Theses of Doctoral (PhD) Dissertation

Effect of copper-complex compounds, applied as foliar fertilizer, on the yield and chemical contents of winter wheat (*Triticum aestivum* L.)

Written by:
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Mosonmagyaróvár
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1 INTRODUCTION AND OBJECTIVES

1.1 Introduction

Farmers have to face the major challenge of supplying the growing population with safe foodstuff in adequate quantity and quality. Beside intensive crop-growing practice, natural exposure of soil nutrients is no more enough. Subsequent delivery of the extracted nutrients is necessary to reach the planned yield safely. Agricultural practice usually uses the method of macro-elements' replenishment (mainly nitrogen, or eventually phosphorous and potassium). Replenishing these elements cannot provide a balance of nutrient supply, only. Research results show that the deficiency of microelements, like the relative depletion of copper can hinder the growth of yields.

The author at al. investigated the effects of the applied nutrient elements and their mixtures on the yields and quality components of crops in their research work with different crop cultures recently. Among other elements, the microelement copper was the main objective of research, because of its biochemical role.

Copper is very immobile in the soil, its leeching happens only at very extreme conditions, but its bind can hinder adequate copper-supply ability of the soil. Research data and results of several soil analyses carried out by the author in Hungary very often show soil features that refer to copper deficiency especially for copper demanding crops. Adequate copper replenishment is very important for the agricultural practice. There are more chances to return copper to the soil, e.g. through seed treatment or in soil or foliar applications. Not the low copper content of the soil but more often the inadequate mobilisation of nutrients lead to inadequate copper supply. Therefore, it is reasonable to replenish nutrients through foliar application to avoid binding the applied nutrients. Foliar fertilizers in chelate form can be applied in lower doses than products containing inorganic salts, too.

Copper, being an important micronutrient element for plants is also a well-known fungicide agent. Plant-pathogens cause severe problems in crop growing through yield loss. *Fusarium* species cause extra problems by producing toxins. Consuming them can cause harm both to human and animal organisms. Accordingly, they are important from the point of view of human and animal health. Toxin contamination reduce the value and marketability of the crop and causes direct economic loss as well.

Foodstuff can only find a place on the market of agricultural products if they can meet the high and diverse expectations of consumers and are of high quality and if they are safe and satisfy the ever-tightening regulations set by food authorities. High quality crops can only be grown if balanced plant nutrition supply and adequate plant protection are practised. Applying products of different copper content have a special role in both fields. Environmental aspects are particularly pronounced, therefore combining and harmonizing individual applications gain on importance. Copper-containing products are especially applicable due to their twin benefits.

1.2 Research objectives

Aim of the present work is to investigate whether the application of copper as a foliar fertilizer can influence the yield and chemical parameters of winter wheat (*Triticum aestivum* L.), a special important crop in the Hungarian agriculture.

The following objectives were set:

- Examining the applicability of carbamide containing copper-saccharose-type solutions on the surface of leaves for copper nutrient replenishment.
- Examining the manufactured copper containing product whether it can influence the yield of winter wheat if applied on copper deficient soil.
- Examining the manufactured copper containing product whether it can influence the chemical parameters of winter wheat (raw protein, gluten, starch and copper content).
- Examining the application of copper containing product if the plant protecting effect of copper can be detected on the strains of *Fusarium graminearum* Schwabe (1839), and *Drechslera sorokiniana* (Saccardo) Subram. & B.L. Jain (1964), lately called as *Helminthosporium sativum* Pammel C.M. King & Bakke (1910), *Bipolaris sorokiniana* (Sacc.) Shoemaker (1959).

2 MATERIALS AND METHODS

2.1 Small plot field experiments

Experiments on small field plots in four replications and random block arrangements were launched to test the effects of copper-saccharose type product on the fields of Solum Mezőgazdasági, Ipari és Kereskedelmi Zrt (Solum Agricultural and Trading Co.) in Komárom in the years of 2011/2012, 2012/2013 and 2013/2014. According to the applied crop rotation the experiments were launched on the field marked as 7a in the years 2011/2012 and 2012/2013 and on the field marked as 10b next to 7a in the years 2013/2014. The test plots sized 10 m^2 .

