

# Influence of Some Fertilizers with Microelements such as Metal-ammonium Phosphates on Germination of *Triticum Aestivum*

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*This paper presents the influence of some fertilizers with microelements such as metal-ammonium phosphates on germination of Triticum aestivum seeds. The study presents experimental results regarding the effect of the solutions of different concentrations of copper-ammonium phosphate, iron (II)-ammonium phosphate, and zinc-ammonium phosphate on germination of Triticum aestivum seeds. Experiment aims to further the process of germination and development of caryopses, at histology level. To obtain chemical fertilizers with microelements such as metal-ammonium phosphates were used waste solutions from galvanic industry, diammonium phosphate and ammonia solution 25% [1,2]. Solutions were prepared on concentrations 0.1, 0.05, 0.025 and 0.0125% of each metal-ammonium phosphate. Lots of 30 seeds of Triticum aestivum L. were set for germination in Petri plates on filter paper, soaked with common water boiled and cooled. Seeds were kept in the dark 12 h at temperature 20-24°C, during which germinates and the length of the rootlets is maximum 1-2 mm. After 12 h of germination, water is removed, the seeds are put in contact with the treating solutions, with the concentrations ranging from 0.1 to 0.125%. 24 h after the treatment of the seeds, there was read the length of the rootlets and calculated the inhibition coefficient and also it was determined the influence on mitotic cell division by microscopic examination [3].*

**Keywords:** fertilizers with microelements, metal-ammonium phosphates, inhibition coefficient.

The metal-ammonium phosphates can be used as primary complex fertilizers with microelements, to obtain solid fertilizer with microelements and as phosphates techniques. Phosphorus compounds have a very important role in plant growth. Without this element, or when it is insufficient, plants do not grow normally and the seeds are not fully germinate. Supplement phosphorus required by poor soils can be achieved by adding ammonium phosphates and metal-ammonium phosphates, which are sources of microelements [4].

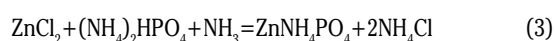
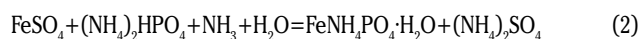
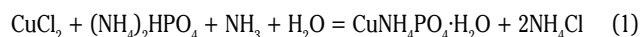
This paper presents laboratory investigations on the influence of copper-ammonium phosphate, iron (II)-ammonium-phosphate and zinc-ammonium phosphate on the seed germination of *Triticum aestivum* L.

Phytobiologic tests are very sensitive and the results obtained offer information about the mechanism of action of certain substances on metabolic processes taking place in cells or tissues [5].

This experiment seeks to survey the process of germination and development of caryopses at histological level. Setting effect on germination was determined by compared bioassay, with the solutions of different concentrations of  $\text{CuNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{FeNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$  and  $\text{Zn NH}_4\text{PO}_4$ .

## Experimental part

The fertilizers with microelements such as metal-ammonium phosphates were obtained by processing waste solutions of chlorides and sulphates with diammonia phosphate and ammonia solution 25% [1, 2, 6], according to reactions:



Lots of 30 seeds of *Triticum aestivum* L. which were set for germination in Petri plates on filter paper, soaked with common water boiled and cooled were used. Seeds were kept in the dark 12 h at temperature 20-24°C, during which germinates and the length of the rootlets was maximum 1-2 mm [1, 7].

There were prepared solutions of  $\text{CuNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{FeNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$  and  $\text{Zn NH}_4\text{PO}_4$  in different concentrations: 0.1, 0.05, 0.025 and 0.0125%.

After 12 h of germination, water is removed, the seeds are put in contact with the solutions examined, with concentrations ranging from 0.1 to 0.125%. After 24 h of treatment, the seeds were passed through a preservative solution plus 0,1% neutral red for staining roots to the package, operation designed to highlight the meristematic tissues. On this biological material it was read the length of the rootlets and calculated the inhibition coefficient and determined the influence on mitotic cell division by microscopic examination [8].

Measurement of root length is made on a glass plate with millimeter graduations and magnifying glass. The inhibition coefficient is calculated according to the formula:

$$I\% = 100 \times (L_m - L) / L_m \quad (4)$$

in which:

I% - inhibition coefficient;  $L_m$  - the average length of the control; L - average length of the test compounds.

