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Research Article

Effects of Different Nanoscale Microelement-Containing Formulations for Presowing Seed Treatment on Growth of Soybean Seedlings

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Effects of nanoscale microelement-containing formulations for presowing seed treatment on germination and growth of soybean were studied. The formulations were prepared based on some well-known commercial products such as Doktor Tarsa Company and Wuxal Company. The results showed that all the tested formulations demonstrated noticeably higher stimulation effects on germination and soybean seedlings growth, among which the nanosized Co- and MoO_3-containing formulations exhibited the highest growth indices compared with the control (overpassed by about 10%), asserting the role of Co and Mo together with biostimulants as an effective nutrient mixture for growth and development of soybean plants. Enzymatic analysis of the nanotreated soybean shoots also demonstrated a similar feature; the studied formulations exhibited higher amylase and lipase activities compared with those of the control.

1. Introduction

Soybean as being rich in albumin is one of the worldwide main industrial crops. At the same time, pathogenic bacteria carried by soybean seeds and many other environmental factors can all affect emergence of seeds. Therefore, soybean seeds are usually coated by seed-coating agents before being planted [1]. The agricultural practice has shown that seed treatment reduces production costs of seedlings and seeds consumption; facilitates seedling establishment by incorporating nutrients, growth regulators, moisture-attractive or moisture-repulsive agents, and other agrochemicals; and provides protection during the critical germination and vigour establishment stages when the seed and emerging seedlings are unable to protect themselves from invasive pathogens [2, 3]. However, most of the seed-coating agents currently used are bringing serious damage to the environment and human health. Agents such as carbofuran and thiram all have great toxicity and cause serious pollution. In addition, it leaves a persistent pollution that has been difficult to eliminate from the environment for many years, and it has become a great hidden danger in ecological agriculture [4].

Zeng and Zhang [5] used a novel soybean seed-coating agent based on carboxymethyl chitosan as the main component, complemented with trace elements, growth regulators, trace fertilizers, penetrating agents, etc., for stimulating germination and growth of soybean seedlings. Results of the tests showed that the crop yield of soybean seeds coated by the novel seed-coating agent increased by 17.95%, and at 25.75% less cost, as compared with a traditional soybean seed-coating agent. The antifeedant test of the novel seed-coating agent showed that it had an excellent antifeedant effect as determined by the analysis of feeding area of the leaves and selective antifeedant rate. The authors claimed that the self-made seed-coating agent 55-F-1 had a unique efficacy in pest control and provided a fundamental protection of coated crops from the erosion of pests. Furbank et al. [6] demonstrated that natural polysaccharide film

is also considered to have a good selective permeability, which can prevent oxygen from entering the film, restrict loss of CO_2, and maintain a high concentration of CO_2 in the film, so as to restrain the seed's respiration, and thus to make the internal nutrient consumption of seeds fall to the lowest possible level.

In agriculture, nanoparticles (NPs) are used as biological stimulants due to their specific advantages over traditional solutions: they do not break up by heat and light, easily penetrate biological cell membrane and can be fully absorbed by plants, and take part in maintenance of microelements balance, which indicate their high biological activity. Moreover, NPs of copper, zinc, iron, B, Se, etc. unlike their salts are less toxic (copper, zinc, and iron NPs are, respectively, seven times, 30 times, and 40 times less toxic compared to their corresponding sulfate salts) [7]. Natarajan and Sivasubramaniam [8] showed that coating seeds with nanomembranes enables them to sense the availability of water and to allow seeds to imbibe it only when time is right for germination. Khodakovskaya et al. [9] reported the use of carbon nanotubes for improving the germination of tomato seeds through better permeation of moisture. Their data show that CNTs serve as new pores for water permeation by penetration of seed coats and act as a passage to channelize the water from the substrate into the seeds.

Effects of various selected nanoparticles on seed germination and growth rate of a paddy variety MR263 were studied by Said et al. [10] using different types and sizes of nanoparticles, i.e., carbon-based materials (10–20 nm single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs)), ~100 nm nanosilicon and nano metal oxides (70–100 nm CuO). Germination rate and root length and shoot length of seedlings were measured on the seventh day. The result showed that MR263 paddy seeds treated with 1 mg/mL nanosilicon particles demonstrated the highest germination rate, which increased from 13% (second day) up to 95% (seventh day). In terms of the growth kinetics, nanosilicon, SWCNTs, and MWCNTs exhibited 34–41% root length growth for MR263 variety compared to controlled (untreated) seeds; meanwhile, CuO nanoparticles were found to suppress the paddy seeds growth, presumably due to its toxicity. It is interesting to note that the growth rate of the seeds is inversely proportional to the nanomaterials' size. In this case, both SWCNTs and MWCNTs have dimensions of 10–20 nm in comparison with nanosilicon of about 100 nm in size, resulting in the fact that smaller nanoparticles penetrate the seeds' pores better and subsequently increase the germination rate by increasing water uptake of the seeds.

