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REVIEW

on a dissertation thesis for awarding the educational and scientific degree "Doctor of Philosophy (PhD)" in the Higher education area (cipher 4). Natural sciences, mathematics and informatics; Professional area (cipher 4.4). Earth sciences; Scientific specialty Ecology and Environmental Protection

Author: Nguyen Cao Nguyen, Part-time PhD student at the Department of Chemistry and Phytopharmacy, Agricultural University of Plovdiv

Topic: Study of the technological processes of zinc hydroxide nitrate nanocrystals production applying for foliar fertilizer for important agricultural plants

Reviewer: Prof. Andon Vassilev Andonov, PhD, Member of the Scientific Jury by Order No RD-16-1089 from 26th of November 2020 of the Rector of the Agricultural University of Plovdiv

1. Brief biographic data of the doctoral student

Nguyen Cao Nguyen graduated with a bachelor's degree in Agriculture from the University of Technical Education in Hochiminh City, Vietnam, in 2006 and a master's degree in Crop Science from the University of Agriculture and Forestry in the same city in 2010. His professional career is entirely related to the testing and implementation of new mineral fertilizers in practice. He has been working in this manufacturing sector for 15 years, holding the positions of researcher, consultant and manager in various government units. He is currently the manager of a consulting center at the Research Institute of Oil and Oil Plants of Vietnam.

Nguyen Nguyen is a well-established specialist in the field of agrochemical services for agriculture. He has good management skills, speaks English and works with modern software products. These skills, along with his high curiosity and strong motivation for professional development, logically lead him to the decision to continue his studies in a doctoral program. In 2015, by Order No RD-16-1249 (December 18, 2015) of the Rector of the Agricultural University - Plovdiv, Bulgaria, Nguyen Nguyen was enrolled as a part-time doctoral student with research supervisor Prof. DSc Krassimir Ivanov.

2. Significance of the studied problem

The studied problem in Nguyen Cao Nguyen's dissertation is zinc (Zn) deficiency in several agricultural crops and the possibility for its overcoming through foliar fertilization. This problem is actual for many regions of the world due to the low content of mobile zinc forms in most agricultural soils, as well as a result of their difficult uptake by plants in adverse climatic conditions.

Zinc is an essential element for plants, being involved in a number of physiological functions (enzymatic stabilization and activation, protein synthesis, photosynthesis, etc.). Therefore, Zn deficiency causes a number of structural and functional disorders in plants, which

greatly reduce crop yields. Along with its vital need for plants, Zn is extremely important for human health. Zn deficiency in food causes diseases that are a current problem for more than 1/3 of the world's population.

The correction of zinc deficiency in plants is traditionally done by mineral fertilization. However, in a number of soils, for example in those with an alkaline reaction, soil fertilization with Zn is not effective due to high chemical fixation of the element. In such cases, foliar feeding with Zn is a rational alternative, but it also has some disadvantages. These are the low solubility of some Zn-containing fertilizers (ZnO), the possible phytotoxicity accompanying the feeding with highly soluble zinc salts [ZnSO_4 , $\text{Zn}(\text{NO}_3)_2$], the high cost of chelated forms (Zn-EDTA) and others. These reasons motivate the search for solutions to optimize Zn-containing foliar fertilizers.

One of the modern approaches for improving foliar fertilizers is the synthesis of slow-acting nanoscale products that could have the following important characteristics: (1) moderate solubility in water, (2) high retention capacity on the leaf surface, (3) low phytotoxicity, etc. Recently, reports for synthesis of Zn-containing hydroxy nitrates have appeared, which have appropriate properties (Li et al., 2013), but they have not been formulated as foliar fertilizers and have not been tested in precise biological and agronomic experiments.

Based on the above, I believe that the studied problem in the dissertation of Nguyen Cao Nguyen is relevant, both in theoretical and applied aspects.

3. Analytical characteristics of the dissertation thesis

3.1. Volume and structure

The dissertation is written in English in a total volume of 160 pages. A summary of 2 pages in Bulgarian is attached to it. It contains 5 sections that are logically structured and well balanced in volume. The work fully meets the requirements described in the Regulations of the Agricultural University of Plovdiv for the implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), and at the same time is characterized by some features that correspond to the accepted form of dissertation in a number of Western European universities. The main difference from the traditional dissertation structure is the presentation of a part of both scientific and methodological information to the sections with results. Having in mind the interdisciplinary nature of the dissertation, which includes very different chemical, biological and agronomic research, I find this structural organization appropriate.

