

## **EFFECT OF DIFFERENT NANOSCALE MICROELEMENTS-CONTAINING FORMULATIONS FOR SEED TREATMENT ON GERMINATION AND GROWTH OF MAIZE SEEDLINGS**

**Nguyen Hoai Chau<sup>\*</sup>, Chu Thi Hao, Doan Quang Ha, Dao Trong Hien,  
Le Thi Phuong, Nguyen Thi Thuy, Ngo Quoc Buu**

*Institute of Environmental Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Ha Noi*

<sup>\*</sup>Email: [nhchau.iet@gmail.com](mailto:nhchau.iet@gmail.com)

Received: 3 January 2018; Accepted for publication: 11 August 2018

### **ABSTRACT**

Effect of the nutrient formulations for pre-sowing seed treatment on germination and growth of maize seedlings was studied. The formulations comprised separate copper nanoparticles (NanoCu), IET1- based on a Novalon Seed Treatment product, IET2 - based on humate metal-organic-coordination complexes (MOC), Nualgi – based on nano silica, wherein microelements were adsorbed on the surface of the silica nanoparticles, and formulations with microelements in a form of Metal-EDTA complexes, based on commercial fertilizer products of Novalon, Wuxal and Comex companies.

Results of the study showed that all the tested formulations, except for the separate nanocopper formulation, demonstrated visibly higher stimulation effects on the maize seedlings growth, among which the nanoscale microelements-containing seeds treatment formulations produced in Institute of Environmental Technology (IET1 and IET2) exhibited the best growth indices although non-significantly differed as compared with the control. The experimental data presented also proved that for obtaining stable stimulation effects on the plant growth kinetics instead of using separate nanosized microelement it is preferable to use complex formulations, wherein besides of microelements there are other components included such as plant regulators, amino- and carboxylic acids, plant protection agents etc.

*Keywords:* nanoscale microelement, seed treatment formulation, EDTA complex, nanochelate.

*Classification numbers:* 2.5.1; 2.9.1; 2.10.2

### **1. INTRODUCTION**

In recent decades, in the context of general development of agricultural science and technology the presowing seed treatment has been widely studied for crops and vegetables, and increasingly used as an indispensable agrotechnique for growing various plants from seeds. There is a great diversity in composition of commercial products for seed treatment, which

gradually changed from single substances to complex ones. Numerous results from Russian researchers [1-4] indicated that all sonication-assisted superdisperse single metals exhibit stimulating effect to increase the growth and crop productivity. However, this approach is not enough when the plant growth and development were processed under different stress factors such as hot or cold temperature, salinity, drought etc. A large body of publications in the world [5-14] showed the necessity to extend the chemical composition of fertilizer formulations. That is, besides microelements, it is necessary to add bioactive substances (referred to as biostimulants) and plant protection agents to the seed treatment formulations [7-11].

A popular fertilizer product containing trace amounts of microelements used for seed treatment, Nano-Gro [5], has been recommended as a stimulator capable of activating the plant protection mechanism due to the formation of natural growth hormones such as auxin, cytokinin and gibberellin. Nano-Gro stimulator contains oligosaccharide microparticles with a diameter about 3-4  $\mu\text{m}$  and nanomolar concentrations of embedded microelement sulfates (Fe, Al, Mg, Mn, Ni, Co and Ag). Upon contact with seeds or a plant's root matrix Nano-Gro begins to communicate with the plant, and with an almost untraceable quantity of microelements nevertheless the plant could sense the presence of the product and immediately gives a response, that means its survival may be threatened. Instead of wilting, the Nano-Gro-treated plant would initiate natural survival mechanisms that allow them to combat the threat [10, 11].

Effect of Nano-Gro stimulator on germination and initial growth kinetics of grasses species was investigated by Jankowski et al. [5]. The results proved that the applied factor significantly accelerated the germination rate and seedlings length in the initial growth phase, but these parameters were reduced at a later time compared with the control. The study of the effect of Nano-Gro stimulator on tomato plant [6] demonstrated a considerable difference in physical and biochemical properties (plant height, root and apical trunk diameters and fruit quality indices) between the Nano-Gro-treated tomato and the control. The research also indicated the fact that this difference gradually declined over time and disappeared after two years of experiment.

According to the research works [7, 8], there were thousands enzymes in seeds, among which many can affect the seed germination process. Therefore if the seeds were impacted only by a limited number of stimulating factors some increase in the seed germination could be seen for some early growth phases, but in general this priority gradually lost when the plant switched to an another development phase. On the other hand, if temperature, humidity and presence of pathogenic fungi in soil were in unfavorable conditions for plant, the difference in the germination phase caused by the seed treatment with the stimulators will disappear.