The purpose of modern technology is not changing plant nutrition processes by increasing the number of fertilizers used but optimizing plants' consumption processes and nutrients fixation. In this connection, in the last decade on the mineral fertilizers market, there were a lot of preparation forms, containing macro- and microelements, including also other plant protection agents and biostimulants. Israeli researchers [11] have developed nanochips of various structures for presowing seeds treatment. This apparatus represents nanostructural polyfunctional systems formed out of nanosorbents based on modified porous mineral vermiculite, which can contain more than 30 various plant nutrition elements, fungicides, and insecticides. By using nanochips, Ruban et al. [12] claimed that presowing seed treatment with the developed efficient multicomponent polyfunctional nanochips provides an obvious yield increase. For example, in comparison with rice yield control, the yield index increased to 26.9 c\ha, and in comparison with mung bean yield control, it increased to 8.6 c\ha.

In this report, the effect of nanosized microelement-containing seed treatment formulations on germination and growth of soybean seedlings was studied. Five nutrient formulations have been investigated in order to elucidate the effects of nanosized microelements and the role of nutrients composition in the seed-coating material on germination and growth of soybean seedlings.

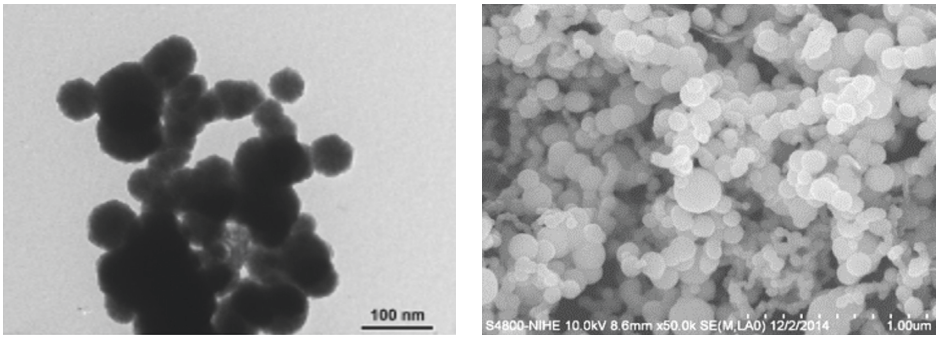
2. Materials and Methods

2.1. Preparation of Nanoparticles. All the microelements and Me-EDTA complexes used for preparing seed-coating formulations were procured from Merck. Cobalt NPs were produced by the chemical reduction method [13] using sodium borohydride as the reducing agent and carboxymethyl cellulose as the stabilizer, while molybdenum oxide NPs were produced by the sol-gel method [14] using a concentrated nitric acid medium. The produced Co and MoO_3 NPs were then transformed into a powder form by centrifugation followed by vacuum-drying. TEM and SEM images of Co and MoO_3 NPs illustrated in Figure 1 showed that their NPs were basically spherical, although the particles' size was rather scattered, with an average value of about 60–80 nm. XRD spectra of the samples presented in Figure 2 confirmed that the produced nanoscale microelements are Co and MoO_3. The XRD spectrum of Co NPs showed that 2θ peaks in the region from 45 to 60° are not enough clearly expressed, because according to the Song et al. [15], when the crystal structure of Co NPs possesses small particle size (less than 5 nm), a broad peak appears between 45 and 60° (2θ), which can be a composite of numerous crystalline lines.