2.1.1 Describing the soil of the growing area

Based on the soil analyses results the soils on the experimental area are slightly alkaline, medium high in calcium and humus. Results received from the analysis of EDTA-KCl extractions show an inadequate copper supply. Besides the slightly alkaline pH and the contents of carbonate and humus that play a role in binding copper and because of the good phosphorous supply, we can expect copper binding that reduces the availability of copper for the plants.

2.1.2 Describing the climate of the growing area

Highly changing rainfall featured the years of experiments. The amount of rainfall in the area was extremely behind the multi-annual average in the economic year of 2011/2012 and it was mere 337mm in the vegetation period. The year 2012/2013 was extremely wet on the contrary. The 624mm rainfall that fell on the surface in the vegetation period and the amount of 216mm in the spring period greatly exceeds the amount featuring the area. The amount of rainfall in 2013/2014 was adequate (478 mm) but its distribution, especially the 14mm in March and 29mm in April, was unfavourable.

2.1.3 Grown crops and agrotechnology

The following varieties were sown on the test fields: GK Csillag in 2011 and 2012, and Hystar in 2013. Solum Co. regularly replenished nutrients in the fields according to the intensive farming practice. During the years of experiments (2011) dung was applied once, and green manure was applied once (2012 – mustard). In the other two years, the green-crop was harvested. Furthermore, fertilizers (NPK complex, MAS, DAM, and Nikrol-28) were regularly applied. Plant protection applications were carried out three times in all the three years.

2.1.4 Applied treatments

Treatments were launched at the end of ear-emergence/at the beginning of flowering in all the three years. Copper was applied in the doses of 0 – 0.1 – 0.3 – 0.5 – 1.0 – 2.0 kg ha^{-1} on the test plots. The treatment-yield curve did not show any maximum values in the first test year, therefore we applied further 4.0 kg ha^{-1} in the second and third years of the tests. Treatments were applied with a high-pressure hand-sprayer of 2.5 litres. The concentration of the applied solution was set so that the quantity of the solution applied on each plot averaged 0.6 dm^3. We used ion-exchanged water to prepare the solutions for the control plots.

The product contained carbamide additive in order to secure the stability necessary for storage and application. In the applied doses the concentration of copper varied 0.17 g L^{-1} and 6.67 g L^{-1} according to the doses. The solutions contained carbamide in a mass ratio of 1:1. Based on the data received, we could disregard the quantity of nitrogen compared to the rise in the nitrogen quantity we got in comparison with the control treatment.

2.1.5 Harvest and crop control

We harvested the crop on 2.5 m^2-of each plot by hand.

The wet gluten and starch content of the harvested crop were analysed at the Chemistry Department of the Institute of Environmental Sciences at the University of West Hungary, Faculty of Agricultural and Food Sciences. Raw protein and copper content were analysed in the laboratory of Synlab Umweltinstitut Ungarn Kft. in Mosonmagyaróvár.

2.2 Fungicide effect-test with the agar-diffusion hole test method

The author at al. selected two strains of plant pathogens, ***Fusarium graminearum*** Schwabe (1839) *NCAIM F.00730* and ***Drechslera sorokiniana*** (Saccardo) Subram. & B.L. Jain (1964) *NCAIM F.00745*, and with the use of the agar diffusion method they analysed if the copper-saccharose type products being important for plant nutrition had an antifungal effect. Aim of the experiments were to analyse *in vitro* the inhibiting effect of the treatments in field applications.

2.2.1 Applied cultures

National Collection of Agricultural and Industrial Microorganisms – NCAIM offered the selected two strains for the experiments. The commercial strains were in lyophilized form and double ampoules available.

2.2.2 Culture preparation

Reconstitution of the lyophilized strains followed the protocol suggested by strain collection. After plating the reconstituted strains on potato-dextrose agar (PDA) they were incubated at 24±1°C- for 72-120 hours under aerobe circumstances lyophilisation was carried out in 10-10 ampoules of cultures containing protective colloids. The second sub-culture created the basis for further analyses.

