THE EFFECT OF GLYCEROL BY-PRODUCT ON THE DEVELOPMENT OF MAIZE AND ZINC UPTAKE

Gabriella RÉTHÁTI – Adrienn VEJZER – Barbara SIMON – Zsolt VADKERTI – László TOLNER

Department of Soil Science and Agricultural Chemistry, Institute of Environmental Sciences, Faculty of Agricultural and Environmental Sciences, Szent István University, H-2103, Gödöllő, Páter K. u. 1. Hungary, Rethati.Gabriella@mkk.szie.hu

Abstract: Biodiesel production belongs to the environmentally sound technologies supported by the EU. During the production significant amount of (crude) glycerol is produced, as by-product. The glycerol content of this by-product is nearly 50%. Beside, it contains macro and microelements valuable for plants, methanol and potassium hydroxide in high amounts. With appropriate cleaning technology it is utilized in agriculture (in composts, mixing it with liquid manure, or forage), however, there is only a few studies made about its direct effect mixed into the soil. Our objectives were to find out 1) the effect of glycerol by-product on the Zn uptake of maize, and 2) to examine if it has any effect on the different developmental stages of maize. In pot experiments, soil (acidic brown forest soil, Gödöllő) were incubated for two weeks with glycerol and known amount of Zn. Treatments (3 replicates): (I) control, (II) 1% glycerol, (III) Zn: 100 mg kg^{-1}, (IV) 1% glycerol + 100 mg kg^{-1} Zn. The experiments were carried out in several developmental stages: swelling, germination, emergence, 2-3 and 3-4 leaf-stages. Dry matter yield, dry matter %, Zn concentration and uptake by plant (shoot, root) were examined. Conclusions: small-scale inhibiting effect of glycerol by-product was found in the beginning of the development of maize (seedling formation, emergence). However, this effect decreased by time and it did not show significant difference at 3-4 leaf-stage between the control and the treated plants. The Zn uptake by maize was not inhibited by mixing the glycerol by-product into the soil, however, the Zn uptake of samples (IV) treated with Zn and glycerol at the same time, was significantly lower than the samples that had only Zn (III), but not glycerol treatment.

Keywords: glycerol by-product, maize, Zn uptake, development of maize

Introduction

Glycerol that is produced as by-product of biodiesel production contains methanol, potassium hydroxide, macro and microelements valuable for plants. After different cleaning procedures it is utilized in agriculture as forage, in food and cosmetic industry. The purification procedure is long and expensive, however, due to the organic carbon content, and the significant amount of macro and micro elements of the by-product, it could be utilized to increase soil fertility. Based on experiments, the glycerol by-product can have positive effects on certain soil properties, however, definite answer can only be given about its applicability if it is examined in the soil-plant system (Tolner et al., 2010). Glycerol, as an organic compound, can be well utilized by soil microorganisms through aerobe and anaerobe metabolism (Németh and Sevella, 2007). Diánez et al. (2007) found the inhibiting effect of glycerol by-product on the reproduction of certain plant pathogenic fungi and bacteria. Zinc, as an essential micro element, is crucial for the plants in certain amounts. In several cases, the reason of problems arises due to the lack of Zn and not its high concentration, for example in areas where the soil phosphorous concentration is high. In Hungary, the plants show shortage of Zn supply on increasing areas, and thus the average Zn content of human food products and the forage decreases (Prasad, 1966). Total Zn content of soils is generally 10-300 mg kg^{-1}, the average content is 50 mg kg^{-1} (Bowen, 1966; Alloway, 1990). In plant leaves, the 27-150 mg kg^{-1} Zn content is normal, however, above 400 mg kg^{-1} (on a dry matter basis) is considered toxic (Kabata-Pendias, 2001). Abdel-Sabour et al. (1988) found that

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the Zn content of maize shoot was 45 mg kg^{-1}, root 36 mg kg^{-1} due to 50 mg kg^{-1} Zn load in silty loam soil. As a consequence of 270 mg kg^{-1} Zn load, at 4-6 leaf-stage of maize, in roots: 80-150 mg kg^{-1}, and in stems: 40-50 mg kg^{-1} micro element increase can be expected on calcic chernozem soil (Kádár, 1995).