Nowadays among abundant commercial fertilizer products for seed treatment one can see a combination of nanosized microelements (M.Es) with different biostimulants that Kauffman [14] defined as materials which are not fertilizers but able to activate the plant growth and development when used with small doses. Based on their origin and concentration biostimulants were essentially divided into 3 groups: humic substances (HS), hormones-containing products (HCPs) and amino acids-containing products (AACPs). Aside from these groups the biostimulants may also include plant protection agents such as fungicides, pesticides, bactericides etc.

Among other fertilizer products for seed treatment which can be mentioned are such as Albit, Fertigrain Start, Wuxal, Regoplant, which are more plentiful in composition, involving vitamins C, PP, B<sub>6</sub>, polyvitamins E, B<sub>1</sub>, B<sub>2</sub>, B<sub>12</sub>, nicotinamides, pentotenol; saccharose, glucose, fructose, SAS Tween 60, fungicides Tebu 60 and Packcil Ultra.

This report presents results of the study on the effects of nanoscale microelements-containing formulations for seed treatment produced in Institute of Environmental Technology (IET) on the growth kinetics of maize compared with other ones based on some commercial fertilizer products such as Nano Copper formulation, Novalon Seed Treatment, Nualgi, Wuxal, Omex etc.

## **2. MATERIALS AND METHODS**

### **2.1. Preparation of nanoscale microelements**

M.Es nanoparticles (NPs) used for making the seed treatment formulations were prepared by using following methods: Chemical reduction methods were used for preparation of NPs of Fe, Cu, Co, Mo and Ag using sodium borohydride as a reducing agent, and chitosan as a stabilizer for silver NPs [15-19], while for boron and selenium NPs metallic magnesium and ascorbic acid were used as reducing agents, respectively, and PVP as a stabilizer for selenium NPs [20-22]; hydrothermal method was used for preparation of ZnO and MnO<sub>2</sub> NPs [23, 24]. Nanoscale M.Es immobilized on nanosilica particles were produced according to [25], as per a commercial fertilizer product of Nualgi company.

M.Es-containing humate nanochelated complexes were manufactured according to [26]. Nanoscale M.Es Fe, Cu, Co, Mo, B, MnO<sub>2</sub> and ZnO produced and preserved in a powder form were undergone sonification before use. Average particle size of these microelements was  $\leq 50$  nm, while nano Se (particle size  $\leq 80$  nm, concentration 300 mg/L), nano Ag (particle size of about 20 nm, concentration 500 mg/L), M.Es immobilized on nanosilica (average particles size 40 nm, concentration 20,000 mg/L), and M.Es-containing nanochelates were maintained in solution (average particles size 20 nm, concentration 28,000 mg/L).

### **2.2. Seeds treatment and sowing experiment**

For the seed treatment the seeds were selected in order to reach morphological uniformity and tested on germination to ensure that at least 85% of the seeds were germinated. Seed treatments were the control (seeds were treated with water) and 7 formulations as illustrated in Table 1, including:

CT1: Formulation (referred to as NanoCu) as per Riazan Agrotechnological University, Russia, where only nano copper particles which were sonicated before presowing seed treatment was used [27];

CT2: Formulation (referred to as IET1) based on a Novalon Seed Treatment one, DOKTOR TARSA Inc., except that the M.Es were in a form of metal nanoparticles;

CT3: Formulation (referred to as IET2) based on a Novalon Seed Treatment one, DOKTOR TARSA Inc., except that the M.Es were in a form of Me-Humate nanochelates [26];

CT4: Formulation (referred to as Novalon) based on a Novalon Seed Treatment one, DOKTOR TARSA Inc., in which M.Es were Metal-EDTA complexes;

CT5: Formulation (referred to as Nualgi) as per NUALGI company, based on the procedure discribed in [25];

CT6: Formulation (referred to as Wuxal) as per WUXAL company, where M.Es were Metal-EDTA complexes;

CT7: Formulation (referred to as Omex) as per OMEX company, where M.Es were Metal-EDTA complexes.

Table 1. Composition of the seed treatment formulations used for experiments.

