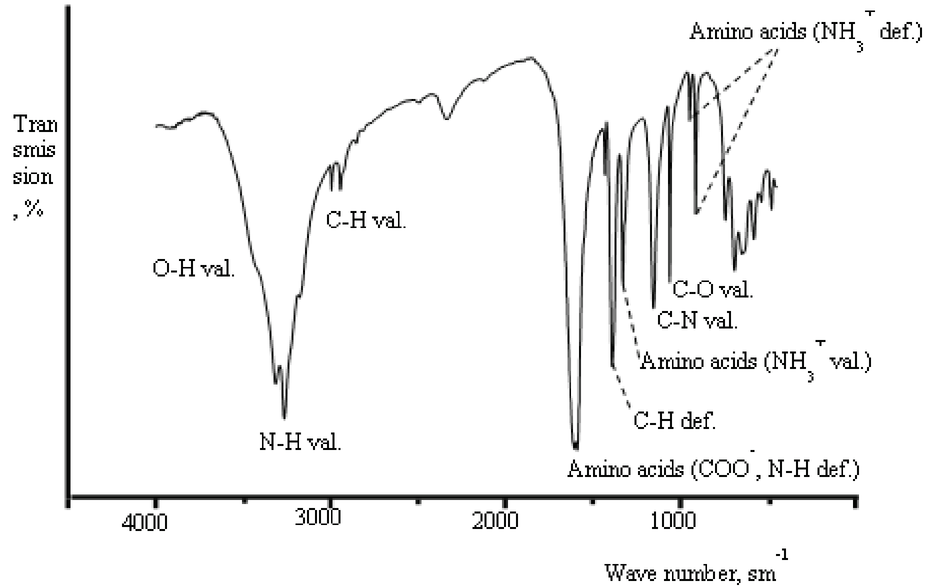

Fig. 2. IR spectrum of cuprum glycinate oscillations: val. – valence, def. – deformable

Table 1
Results of the synthesis cuprum glycinate product yield determination

Theoretical number of moles, mol	Actual number of moles, mol	Product yield, %
$2.60·10^{-4}$	$2.54·10^{-4}$	97.7

The content of crystallization water was determined. One molecule of cuprum glycinate has one molecule of water: $Cu(NH_2CH_2COO)_2 • H_2O$, M = 230 g/mol.

The influence of cuprum glycinate ($Cu(Gl)_2$) on the germination of winter barley seeds was studied. Seeds of this agricultural crop treated with distilled water served as the control. Also, for comparison, some of the seeds was treated with a complex compound of copper with ethylenediaminetetraacetate (Cu-EDTA).

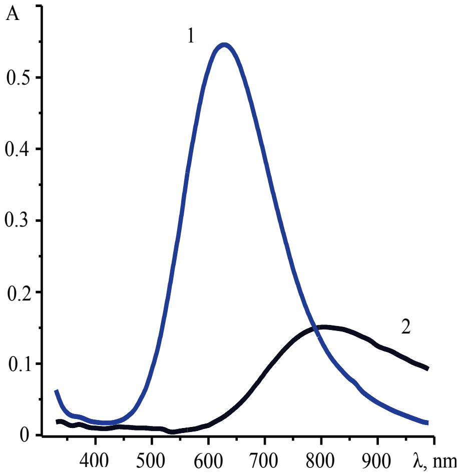

Fig. 3. Atomic absorption spectrum of aqueous solutions of 0.003 M copper glycinate (1) and 0.01 M copper sulfate (2), l = 1 cm