Materials and methods

The examined parameters of the glycerol by-product used for the experiments can be seen on *Table 1.* Maize seeds (*Zea mays*) were used for the experiments, which were put into solution containing 0.5% and 1% glycerol by-product, and glycerol. The seeds were taken out of the solution every hour in the first six hours of the experiment, then 12, 24, and 48 hours later, the seeds were taken out, wiped and measured. After this, the swollen seeds were put onto cotton wools, that were soaked with the previously mentioned solutions, and then the exact time of the turnout of the sprout was detected.

Table 1. Parameters of the applied glycerol by-product (on a dry matter basis)

pH_{H2O}	pH_{KCl}	Dry matter %	LOI % (Loss on ignition)	C %	N %	Total P mg cm^{-3}	Total K mg cm^{-3}	Zn-EDTA µg cm^{-3}	Zn-H_2O µg cm^{-3}
10,7	9,6	36	92,6	53,7	0,04	0,92	14,81	28,35	1,4

In order to examine the further development stages of the plant, pot experiments were set on brown forest soil from Gödöllő-Szárítópuszta (*Table 2.*), which K_A value was 25. (K_A stands for János Arany's method (Buzás, 1993), in which the amount of distilled water (mL) that the 100g of ground soil can take up, during constant stirring, until it reaches the upper limit of plasticity - "thread like" phenomenon.).

Table 2. Parameters of the brown forest soil (Gödöllő-Szárítópuszta) used in the experiment

	pH_{KCl}	pH_{H_2O}	LOI %	C : N	Total P mg kg^{-1}	Total K mg kg^{-1}	Zn_{EDTA} mg kg^{-1}	Zn_{H2O} mg kg^{-1}
Control	4,17	5,74	2,08	15	760	4429	1,56	0,15
Gly bp	4,83	5,68	2,57	18	900	5092	2,02	0,29
Zn	4,41	5,47	2,08	15	800	4400	136,8	2,94
Gly bp + Zn	4,73	5,53	2,54	15	960	5100	118,6	1,81

The soils, used in the pot experiment, have been incubated on room temperature for three weeks with glycerol by-product (1%) /Gly bp/; Zn (100 mgZn kg^{-1} as $ZnSO_4$); and together with glycerol and Zn /Gly bp + Zn/. After this, 200-200g of soil and 8-8 pieces of maize seeds were put into pots. The soil moisture was set to field capacity. The parameters of the incubated soil are presented in *Table 2.*

The emergence time and the increase of length until the 4 leaf-stage were determined. At this point, the plants were taken out of the soil, then dry matter, Zn concentration and Zn uptake of the shoots and roots were determined. The shoots and roots were hydrolysed by HCl at 105˚C temperature. The Zn content was determined by Perkin-Elmer AAS. The experiment was carried out in 3 replicates.

Results and discussion

It can be seen on *Figure 1.* that the glycerol by-product and the glycerol did not have affect on the swelling of maize seeds after 48 hours. During swelling, the macromolecules (starch, protein) become hydrated, their volume increases and the seed swells. This physical-chemical process does not mean the germination potential of the seeds, for this the enzymatic system has to be activated.