**Control- *Triticum aestivum* L.** seeds germinated on filter paper soaked with distilled water

**Sample test- *Triticum aestivum* L.** seeds germinated in the presence of test solutions with concentrations of 0.1, 0.05, 0.025 and 0.0125 %.

It was investigated the amount of inhibition of root growth, taking into account the environmental pH, because values obtained at a too high pH are not conclusive.

For determinations of the influence on mitotic cell division, the method applied was the rapid Feulgen method

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**Table 1**  
ROOTLETS PARAMETERS CORRESPONDING CONCENTRATIONS SOLUTIONS OF METAL AMMONIUM-PHOSPHATES WHERE THE MORPHOLOGICAL CHANGES ARE MAXIMUM

Solution	Number of rootlets	Color / Appearance	Dimensions (in mm)
Control	2 - normal coleoptiles	White-gray and right-elongated	13,04
CuNH <sub>4</sub> PO <sub>4</sub> ·H <sub>2</sub> O 0,0125%	2 - normal coleoptiles	Yellow-brown / bent	11,05
FeNH <sub>4</sub> PO <sub>4</sub> ·H <sub>2</sub> O 0,025%	2-3 reduced coleoptiles	Yellowish white / straight- slightly bent	12,95
Zn NH <sub>4</sub> PO <sub>4</sub> 0,05%	1-2 green-yellow coleoptiles	White-yellowish / modified	11,60

of recognizing of chromosomes in plants. By this method, DNA is selectively stained, while the other cellular components are not stained. To enhance colour, microscopic preparations will be displayed in the solution of acetic orcein.

Microscopic samples are obtained by displaying the material between blade and slide in a drop of acetic water or acetic acid carmine. By the optical microscope with 40X objective it was observed the nuclei and chromosomes in red-purple color and almost colorless cytoplasm. The samples were analyzed by means of photographes shots with a Carl Zeiss microscope [8].

## Results and discussions

The influence of fertilizers with microelements solutions on *Triticum aestivum L.* caryopses germination and the rootlets parameters corresponding concentrations solutions of copper, zinc and iron (II)-ammonium phosphates where the morphological changes induced by the treatments are maximum, are presented in table 1.

The value of the inhibition coefficient of root growth is a criterion for assessing how the test solutions influence organogenesis. If the values of inhibition coefficient of root growth is over 50%, consider that the solutions obtained from test compounds inhibit root formation, affecting cell division. If the values of inhibition coefficient are less than 50%, we consider that the solutions obtained from test compounds can be used as chemical fertilizers, so can be recommended for next steps of screening. The results obtained are given in figures 1, 2 and 3.

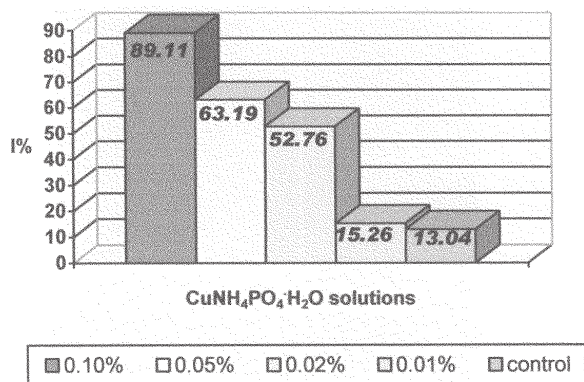


Fig. 1. Variation of inhibition coefficient (I%) reported to concentration solutions of CuNH<sub>4</sub>PO<sub>4</sub>·H<sub>2</sub>O

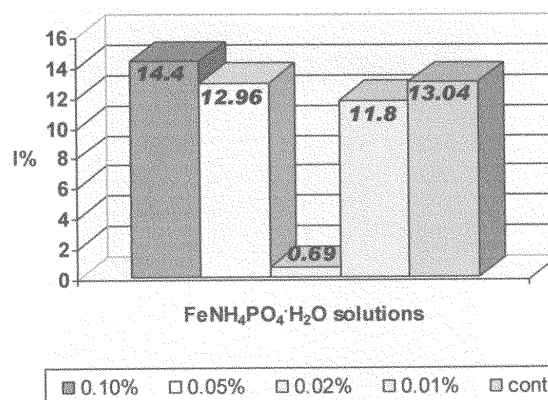


Fig. 2. Variation of inhibition coefficient (I%) reported to concentration solutions of FeNH<sub>4</sub>PO<sub>4</sub>·H<sub>2</sub>O