Any other analysis was initiated from new, lyophilized cultures that were plated on PDA agar incubated at 24±1°C-on 72-120 hours under aerobe conditions. Mycelia developed on the surface of agar were washed and filled into sterile test-tube. Spore numbers of inoculum were set – if needed by dilution – at 10^7 mL^{-1} for both tested strains in every experiment.

2.2.3 Agar-diffusion hole test preparation

Plates used for the analyses were prepared from suspensions having a pre-set cell number with the use of potato-dextrose agar. Taking into consideration the dilution at inoculation the final concentration in the petri dish reached 10^5 mL^{-1}. After the plates congealed, we made 4 holes of 12mm with a sterilized agar-drill under a laminar cabinet. Identical quantities (200 µL) were put into the holes from the dilution series of the aqueous solution of the test substance.

Petri-dishes were kept in a fridge at a temperature of 0-8 °C for 4 hours to let the substance to diffuse into the agar during a slowed down reproduction cycle. The plates were placed into a thermostat with a temperature of 24±1°C.

We measured the diameter of the inhibiting or enhancing zones around the holes with a nonius scale meter to conclude the antifungal effect of the inhibiting substance after incubating them for 48-72 hours (after 48 hours we inspected them every day).

Experiments were launched in three replications.

2.2.4 Applied substances

Among the commercially available copper containing plant protection substances, we applied a copper-oxychloride product (Rézoxiklorid 50WP /Agroterm Kft/, 50 % effective copper content) as agent control. Copper-oxychloride ($3*Cu(OH)_2*CuCl_2$) is a contact fungicide with 88 % copper-oxychloride active substance content that equals 50 % copper content. Concentrations of products applied in the tests were 3000 mg L^{-1}, 2000 mg L^{-1} and 1000 mg L^{-1} referring to the copper content.

Concentrations of the active substance in the products used in the field experiments were set according to the concentrations of solutions used in field nutrition experiments. Concentrations of the applied solutions 0 – 0.17 – 0.50 – 0.83 – 1.67 – 3.33 – 6.67 g L^{-1} equalled the copper concentrations. Solutions were prepared with analytical reagents or chemicals of equal purity.

3 RESULTS AND DISCUSSION

3.1 Small plot field experiments

3.1.1 A summary of the results in 2012

Because of the applied treatments we managed to achieve 90% significant yield increase with a doses of 1 kg ha^{-1}, and applying 2 kg ha^{-1} we achieved a significance level of 95 %. Applying the highest dose of 2 kg ha^{-1}, we could achieved the maximum yields.

We reached also significant effects on the raw protein and wet gluten content when we applied the treatments starting doses of 0.5 kg ha^{-1} as well as 1 kg ha^{-1}. Applying copper in the doses of 2 kg ha^{-1} resulted in the highest increase in both treatments similar to the yield increase. Compared to the control the highest raw protein content was 12.5±0.5 % showing an increase of nearly 10%.

We could not detect any significant difference in the starch and copper contents.

3.1.2 A summary of the results in 2013

In the second year of the experiment, we detected significant increase in the yield and we got a maximum of the yield if applying the dose of 2 kg ha^{-1}. The increase became significant after applying 1 kg ha^{-1} in the treatments. An increased dose of 4 kg ha^{-1} caused yield depression compared to the maximum achieved yield. Among the 3 experimental years, this year showed the highest measurable yield and increase in percentage (18.6 %) compared to the control.

We managed to reach significant effects on the raw protein and wet gluten content if we applied doses higher than 1 kg ha^{-1} or 0.5 kg ha^{-1}. We could achieve the highest increase in raw protein with copper doses of 2 kg ha^{-1} similar to the yield increase. The highest raw protein content reached 13.4±0.8 %. If we applied the highest doses, the measured raw protein content was slightly lower, but we could not detect a significant reduction similar to the yield. The two highest rates of doses resulted in gluten values next to each other, but we could only detect the highest value if we applied copper doses of 4 kg ha^{-1}.