2.2. Seeds Treatment and Sowing Experiments. For seed treatment, soybean seeds (cultivar DT-51) procured from the Center for Research and Development of Bean, Department of Agriculture and Rural Development of Vietnam were carefully selected in order to attain morphological uniformity and tested on germination to ensure that at least 85% of seeds were germinated. 120 g of seed grains was selected and divided into 6 equal fractions as shown in Table 1, which are as follows:

(i) F1 was a control formulation where the seeds were treated only with RO water

(ii) F2 and F3 were prepared as per a Wuxal® Company commercial formulation (https://wuxal.com/products.html), in which Co and MoO_3 were in the form of NPs, and F2 was prepared without using



(a) (b)

Figure 1: (a) TEM micrograph of Co and (b) SEM micrograph of MoO_3 produced by the chemical reduction method.

biostimulants, while F3 was prepared using biostimulants (growth regulators)

(iii) F4 and F5 were based on a Ryazan Agrotechnological University's product [16], in which F4 was prepared without using biostimulants, while F5 was prepared using growth regulators

(iv) F6 was based on a Novalon Seed Treatment's formulation where microelements were in the form of Me-EDTA complexes (https://www.doktortarsa.com/products.aspx?page=novalonseedtreatment), except for boron which was used as $Na_2B_4O_7$.

For the formulations F2, F3, F4, F5, and F6, the nutrients concentration of their solutions were calculated and prepared so that 1 L of the solution should cover 40 kg of seeds. Subsequently, 20 g of selected soybean seeds was placed in a Petri dish and 0.5 ml of each formulation was sprayed on the seeds and stirred with a glass stick to obtain evenly distributed nutrient material on the grain surface. The dishes were dried on air for 2 h at ambient temperature. The sowing was conducted in Petri dishes on a 6% agar medium (10 grains/dish) for determination of germination rate and in sandy pots (5 grains/pot) using construction sand, which was sterilized at 160°C for 2 h before use. The pot experiment was carried out in a shade grid house. The agar Petri dish and pot experiments were laid out as a randomized complete block design (RCBD) with 5 and 10 replicates, respectively.

Measurement of root and shoot lengths of the seedlings as well as their fresh and dry weights were conducted 3 and 7 days after sowing, respectively. To determine the root length of seedlings for obtaining the mean value, the 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 of roots and shoots was conducted for a total number of seedlings in each separate pot. The growth kinetics of individual species was studied by applying polynomial regression analysis, while significance of polynomial coefficients was tested by Student's t-test at 5% probability level. The experimental data were statistically tested using the software IRRISTAT 5.0.

2.3. Enzymatic Activity Determination. The activity of lipase was determined by the automatic pH titration method [17], which was based on the lytic capability of lipase, to determine the quantity of fatty acid formed in 10 min through the quantity of NaOH added to maintain a constant pH. A substrate mixture, including 5% (w/v) refined oil and 1% (w/v) gum arabic, was emulsified for 15 min in a liquidizer. 20 mL of the substrate emulsion was put in a glass beaker, which was placed on a magnetic stirrer coupled with a pH measuring instrument. The glass beaker was equipped with a water jacket through which warm water was circulated from a thermostat to maintain the reaction mixture at 40°C. After the substrate solution was set at pH 8.0 and 40°C, the hydrolytic degree of the oil was measured for 10 min. Hydrolytic degree was calculated through the quantity of 10 mmol NaOH solution used to stabilize pH 8.0. After 10 min, 10 μL of the enzymatic solution was injected and the added quantity of NaOH 10 mmol solution used for maintaining pH 8.0 was determined. Enzymatic activity of lipase in 1 mL of the substrate was determined as follows:

$$A_L = \frac{\text{NaOH quantity spent after adding enzyme} - \text{NaOH quantity without adding enzyme}}{\text{measuring time (min)} \times \text{enzyme volume added } (\mu\text{L}) \times 100} \text{ (IU/ml)}. \quad (1)$$

The activity of amylase was determined by using a method based on the presence of free carbonyl group (C=O) ([18]; reaction color depends on the precise quantity of reducing sugar in the solution). A unit of α-amylase is a quantity of the enzyme required for hydrolyzing starch to obtain 1 μM of sugar (as per glucose) at the optimal temperature and pH for 1 minute. The reaction lasted 10 min and then 3 ml of DNSA (3,5-dinitrosalicylic acid) was added,