No	Formulations	Nutrient content (mg/kg of seeds)							
		Ctrl	CT1	CT2	CT3	CT4	CT5	CT6	CT7
I	Group 1. Macronutrients	0	0	121.5	121.5	187.5	5.9	86.3	0
1	Urea	0	0	30	30	45	0	11.3	0
2	P <sub>2</sub> O <sub>5</sub>	0	0	60	60	76.5	2.3	0	0
3	K <sub>2</sub> O	0	0	22.5	22.5	60	2.3	75	0
4	S	0	0	6	6	5.25	0.7	0	0
5	CaO	0	0	1	1	0.75	0.3	0	0
II	Group 2. Microelements	0	3.3	3.3	3.3	33.08	3.3	3.3	3.3
1	Fe	0	0	0.40	0.40	7.5	2.512	0.059	0
2	Cu	0	3.3	0.40	0.40	3.75	0.139	0.295	0
3	Co	0	0	0.26	0.26	0	0.007	0	0
4	Zn	0	0	1.19	1.19	18.75	0.209	0.884	3.3
5	Mn	0	0	0.53	0.53	2.25	0.419	0.589	0
6	B	0	0	0.26	0.26	0.75	0.007	0.589	0
7	Mo	0	0	0.13	0.13	0.08	0.007	0.884	0
8	Se	0	0	0.13	0.13	0	0	0	0
III	Group 3. Biostimulants	0	0	19.95	19.95	82.5	36.8	0	167.6
1	Growth Regulators:	0	0	0.4	0.4	0.37	0	0	0.4
1.1	Auxin (NAA, 46%)	0	0	0.3	0.3	0.28	0	0	0.3
1.2	GA3 (50%)	0	0	0.1	0.1	0.09	0	0	0.1
2	Amino Acids (50%)	0	0	4.5	4.5	2.25	0	0	4.5
3	Nano SiO <sub>2</sub>	0	0	3.3	3.3	1.13	36.8	0	3.3
4	Oligosaccharide	0	0	6	6	1.13	0	0	6
5	Humic acids	0	0	5.35	5.35	77.25	0	0	0
IV	Group 4. fungicides	0	0	1.25	1.25	0	0	0	1.25
1	Nano silver	0	0	1.25	1.25	0	0	0	1.25

For each experiment, 20 g of seeds were taken in a petri dish with a cover. 1 mL of each formulation was added (based on the recommended treatment dose: 1 L of the stock solution of a formulation corresponds to the treatment of 20 kg of seeds) and shook to ensure that all the seeds

were evenly covered with the nutrients. The seeds were removed and dried on air for 1 - 2 h, then ready for the sowing. The soil used for experiments was of organic origin (65 % silt, 30% organic substances, 1.2 % urea, 1.2 % P<sub>2</sub>O<sub>5</sub>, and 0.7 % K<sub>2</sub>O) and procured from Ornamental company Nam Anh. This soil was mixed with sand in a proportion: 50 % soil + 50 % sand.

Sowing experiments were carried out in pots in a shade grid house. Each portion of 5 seeds coated with one formulation was placed in each pot. The experiment was laid out as a factorial based on a randomized complete block design (RCBD) with 3 triplicates. Measurement of root and shoot lengths of the seedlings as well as their fresh and dry weights were conducted after 7, 10 and 12 days of sowing. To measure the root length for obtaining mean values a longest root filament from each seedling was chosen. Fresh roots were washed with tap water and blotted with paper towels before weighing, while dry weight of roots and shoots were determined after drying in a hot-air oven at 70 °C for 24 h. Because of small biomass of the seedling roots, the weighing roots and shoots was done for the seedlings altogether in each separate pot. The growth kinetics of individual species was carried out using polynomial regression analysis, while significance of polynomial coefficients was tested by Student's t-test at 5 % probability level.

### 3. RESULTS AND DISCUSSION

Effects of seed treatment formulations CT1, CT2 and CT3 (referred to as NanoCu, IET1 and IET2, respectively) on the maize seedlings growth after 7 days of sowing were presented in Table 2 and Fig.1. The experimental data showed that the formulations used for the seed treatment recorded in general statistically ( $p \leq 0.05$ ) non-significant effect on the seedlings growth. But all of them showed the growth rate slightly higher than the control, among which CT2 and CT3 give significantly differed values of shoot and root lengths by 6.8 % and 10 % as compared to the control, respectively, shoot and root dry biomass values for CT2 – by 12.5 % and 6.1 %, respectively, while for CT3 – by 2.81 % and 11.8%, respectively, as compared to the control. These data showed that formulation IET1 favors the shoot growth, while IET2 – the root growth.