We could not detect any significant difference in the measured starch and copper contents this year either.

3.1.3 A summary of the results in 2014

In the third year of the experiment, the applied treatments showed a significant rise in the yield. As a result, treatments with doses of 1 kg $ha^{-1}$and 2 kg ha^{-1}, but the unfavourable distribution of rainfall we could not repeat the high yields of the second year again. Treatments with doses of 2 kg ha^{-1}- resulted in maximum yields this year, too and treatments with 4 kg ha^{-1}k showed yield depression compared to the maximum yields as well.

We also managed to reach significant effects on the raw protein and wet gluten contents if we applied treatments of 0.5 kg ha^{-1}-or higher. Similar to the second year there were minimal differences in the raw protein content between the two treatments with the highest dosage, but we got the highest value if we applied a copper dose of 4 kg ha^{-1}. We could measure similar values in the gluten content if we applied treatments with the two highest copper doses, but this year we measured the highest value if the copper dose amounted 4 kg ha^{-1}. Gluten content was the highest this year (34.1±1.4 %) minimally exceeding the minimum value of quality improving wheat (34 %).

There were no significant differences in the measured starch and copper contents this year.

3.1.4 A summary of the three experimental years

Averaging the statistical evaluation of the experimental results of the three years treatments with doses of 0.5 kg ha^{-1} and higher induced significant yield increase at 95%-level of significance. Treatments with 2 kg ha^{-1} resulted maximum yields, treatments with 4 kg ha^{-1} showed yield depression compared to the maximum yield, but the difference of the two treatments was not significant. The highest yield amounted 6.43±1.30 t ha^{-1}, which showed a yield increase of higher than 15% compared to the control treatment. The yield function reached its maximum at copper doses of 2.89 kg ha^{-1}.

The raw protein content reached its significant increase when we applied doses of 0.5 kg ha^{-1}-or higher at significance value of 95 %. We achieved the highest measured raw protein content at treatments with copper dosage of 4 kg ha^{-1} (13.2±0.6 %), but this value slightly exceeded the value 13.0±0.9 % received in treatments with 2 kg ha^{-1} copper doses. The raw protein function of the treatments reached their maximum at copper doses of 3.26 kg ha^{-1}.

The highest gluten content was shown at treatments with 4 kg ha^{-1} copper dosage. The increase in gluten content got significant after treatments with 0.5 kg ha^{-1} or higher compared to the control. The highest measured gluten content of 34.0±2.0 % showed an increase higher than 15%. The function fitting the gluten content that was measured after the treatments, reached its maximum at copper doses of 3.18 kg ha^{-1}.

Averaging the results of the 3 years, we could not show any significant effects on the starch and copper content regarding the analysed parameters and the applied treatments.

3.2 Results of agar diffusion tests

In the analyses, we evaluated the effect of the compounds and the concentrations applicable in the small plot field crop growing experiments based on the diameter of the inhibiting rings. Inhibiting zone is the area that is not covered by mycelia around the test holes, and there were no signs of growth detectable by stereo microscopic analyses.

3.2.1 A summary of the results of agar diffusion tests

Analysing copper-oxychloride and *Fusarium graminearum* we could observe the development of inhibiting zone at the three examined levels of concentration. As a result of the increasing copper concentration the diameter of the inspected inhibiting zones increased, and we got a linear correlation (r^2=1.0000) based on the results of analyses. Products, in the field experiments did not show any inhibiting zones at the three lowest levels of concentrations (0.1 – 0.3 – 0.5 kg ha^{-1}) only at the three higher levels of concentrations (1 – 2 – 4 kg ha^{-1}). We could observe significant inhibiting effect at the two highest levels of concentrations. The dependence of the concentration – at the measurable 3 levels of concentration – was also linear (r^2=0.9935).