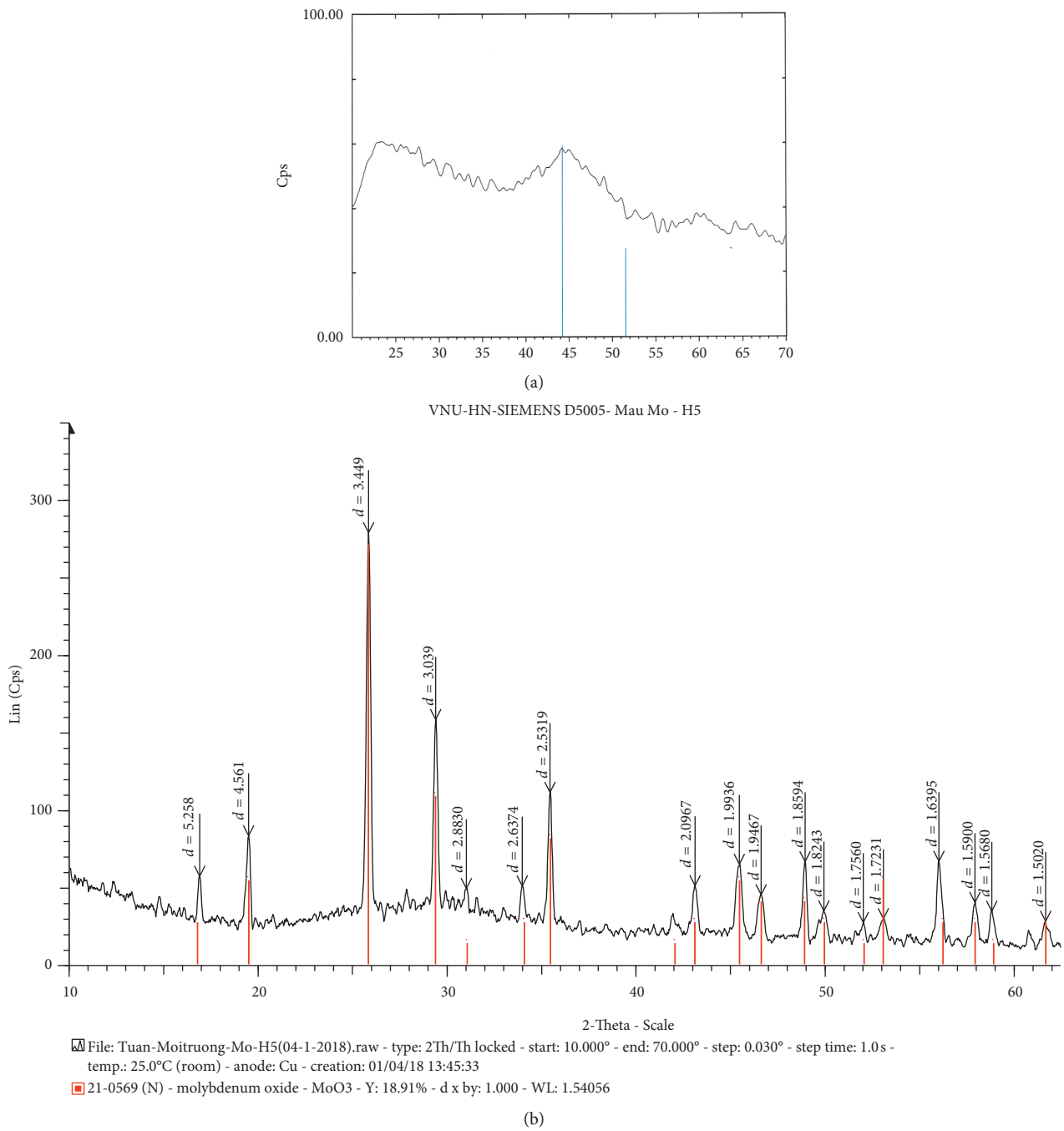


Figure 2: XRD spectra of nano Co (a) and nano MoO_3 (b).

the mixture was boiled for 10 min, and the reaction was stopped by rapid ice freezing. Finally, 6 ml of water was added and absorption was measured at 540 nm, and the amylase activity was calculated through a calibration curve:

(i) Step 1. A test tube containing 125 μL of the enzyme solution (centrifuged before to remove cells and diluted to appropriate activity) and 250 μL of 1% casein solution was shaken and incubated at reaction temperature (50°C) for 30 min. Then, 625 μL of TCA 1% was added to stop the reaction followed by centrifugation to obtain the transparent solution. A mixture of 0.5 mL of the solution with 2 mL of 6% Na_2CO_3 and 0.5 mL of 5 times diluted Folin-Xiocanto reagent was prepared and shaken. After 30 min, the mixture was photometrically measured at 750 nm wavelength.