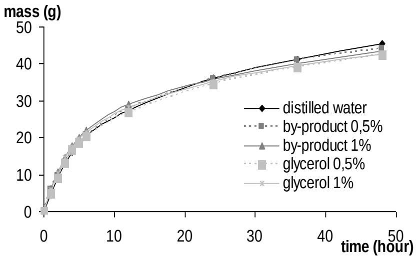

Figure 1. Swelling of maize seeds

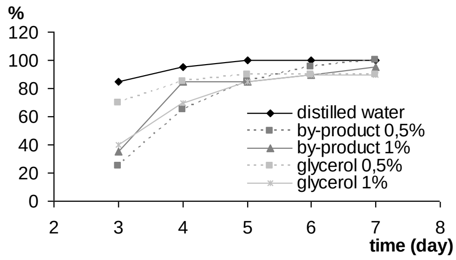

Figure 2. Germination of maize seeds

The start of germination was considered when the rootlet appeared. It can be seen on *Figure 2.* that, due to the glycerol treatments, the germination of maize seeds started later and was slower compared to the control.

The emergence of maize seeds is presented on *Figure 3.* One hundred percent emergence means that the rootlets of all the eight maize seeds rise above the soil surface. Regarding the emergence intensity of the seed, neither the glycerol, nor the Zn caused any significant difference compared to the control. However, the glycerol and the Zn together in the soil elongated the emergence time significantly (*Figure 3.*).

During the pot experiment, we noted that Zn treatment had positive effect on the shoot growth. The glycerol by-product had a growth inhibiting effect until the 2-3 leaf-stage, however, at this effect was not noticeable at 3-4 leaf-stage. The glycerol by-product and Zn together caused the decrease of the plants' height at both the 2-3, and 3-4 leaf-stage (*Table 3.*).

Based on the results of the dry weight of roots and shoots, the Zn treatment had a positive effect on both the shoot and root yield. The root yield increased due to the glycerol by-product and the Zn treatment compared to the control. At the original Zn content of soil (control and glycerol treatment), the glycerol treatment did not have any effect on the Zn content of root and shoot.

However, soils that were treated with 100 mg kg^{-1} Zn, the Zn content of plants showed significant increase compared to the control, furthermore, the presence of glycerol beside Zn decreased the Zn content of plants. It can be stated regarding the Zn uptake, that the root took up more Zn from soil than the shoot, which is probably due to the higher dry matter weight of root.

Conclusions

Examining the affects of glycerol by-product on maize, we can state that contaminated and uncontaminated glycerol in different concentrations does not have any effect on the swelling of maize seed after 48 hours.

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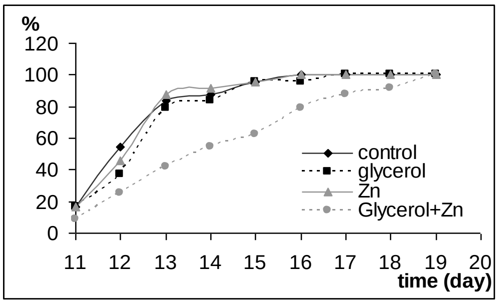

Figure 3. Emergence of maize seeds

However, its inhibiting effect can be noticed after the physiological processes start within the seed (germination, emergence, growth), especially with Zn treatment, which effect decreases by the development of plants (3-4 leaf-stage). The Zn concentration of maize was not influenced by the 1% by-product treatment.

Table 3. The effect of glycerol by-product on the parameters of the maize in different developmental stages

	Height (2-3 leaves) cm	Height (3-4 leaves) cm	Dry weight (3-4 leaves) g		Zn concentration (3-4 leaves) mg kg^{-1}		Zn uptake (3-4 leaves) µg pot^{-1}	
			root	shoot	root	shoot	root	shoot
Control	5,15	12,83	0,65	0,31	46,4	55,7	30	17
Gly bp	3,83	11,58	0,81	0,35	46,4	57,2	25	20
Zn	5,60	14,60	0,85	0,43	299,8	253,7	255	109
Gly bp + Zn	2,83	10,94	0,72	0,28	196,4	121,9	142	34
$SD_{5\%}$	**0,76**	**1,35**	**0,14**	**0,08**	**28,6**	**28,4**	**42**	**16**

However, applying the by-product with Zn together decreased the Zn content of shoots. Further investigations are needed in order to understand and discover the processes in the background (effect of glycerol and Zn together; mixing of glycerol into soil).

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