All three levels of concentrations of copper-oxychloride proved to be effective against *Drechslera sorokiniana,* but similar to *Fusarium graminearum* we could observe the inhibiting effect of copper-saccharose type product at the three highest levels of concentrations (1 – 2 – 4 kg ha^{-1}). The observed inhibiting effect was only significant at the two highest levels of concentrations. The inhibiting effect was in both products linear with the applied coper concentration (r^2=0.9980 and r^2=0.9552).

4 CONCLUSIONS, SUGGESTIONS

The results proved that the applied copper treatments positively influenced the yield and chemical parameters of winter wheat at copper doses of 0.5 kg ha^{-1} and higher at a significance level of 95%. We could detect 15% increase in yield, 10% in raw protein and 15% in gluten content. We could not show any significant difference in the starch and copper content in the analyses. To achieve the maximum yield under experimental circumstances we needed a dose of 2.89 kg ha^{-1}.

In vitro experiments proved that the product effective in field experiments acted as an efficient fungicide if applied with higher copper doses on *Fusarium graminearum* Schwabe (1839) and *Drechslera sorokiniana* (Saccardo) Subram. & B.L. Jain (1964) strains. Based on the results the effect was lower than that of the copper-oxychloride substance, selected as a control substance, which is commercially available for a long time.

Based on the applied experiments we can make the following conclusions and suggestions:

- The experiments prove that the copper containing product applied in field experiments is capable to ease the copper deficiency and to increase the yield in copper-depleted soils. Experiments showed that 2 kg ha^{-1} is the optimal dosage for winter wheat.
- Based on *in vitro* examinations we can suggest counting with the plant protecting efficiency of the product along with the treatments aiming plant nourishment. As a result, a harmonized nutrition treatment can complete the plant protection practice that is very important for the environment protection and can reduce the costs as well.

5 NEW SCIENTIFIC RESULTS

1. The scientific work proved that we can make a product from copper-saccharose type substance through adding carbamide additives that can increase the **yield, raw protein** and **gluten** content of winter wheat if applied on the leaves with a copper dose of 0.5 kg ha^{-1} or higher at a significance level of 95%.
2. In small plot experiments on copper depleted soils foliar treatments proved to produce significant **yield increase** at the end of ear emergence/ at the beginning of flowering. At the launched levels of treatments, we reached 16.7 % increase with doses of 2 kg ha^{-1}. Under the experimental circumstances, we needed a dose of 2.89 kg ha^{-1} to produce the maximum yield.
3. As a result of the treatments, we achieved significant increase in the **raw protein** and **gluten** content compared to the control and through improving the quality of wheat; we contributed to its exportability. With treatments of 4 kg ha^{-1} we achieved 10.9 % increase in raw protein content, and 16.0% increase in gluten content. The treatment with 2 kg ha^{-1} that showed the highest yield increase produced slightly less increase in the results of the two parameters 9.2 % and 13.0%.
4. *In vitro* experiments proved that the product effective in field experiments acted as an efficient fungicide if applied with higher copper doses on *Fusarium graminearum* Schwabe (1839) and *Drechslera sorokiniana* (Saccardo) Subram. & B.L. Jain (1964) strains. The product used as nutrient replenishment harmonized with plant protection practices as a complementary treatment can reduce the environment impact and costs as well.

6 LIST OF PUBLICATIONS

6.1 Publications related to the doctoral dissertation

6.1.1 Paper published in a foreign-language peer-reviewed hungarian journal

Kalocsai R., Schmidt R., Szakál P., ***Giczi Zs.****, Glatzer W.* (2004): Effect der Blattdüngung auf den Zuckergehalt der Weintraubensorte Zweigelt. Acta Agronomica Óváriensis, **46**, 151-162.