(ii) Step 2. 125 μL of the enzyme solution and 625 μL of 5% TCA solution were added to a control tube and shaken vigorously. Then, 250 μL of 1% casein was added and the procedure was continued as in Step 1.

(iii) Step 3. For obtaining the tyrosine calibration curve, 100 mL of 1 mmol of tyrosine stock solution was

Table 1: Nutrient formulations for presowing soybean seed treatment.

N	Formulations	Nutrient doses (mg/kg of seed)					
		F1	F2	F3	F4	F5	F6
I	*Macronutrients*	*0*	*0*	*0*	*0*	*0*	*187.6*
1	Urea	0	0	0	0	0	45.0
2	P_2O_5	0	0	0	0	0	76.5
3	K_2O	0	0	0	0	0	60.0
4	CaO	0	0	0	0	0	0.8
5	S	0	0	0	0	0	5.3
II	*Nano microelements*	*0*	*3.3*	*3.3*	*0.165*	*0.165*	*33.3**
1	Fe	0	0	0	0	0	7.5
2	Cu	0	0	0	0	0	3.8
3	Co	0	0.3	0.3	0.165	0.165	0
4	Zn	0	0	0	0	*0*	18.8
5	Mn	0	0	0	0	0	2.3
6	B	0	0	0	0	0	0.8
7	Mo	0	3	3	0	0	0.1
III	*Biostimulants*	*0*	*0*	*0.2*	*0*	*0.2*	*2.6*
1	Auxin (NAA, 46%)	0	0	0.15	0	0.15	0.3
2	GA3 (50%)	0	0	0.05	0	0.05	0.1
3	Amino acids (50%)	0	0	0	0	0	2.2

*Microelements are in the form of Me^{n+}-EDTA complexes.

prepared and different tyrosine concentrations were made by taking appropriate volumes of the stock solution. After adding 2 mL of 6% Na_2CO_3 solution, the samples were shaken, then 0.5 mL of Folin's reagent was added, and the mixture was kept at ambient temperature for 30 min. The photometric measurement was carried out at 750 nm wavelength.

Enzymatic activity of amylase was calculated according to the following formula:

$$\text{IU/ml} = \frac{x * 1 * a}{30 * 0.125 * 0.5}, \quad (2)$$

where x is the tyrosine concentration found on the calibration curve, 1 is the total volume of the reaction mixture (mL), a is the dilution factor of enzyme, which is 1, 30 is the reaction time (min), 0.125 is the volume of the enzymatic solution used for reaction (mL), and 0.5 is the volume of the reaction solution used for photometric analysis (mL).

3. Results and Discussion

Germination rate of soybean seeds coated with different nutrient formulations containing different nanoscale microelements is presented in Table 2. The data showed that all the tested formulations demonstrated a substantially higher germination rate compared with the control, among which the formulation F3 with Co and Mo NPs exhibited the highest germination value. The results also showed the role of the biostimulants in promotion of the soybean seed germination (compare the germination rate of F3 with F2 and F5 with F4, wherein F3 and F5 contain the growth regulators auxin and gibberellin while F2 and F4 do not). It was noteworthy that the formulation F6, instead of metal nanoparticles, used a lot of biogenic microelements in the form of Me-EDTA complexes and with much a large quantity (10 times in comparison with F3) but gave the same germination rate as F3.

Table 2: Germination rate of soybean seeds treated with different formulations (experiment on agar medium), %.

Formulations	48 h after sowing	60 h after sowing	72 h after sowing
F1	14	54	95
F2	18	60	96
F3	26	64	100
F4	20	56	96
F5	22	60	97
F6	24	62	100

Growth indices of soybean seedlings germinated from seeds coated with different nutrient formulations are presented in Tables 3 and 4 and Figures 3–5. The experimental data showed that the formulation F3 in which the microelement group (Table 1) used cobalt and molybdenum NPs together with growth regulators exhibited the highest growth indices such as shoot and root length of the seedlings and their dry biomass. As mentioned above, adding growth regulators to the nutrient formulations enhanced the growth rate of the soybean seedlings. Indeed, comparing the growth indices of F2 with F3 and F4 with F5 presented in Tables 3 and 4 and Figures 3 and 4, one can see that, for all the cases, F3 and F5 with included growth regulators gave higher values of these indices compared with those of F2 and F4 without growth regulators. These results were in good accord with the work of Israeli researchers [12] who have proved the necessity to use complex composition of nutrient formulations for presowing seed treatment.