Table 2. Effect of seed treatment formulations CT1, CT2 and CT3 on the maize seedlings growth after 7 days of sowing.

Formulations	Mean length, cm/plant		Mean fresh biomass, g/plant		Mean dry biomass, g/plant	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	14.29	12.87	0.524	0.537	0.0427	0.0391
CT1 (NanoCu)	15.01	13.54	0.527	0.546	0.0443	0.0421
CT2 (IET1)	15.26	13.63	0.578	0.543	0.0453	0.0440
CT3 (IET2)	15.10	14.16	0.583	0.578	0.0439	0.0437
LSD <sub>0.05</sub>	0.96	1.10				
CV(%)	11.4	10.7				

*CV (%)*: coefficient of variation; *LSD<sub>0.05</sub>*: least significant difference at 5 % probability level

The seedlings growth affected by the seed treatment formulations CT1, CT2 and CT4 (referred to as NanoCu, IET1 and Novalon, respectively) after 10 days of sowing presented in Table 3 and Fig. 2 showed that formulations IET1 and Novalon demonstrated significantly differed values of shoot and root lengths compared to the control (Fig. 2 a,b). Formulation Novalon exhibited the largest shoot and root lengths of seedlings after ten days of sowing.

However, for shoot and root dry biomass, formulation IET1 gave the largest value. This conclusion coincides with the study [28], which proved that microelement in a form of EDTA complex demonstrates less growth stimulation effect as compared with that in a sulfate or nano form. The data also showed that root dry biomass of formulation NanoCu was even lower than that of the control. These results were in concordance with those of the study [8], which approved that seed treatment formulation with a separate nanosized microelement gives less growth stimulation effect in comparison with the complex formulation, wherein microelement is accompanied with other components such as macronutrients, growth regulators, amino and other carboxylic acids etc.

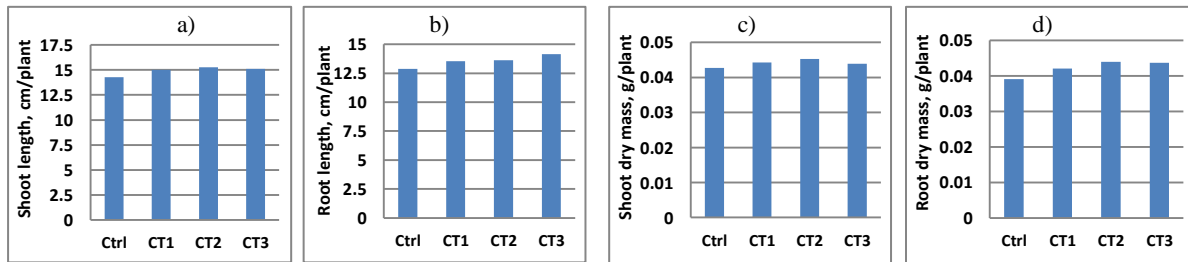


Figure 1. Effect of the seeds treatment formulations on the maize seedling growth after 7 days of sowing: length of shoot (a) and root (b), dry mass of shoot (c) and root (d).

Table 3. Effect of seed treatment formulations CT1, CT2 and CT4 on the maize seedlings growth after 10 days of sowing.

Formulations	Mean length, cm/plant		Mean fresh mass, g/plant		Mean dry mas, g/plant	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	15.74	13.61	1.134	0.996	0.523	0.88
CT1 (NanoCu)	16.14	14.57	1.159	1.090	0.560	0.83
CT2 (IET1)	16.8	15.34	1.275	1.131	0.575	1.00
CT4 (Novalon)	17.4	16.10	1.225	1.108	0.526	0.87
LSD <sub>0.05</sub>	0.924	0.97				
CV	7.5	9.5				

CV (%): coefficient of variation; LSD<sub>0.05</sub>: least significant difference at 5 % probability level