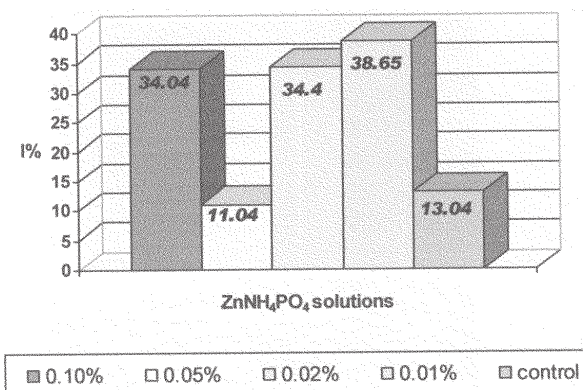
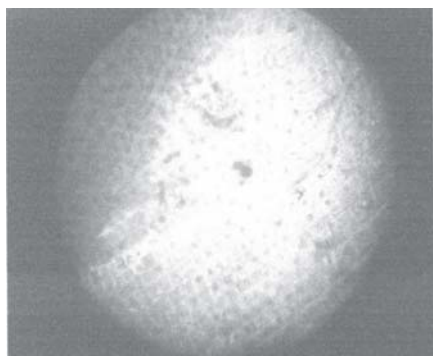


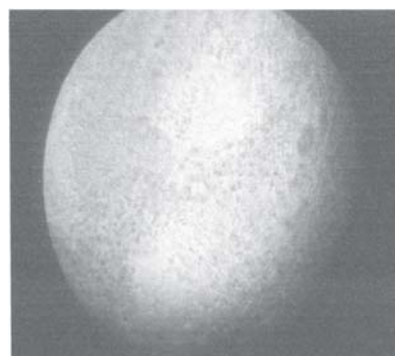
Fig. 3. Variation of inhibition coefficient (I%) reported to concentration solutions of ZnNH<sub>4</sub>PO<sub>4</sub>

Solutions of copper-ammonium phosphate are not recommended for accelerating seed germination since all concentrations tested have higher values than the standard. Moreover, solutions of 0.1, 0.05 and 0.025% present germination inhibition activity in amounts exceeding 50%. 0.1% solution is strongly inhibiting the morpho and hystogenesis.

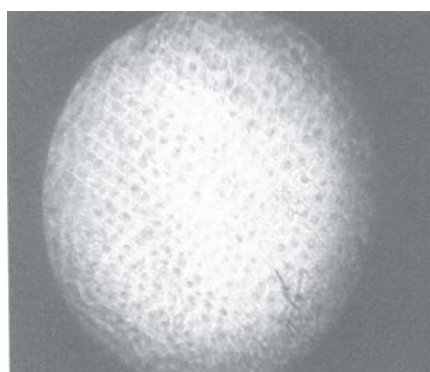
Comparing the analyse, iron (II)-ammonium phosphate solution presents an optimal activity, does not inhibit germination and development of caryopses. None of the solutions tested approach the threshold of 50%. Excepting the 0.1% solution, all the others have a stimulating action,



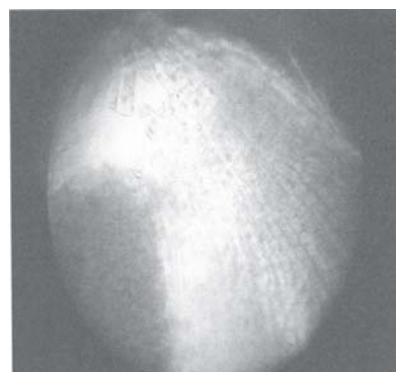
Section through radicle germinated in control solution



Section through radicle germinated in  $\text{CuNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$  solution 0,1%



Section through radicle germinated in  $\text{ZnNH}_4\text{PO}_4$  solution 0,1%



Section through radicle germinated in  $\text{FeNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$  solution 0,1%

Fig. 4. The results of microscopic examination of radicles germinated in 0.1% solutions of copper, zinc and iron (II)-ammonium phosphates

because the inhibition coefficient was below the standard. The most active is the solution of 0.025% concentration, corresponding to 0.69 inhibition coefficient. In conclusion, the tested compounds meet the intended purpose of stimulating the germination.