The data depicted in Figure 4 also showed that F3 which used a mixture of Co and Mo NPs as microelement nutrients have better root and shoot dry matter values compared with F5 which used Co NPs only, asserting the role of Co and Mo together with growth regulators as an effective nutrient composition for growth and development of soybean plants. Thus, it can be seen that the experimental results obtained were in good agreement with the work of Fedotov et al. [19], which confirmed the role of biostimulants together with metal NPs in promoting plant growth and development, contributing to elucidate the impact of complex fertilizer formulations on plant growth. It is interesting to note that the formulation F6 which used microelements in the form of Me-EDTA complex with their dose 10-fold higher than that of Wuxal's formulation F3 exhibited actually the same morphological indices.

Results of enzymatic study on the effects of different nutrient formulations on soybean shoots are presented in Table 5 and Figure 6. The data showed that amylase and lipase activities of the shoots grown from seeds treated with a mixture of Co and Mo NPs (F3) and with Co NPs only (F5) were much higher than those of the control, while the formulation F3 gave better enzymatic values compared with those of the formulation F5, proving the stimulation role of molybdenum in promoting soybean growth.

This result fits well with the results of Azizbekian et al. [20], who considered metal NPs not as a fertilizer but as an

Table 3: Growth indices of soybean seedlings 7 days after sowing the coated seeds.

Formulations	Shoot length (cm)	Root length (cm)
F1	8.28[a]	6.06[a]
F2	8.92[a]	6.65[a]
F3	9.41[b]	6.77[a]
F4	9.35[b]	6.48[a]
F5	9.23[b]	6.54[a]
F6	9.37[b]	6.57[a]
$LSD_{0.05}$	0.69	0.81
CV%	5.5	6.1

CV, coefficient of variation; $LSD_{0.05}$, least significant difference at 5% probability level. Values in the same column with the same letter are not different.

Table 4: Shoot and root dry mass of the soybean seedlings germinated from seeds treated with different nutrient formulations 7 days after sowing.

Formulations	Dry matter (mg/seedling)	
	Shoot	Root
F1	186.5[a]	27.4[a]
F2	188.3[a]	30.0[a]
F3	200.4[b]	37.5[b]
F4	187.4[a]	29.4[a]
F5	189.9[a]	31.3[a]
F6	195.0[b]	33.9[a]
$LSD_{0.05}$	10.4	8.5
CV%	4.5	5.3

CV, coefficient of variation; $LSD_{0.05}$, least significant difference at 5% probability level. Values in the same column with the same letter are not different.

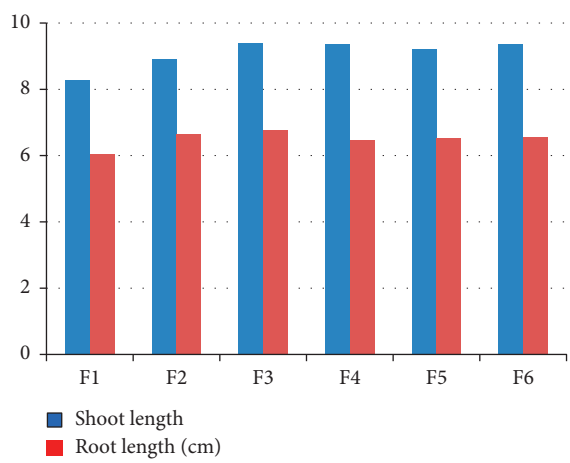


Figure 3: Growth indices of the soybean seedlings germinated from seeds treated with formulations of different compositions 7 days after sowing.

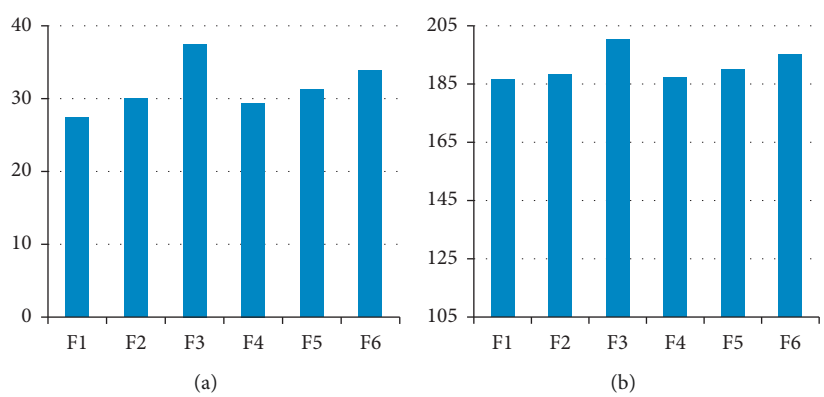


Figure 4: Shoot (mg/plant) (a) and root (mg/plant) (b) dry matter of the soybean seedlings 7 days after sowing.