Discussion

Scientific experience and production practice convincingly demonstrate that the size and quality of the future harvest depends on the quality of seed preparation before sowing. Therefore, timely seed germination is an important stage of plant growth and development at the beginning of their ontogenesis (El-Maarouf-Bouteau, 2022; Chandel et al., 2024). One of the stages of germination is the termination of post-harvest physiological seed dormancy, for which various pre-sowing methods and processes are used, including the effects of temperature, light, hormones, and enzymes (Chin et al., 2021). The post-harvest dormancy of seeds is due to the properties of the seed coats, their impermeability or their function as a mechanical barrier that prevents germination. Usually, freshly harvested physiologically immature seeds of cereal grain crops have dense coats and therefore absorb water more slowly than seeds that have completed post-harvest ripening. Seed coats contain natural growth inhibitors that delay the growth of the embryo. Functions of natural germination inhibitors are performed by water-soluble phenolic compounds – phenolcarboxylic acids, flavonoids contained in cereal seed coats. During the time after harvest ripening, the thickness of the seed coats decreases, the integrity of the lipid layer is disturbed, the level of activity of natural inhibitors decreases, and this correlates with an increase in seed germination. The period of post-harvest ripening of seeds varies for different crops. In winter barley, it is the longest: even after eight months of storage, from 10% to 17% of grains remain in a state of rest (Kramarev et al., 2023). Hence, the germination of seeds from dormancy is of paramount importance to ensure stable germination and cultivation of high-quality crops.

Pre-sowing seed encrustation, which usually includes its treatment with certain stimulants and subsequent drying, serves to increase metabolism and accelerate the germination process. Most often, traditional methods of encrustation are used: hydropriming (Bourioug et al., 2020), hormonal and osmo-priming (Bourioug et al., 2020; Benadjaoud, et al., 2022), and others, each strengthening seed germination. New approaches are also emerging, such as nano-priming (Arnott et al., 2021; Kumar et al., 2022) (using solutions filled with nanoparticles) and magneto-priming (Alvarez et al., 2021) (using magnetic fields), which promote uniform seed germination. A new strategy is seed biopriming, which not only eases seed dormancy, but also increases the efficiency of nutrient uptake by germinating seedlings (Chin et al., 2021). Improving the technology of priming to obtain more effective results in terms of germination, germination time, and sprout strength remains an urgent task.

Table 2
Results of the study of the influence of copper glycinate $Cu(Gl)_2$ and copper ethylene diamine tetraacetate Cu-EDTA on the germination of barley seeds of the winter variety Tutankhamun (control – seeds treated with distilled water; data are presented as the mean of three independent replicates ± standard deviation)

Processing	Amount of copper in g per 1 ton of grain	Germinated, %	Maximum length of the roots, sm	Maximum length of the sprout, sm
Control	–	31 ± 2	3.1	2.1
$Cu(Gl)_2$	40	40 ± 3	4.0	2.4
	20	58 ± 3	3.4	2.7
Cu-EDTA	40	20 ± 2	3.3	2.1
	20	31 ± 3	2.9	2.0

Seed encrustation to promote germination involves pre-treating the seeds, traditionally involving pre-soaking and coating. Such pre-treatment methods improve the appearance of roots and germination energy, while causing positive changes in metabolic processes in seeds (Johnson et al., 2021). Seed encrustation is an approach applied to various crops to increase germination index, reduce dormancy and seed loss, to stimulate plant growth, improve crop quality, and mitigate biotic and abiotic stress. Its cost-effectiveness, practicality, and effectiveness in breaking seed dormancy in various plants make it the best method. Encrustation effectively solves the problems associated with germination, providing timely and friendly seedlings (Chandel et al., 2024).

Taking into account the high efficiency of pre-sowing encrustation, we used this method of pre-sowing seed preparation in this work. Cuprum glycinate was chosen as a germination stimulator due to its natural origin and environmental friendliness. The latest trends in organic farming suggest reducing the use of harmful and dangerous substances, including during encrustation, in order to prevent environmental pollution, reducing the harmful impact on the ecosystem.