Szakál P., Kerekes G., Schmidt R., Barkóczi M., ***Giczi Zs.****, Kalocsai R.* (2005): Influencing the organic matter content of potato by macro and trace element fertilisers. Cereal Research. Communications, **33**, 415-418. (IF 0.320)

Giczi Zs*., Kalocsai R., Schmidt R., Szakál P.* (2006): The effect of N, P, K fertilisation and bacterial inoculation on the oxidation of elemental sulphur in danube alluvial soil. Cereal Research Communications, **34**, 187-191. (IF 1.037)

Barkóczi M., Szakál P., Schmidt R., Kalocsai R., ***Giczi Zs.****, Halasi T.* (2006): Copper ion-exchanged zeolite in plant nutrition. Cereal Research Communications. Proceedings of the V. Alps-Adria Workshop Opatija, Croatia, 6-11 March, 2006, 397-400. (IF 1.037)

Kalocsai R., ***Giczi Zs.****, Schmidt R., Szakál P., Barkóczi M.* (2006): Effect of sulphate fertilisation on the quality of winter wheat. Cereal Research Communications, **34**, 529-532. (IF 1.037)

Giczi Zs.*, Kalocsai R., Vona V., Szakál T., Lakatos E., Ásványi B.* (2020):Study of the antifungal effect of a copper-containing foliar fertilizer. Cereal Research Communications, https://doi.org/10.1007/s42976-020-00108-y (IF 0,811*)

*2019 impact factor

6.1.2 Paper published in a Hungarian-language peer-reviewed journal

Szakál P., Barkóczi M., ***Giczi Zs.*** (2007): Bioetanol-előállítás céljára termesztett búza Mn-trágyázása. Acta Agronomica Óváriensis, **49**, 589-594.

*2019 impact factor

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Tóth E. A., Kalocsai R. , Dorka-Vona V., ***Giczi Zs.*** (2018): Az esszenciális mikroelemek szerepe a növények élettani folyamataiban, Acta Agronomica Óváriensis, **59**, 126-150. (szakirodalmi összefoglaló)

Giczi Zs.*, Kalocsai R., Vona V., Szakál T., Teschner G., Lakatos E.* (2020): Réz kezelések hatása őszi búza (*Triticum aestivum* L) hozamára és nyersfehérje tartalmára. Acta Agronomica Óváriensis, **61**, 23-32.

6.1.3 Conference abstracts in a foreign-language

Schmidt R., Szakál P., Kalocsai R., ***Giczi Zs****.* (2005): The effect of copper and zinc treatments and precipitation on the yield and baking quality of wheat. Réz, cink a környezetben szakmai konferencia. Acta Agronomica Óváriensis, **47**, 195-203.

6.1.4 Conference abstracts in Hungarian

Tóásó Gy., Schmidt R., Szakál P., ***Giczi Zs.****, Kalocsai R.* (2005): A komposzt szeléndúsításának hatása a termesztett csiperke cink-, réz-, vas-, mangán – és nátriumtartalmára. Réz, cink a környezetben szakmai konferencia. Mosonmagyaróvár 2005.09.22, Acta Agronomica Óváriensis, **47**, 159-167.

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6.1.5 Scientific paper not related to the dissertation

Schmidt R., Kerekes G., Szakál P., Kalocsai R., ***Giczi Zs.****, Szlovak G.* (2005): The effect of previous crops on the cellulose decomposition activity of the soil. Cereal Research Communications, **33**, 61-64. (IF 0.320)

Csathó P., Magyar M., Holló S., Német I., ***Giczi Zs.****, Németh T.* (2012): Az algériai nyersfoszfát közvetlen alkalmazásának agronómiai és környezeti szempontú értékelése savanyú talajokon hazai szabadföldi kísérletekben. AGROKÉMIA ÉS TALAJTAN, **61**, 327-344.

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Rajkai K., Koltai G., ***Giczi Zs.*** (2019): Ground water level and moisture regime monitoring of land use types in the Szigetköz. In: Kende Z., Bálint Cs., Kunos V. (szerk.): 18th Alps-Adria Scientific Workshop : Alimentation and Agri-environment : Abstract book. Szent István Egyetem Egyetemi Kiadó, Gödöllő, 134-135.

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*Vona V., Centeri Cs., **Giczi Zs**., Kalocsai R., Biro Zs., Jakab G., Milics G., Kovács A. J.* (2020): Comparison of magnesium determination methods on Hungarian soils. Soil and Water Research, **15**, 173-180. (IF 0,982*)

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