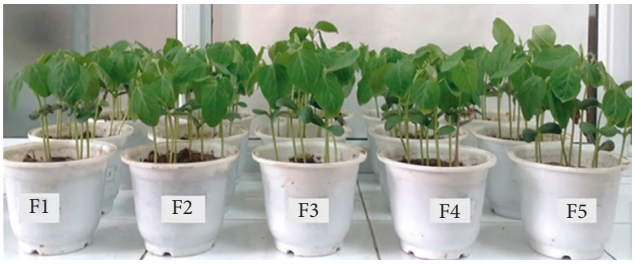


Figure 5: Soybean seedlings grown with formulations of different nutrient compositions 7 days after sowing.

Table 5: Enzymatic activity of the soybean seeds treated with different nutrient formulations (triple experiment).

Enzymes	Formulations	Enzymatic activity (IU/g)			
		Experiment 1	Experiment 2	Experiment 3	Average
Amylase	F1	1794	1610	1617s	1673 ± 104
	F2	ND	ND	ND	ND
	F3	2884	2425	2523	2611 ± 242
	F4	ND	ND	ND	ND
	F5	2212	2121	2229	2187 ± 58
	F6	2006	1849	1877	1911 ± 83
Lipase	F1	0.05	0.07	0.04	0.053 ± 0.015
	F2	ND	ND	ND	ND
	F3	0.106	0.12	0.089	0.105 ± 0.016
	F4	ND	ND	ND	ND
	F5	0.105	0.091	0.102	0.102 ± 0.010
	F6	0.078	0.09	0.082	0.084 ± 0.007

ND, not determined.

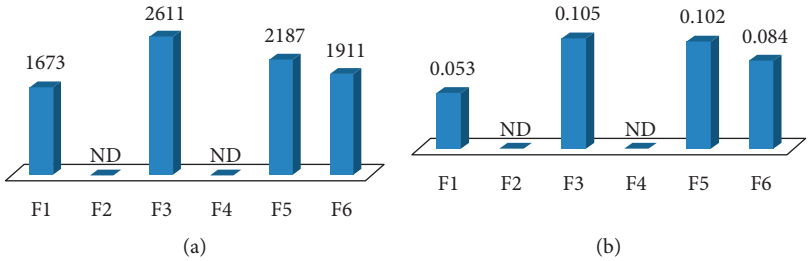


Figure 6: Enzymatic activity of soybean shoots grown from seeds treated with formulations of different nutrient compositions: amylase activity (IU/g) (a) and lipase activity (IU/g) (b) 48 h after sowing.

agent promoting microelements to penetrate the plant cells and take part in the enzymatic reactions for enhancing the rate of plant growth and development. As mentioned above, the formulation F6 which used microelements in the form of Me-EDTA complexes exhibited noticeably lower enzymatic activities compared with F3 and F5 which used microelements in the form of metal nanoparticles with much less quantities (3.3 and 0.165 mg/kg of seeds, respectively). This result was in accordance with the work [21], which showed that mobility of Me-EDTA complex in plant is lower in comparison with that of the formulations containing metals in the nanoform and at the same time indicated the specific role of Co in stimulating soybean growth.

4. Conclusion

The results of the research showed that all the tested formulations demonstrated noticeably higher stimulation effects on germination and growth of soybean seedlings compared with the control, among which the nanoscale Co- and Mo-containing formulations (F2 and F3) exhibited the highest growth indices (overpassed by about 10%), asserting the role of Co and Mo as an effective nutrient formulation for growth and development of soybean plant. It was preferable to use complex formulations, which besides microelements the stimulating components such as growth regulators were also added. Enzymatic analysis of the nanotreated soybean shoots also demonstrated a similar feature: all the studied formulations exhibited higher amylase and lipase activities compared with those of the control. The research results could be considered as a contribution in shedding more light on the effects of nanoscale microelement-containing complex fertilizer formulations on plant growth and development.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This research was implemented in the framework of the project "Applications of nanotechnology in the agriculture" (code: VAST.TĐ.NANO-NN/15–18).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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