The glycine complex of cuprum was synthesized using the two methods given above. During the interaction of copper oxide with aqueous solution of glycine, pink metallic copper was observed in the mixture of reaction products, so another method of obtaining a complex compound was tested – by the interaction of suspension of $Cu_2CO_3(OH)_2$ with glycine during heating. As evidenced by the data in the Table 1, this method is quite effective – the yield of the product was 97%. The chemical analysis of the synthesized compound was carried out and its formula was confirmed: $Cu(NH_2CH_2COO)_2{\bullet}H_2O$. Characteristics of metal complexes with amino acids vibration bands (Zabihzadeh et al., 2024) are present on the IR spectrum of the synthesized complex compound (Fig. 1). At the same time, there are no strong narrow bands of free OH groups' valence vibrations in the region of 3,650–3,590 cm^{-1} due to the formation of covalent bonds with the central atom.

The atomic absorption spectrum of the synthesized substance was also obtained (Fig. 2, spectrum 1). The spectrum of copper glycinate is significantly different from the spectrum of copper sulfate (Fig. 2, spectrum 2). The maximum of the absorption band for copper glycinate was at λ_{max} = 630 nm, ε = 165.76 L/(mol•cm), while for copper sulfate λ_{max} = 805 nm, ε = 13.48 L/(mol•cm). This also confirms the formation of cuprum glycinate.

After treating winter barley seeds with aqueous solution of cuprum glycinate, we observed better germination, and the length of sprouts and roots was greater (Table 2). We should also note that the treatment of grain with copper in the amount of 20 g per 1 ton of grain produced better results than twice concentrated. Treatment with a complex compound of cuprum with ethylenediaminetetraacetate had no significant effect on the germination and characteristics of sprouts (Table 2).

The seed dormancy is regulated by the balance of plant hormones in the seed, molecular interactions, in particular reactions such as oxidation and the interaction of amino acids with reducing sugars. The amount of reactive oxygen species – oxygen ions, free radicals, and organic and inorganic peroxides, as well as the amount of nitrogen oxide (NO) increases during seed germination, and treatment with oxidants and nitrogen compounds contribute to seed emergence from dormancy (Leymarie et al., 2012; Ma et al., 2016; Ne et al., 2017). The pro-oxidative environment in mature seeds suggests the hypothesis that protein redox regulation may be part of the germination mechanism, and reversible redox modifications of proteins may be considered as molecular switches controlling developmental processes (Ne et al., 2017).

Thus, the positive effect of copper ions on seed germination (Table 2) can be explained by its oxidative properties and ability to react with hormones and proteins. The difference between the effects on seeds of complex copper compounds with different complexing agents can be explained by the different degree of digestibility – the complex with the ligand, which is an amino acid, is better digested by living organisms, and glycine can participate in the reaction with reducing sugars (Ne et al., 2017).

Conclusion

When solving one of the main problems associated with the seed dormancy period, seed priming becomes a promising solution for increasing the germination rate and germination energy indicators in the initial phase of plant ontogenesis. Seed encrustation is an optimal means of overcoming germination-related obstacles, is cost-effective and environmentally safe. The seed germination rate is one of the most important indicators of seed quality, as it determines its biological and economic value. Complex compounds of biometal cuprum with organic chelate ligands were used as microfertilizers in chelated form for pre-sowing encrustation of seeds, as they have high stability and sufficient solubility in water, are non-toxic, and are better absorbed by plants.

It is expedient to synthesize the glycine complex of cuprum by the reaction of suspension of $Cu_2CO_3(OH)_2$ with glycine during heating (the yield was 97%), since it forms a complex compound $Cu(NH_2CH_2COO)_2 \cdot H_2O$ of sufficient purity, which was confirmed by chemical analysis. When studying the effect of cuprum glycinate on the germination of winter barley, positive results were observed: germination exceeded the control by 9–27%, unlike cuprum ethylenediaminetetraacetate, subject to which the germination rate did not exceed the control. The best laboratory germination, greater length of sprouts and roots was observed when the seeds were treated with solutions of cuprum glycinate in the amount of 20 g of cuprum per 1 ton of grain.

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