All solutions of zinc-ammonium phosphate are active as regards the influence on germination. There is no inhibition activity, no value reach the threshold of 50%, so these solutions can be used successfully as fertilizers. The most active solution is 0.05% solution, with an inhibition coefficient biologically superior to the control. In conclusion, this substance stimulates germination only in solution concentration of 0.05%.

Phytobiological test determines the changes induced by the tested substance at organogenesis level, detectable on a macroscopic scale. The influence on the caulogenesis could be detected also on the microscopic level, through the changes induced on cell division. The influence on mitotic cell division is monitored at the level of tissues with numerous divisions, such as root meristems [9,10].

The results of microscopic examination are shown in figure 4.

According to microscopic examination results, the fertilizer solutions of metal-ammonium phosphates influenced differently the cell division, during the experiment. The changes were in strict correlation with heavy metal type of combination, and the concentration of the solutions analyzed. The influence of solutions of metal-ammonium phosphates on mitotic cell division was highlighted in all the solutions tested, the most striking results in concentrations of 0.1% [1].

Rootlets germinated in 0.1% solutions were analyzed at the cellular level by showing mitotic cell division. Feulgen method allowed the nuclei, the cytoplasm, the arrangement of genetic material obtained through the

intensity of staining. The results were interpreted in comparison with the appearance of cells in division in the control (distilled water).

Solution of copper-ammonium phosphate 0.1% induced a slight disruption in cell division, changing the appearance of cells that have strangled in the middle and changes in size. There is the small size of cells and a reduced the number of viable cells. The dense cytoplasm, nuclei heavier stands, have small size and parietal position. Chromosomes are in concentration lower than the control. Items mentioned lead to the conclusion of chromatin condensation and inhibition at highlighting the chromosomes. Division in prophase stages are disrupted in all the processes involved.

The appearance of cellular division for the tissues analyzed in the solutions of zinc-ammonium phosphate and iron (II)-ammonium phosphate is characterized by visible chromosomes. Mitotic centers appear, nucleoli become smaller and even disappear completely, and the nuclear membrane is hardly in evidence. Because of the condensed chromatin filaments, is recognized the look of a ball in a large number of cells. All those factors identified suggest that none of the solutions influenced in a negative way the process of mitotic division.

## Conclusions

The studies regarding the influence of some fertilizers with microelements such as metal-ammonium phosphates on *Triticum aestivum* L. germination are very sensitive tests, the results provide information about mechanisms of action at the cellular level and histology. It was intended to obtain the value of the inhibition coefficient of root growth.

Phytobiologic studies regarding the influence of copper-ammonium phosphate solutions  $\text{CuNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$  on

germination at *Triticum aestivum L.* have shown that they are not recommended for accelerating seed germination since all concentrations tested have values of the inhibition coefficient higher than the standard. Thus, copper-ammonium phosphate is not recommended to speed up seed germination.

After the studies on germination at *Triticum aestivum L.*, iron(II)-ammonium phosphate solutions  $\text{FeNH}_4\text{PO}_4 \cdot \text{H}_2\text{O}$  presents an optimal activity, does not inhibit germination and development of caryopses. None of the solutions tested approach the 50% inhibition factor and, except for the solution of 0.1%, all others having a stimulating action, were below the standard. In conclusion, the test compound biologically meets the intended purpose of stimulating the germination quality.

According to studies on germination at *Triticum aestivum L.*, solutions of zinc-ammonium phosphate  $\text{ZnNH}_4\text{PO}_4$  has an optimum activity, inhibition does not work, no amount reaches the 50%, but only one solution, the value of 0.05% being less than the standard. This substance stimulates germination only in 0.05% solution concentration.

At the cellular level, changes induced by the tested solutions are obvious. Solution of copper-ammonium phosphate 0,1% inhibited all processes involved in the prophase mitotic division, thus explaining the small cells, and modified in terms of shape, condensed cytoplasm, nuclei of heavy viewing. Copper-ammonium phosphate is not recommended for use as accelerator of the germination, with cell inhibitory effect. The concentrations tested showed no toxic effect.

The appearance of cellular division in the tissues analyzed in the solutions of zinc-ammonium phosphate and iron(II)-ammonium phosphate suggests that none of the solutions influenced in a negative way the process of mitotic division. Optimal concentration to be an effective fertilizer is depending on the crops they are applied on and the soil type.

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