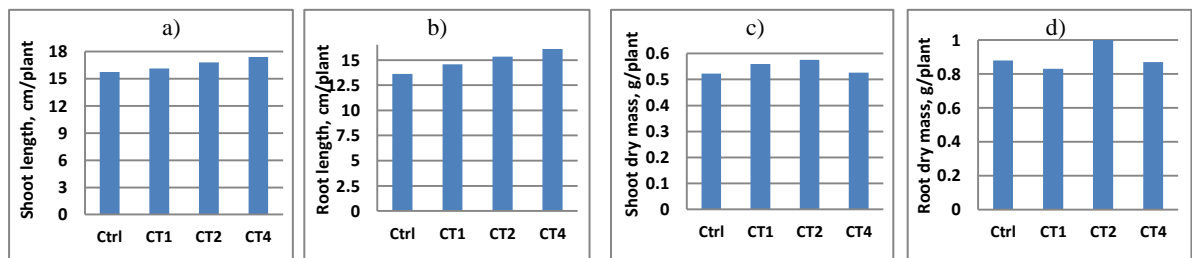


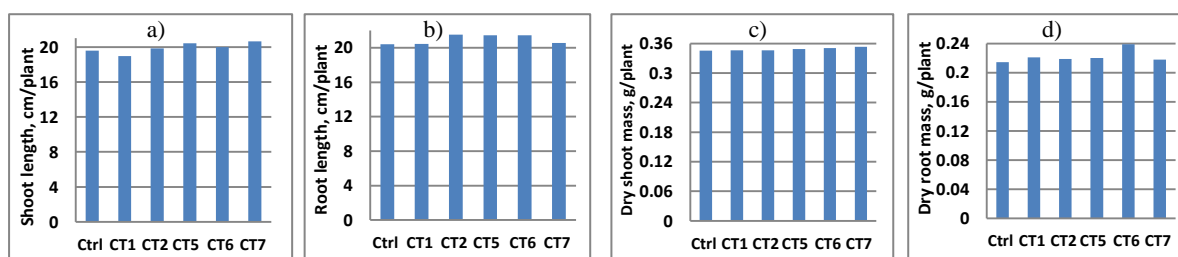
Figure 2. Effect of the seeds treatment formulations on the maize seedling growth after 10 days of sowing: length of shoot (a) and root (b), dry mass of shoot (c) and root (d).

Table 4 and Fig. 3 described influence of the formulations for seeds treatment (NanoCu, IET1, Nualgi, Wuxal and Omex) on the seedlings growth of maize after 12 days of sowing. Comparing Fig. 3 with Fig. 1 and 2 one can see a tendency that the longer the growth time the

less the significant difference in growth kinetics. But even so the experimental results presented in Fig. 3 showed that, except of the shoot length of CT1, the growth indices of all the formulations exceeded the control value, although in general with a statistically non-significant difference as compared with the control.

*Table 4.* Effect of the formulations for seed treatment (CT1, CT2, CT5, CT6 and CT7) on the maize seedlings growth after 12 days of sowing.

Formulations	Mean length, cm/plant		Mean fresh mass, g/plant		Mean dry mass, g/plant	
	Stem	Root	Stem	Root	Stem	Root
Control	19.60	20.39	4.43	3.62	0.345	0.214
CT1	18.98	20.45	4.40	3.70	0.346	0.221
CT2	19.85	21.53	4.64	3.97	0.346	0.219
CT5	20.43	21.44	4.68	3.69	0.349	0.22
CT6	20.0	21.44	4.44	3.85	0.351	0.239
CT7	20.65	20.56	4.58	3.65	0.353	0.218
LSD <sub>0.05</sub>	1.05	1.73				
CV	5.9	9.2				



*Figure 3.* Effect of the seed treatment formulations on the maize seedlings growth after 12 days of sowing: stem (a) and root (b) lengths, dry mass of stem (c) and root (d).

#### 4. CONCLUSIONS

For studying the effects of the nutrient formulations on maize seedlings growth different formulations were used, including separate copper nanoparticles (NanoCu), IET1- based on a Novalon Seed Treatment product, IET2 - based on metal-humate nanochelates, Nualgi - based on nano silica, wherein M.Es were in a form of nanoparticles, and formulations with M.Es in a form of EDTA complexes, based on commercial fertilizer products of Novalon, Wuxal and Comex companies.

It was shown that nanoscale M.Es-containing formulations produced in Institute of Environmental Technology (IET1 and IET2) exhibited the best growth indices although with statistically low-significant difference as compared with the control, and visibly higher than the formulations using M.Es in a form of EDTA complex. The study also proved that for obtaining stable stimulation effects on the plant growth kinetics, instead of using separate nanosized M.E, it is preferable to use complex formulation, wherein besides of microelements there are other components such as plant regulators, amino- and carboxylic acids, plant protection agents etc.

**Acknowledgment:** This research was implemented in the framework of the project VAST.TĐ.NANO-NN/15-18. The authors express their gratitude to the Vietnam Academy of Sciences and Technology for its financial support in implementation of this research.

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