3.2. Literature survey

As I have already mentioned, the literature review on the topic is presented by 2 ways. Section 1 (Introduction) provides a brief general information about (●) the current issues of plant foliar fertilization, (●) maize (*Zea mays* L) nutrition with Zn as well as (●) the plant species turmeric (*Curcuma longa* L) and phyllanthus (*Phyllanthus amarus* Schum), which are important crops for both medical and food industry of Vietnam. The literature review is supplemented and in-depth in the subsections of Part 3 (Experimental Results) with specific information about

(●) the synthesis of nanosized Zn- (and Cu)-containing hydroxy nitrates, (●) the physiological effects of zinc deficiency on plants, and (●) the influence of foliar fertilization with Zn on yields and quality of maize and other crops. I consider that Nguyen Cao Nguyen has a high awareness of the studied problem, as the dissertation cites a significant number (163) of literature sources, reflecting both its historical development and recent scientific achievements.

3.3. Aim and tasks

The main goals of the study is to (1) obtain new scientific information and knowledge that allow controlled synthesis of Zn-containing foliar nanofertilizers and (2) to assess their potential to increase yields and quality of important agricultural and medicinal crops.

The aim is based on a working hypothesis, which suggests that the problem of Zn- and other essential elements deficiency in crops can be more successfully solved by development and application of nanoscaled foliar fertilizers.

The methodological approach of the research is characterized by (a) complexity, due to the wide scope of the research - from the synthesis of products to their application, (b) reliability, based on the use of modern methods of analysis in different scientific fields and (c) integrity - search for relationships between the amount of applied Zn fertilizer, the plant mineral status and the physiological and agronomic effects caused.

To achieve the goal, 4 specific tasks have been set. They are logically interrelated and reflect the main stages of the study, namely:

- First task: synthesis, physical-chemical characteristics of new Zn- (and Cu) -containing nanoscale products and monitoring of their stability over time;
- Second task: comparative testing of the effects of a synthesized Zn-containing nanoscale product and a competitive product on the physiological status and productivity of Zn-deficient maize plants;
- Third task: evaluation of the efficiency of the created Zn-containing foliar fertilizers on the productivity and quality of the important agricultural crop of corn in real field conditions;
- Fourth task: assessment of the influence of foliar fertilizers on the mineral status and quality of turmeric (*Curcuma longa* L.) and phyllanthus (*Phyllanthus amarus* Schum) crops.

3.4. Material and methods

The study was conducted during the period 2016 - 2018. They include laboratory and field experiments, the main of which were performed in the Agricultural University - Plovdiv. Some of the field experiments were carried out in Vietnam.

The experiments with maize were carried out with the hybrids P9241 (Pioneer Corteva) and Kneza 307 (Maize Research Institute - Kneza, Bulgaria). In 2019, the experiments with maize have been enlarged. The effect of the synthesized foliar fertilizers was tested on 10 hybrids with different vegetation period. Seeds from local collections in Lam Dong Province, Vietnam were used in experiments with turmeric (*Curcuma longa* L) and phyllanthus (*Phyllanthus amarus* Schum).

The experimental design of the various laboratory and field experiments is described in detail and demonstrated by 10 photographs. They are performed by standard methods with the

necessary replication of the variants. Due to the complex nature of the research, it includes a wide range of analytical methods, which are selectively motivated, according to the specifics of the tasks.

High-sensitivity instrumental methods such as X-ray diffraction analysis (XRD), scanning (SEM) and high-resolution transmission electron microscopy (HRTEM), thermal analysis (TG, DTG, DTA), chemical analysis (ICP-AES) and others have been used in chemical research. Physiological studies have been performed with modern scientific instruments for the determination of leaf gas exchange (LCA-4), chlorophyll fluorescence (MINI-PAM) and chlorophyll content (CCM-300). The obtained results were statistically processed with the IBM-SPSS software package.

3.5. *Results, discussion and conclusions*

The results of the study are presented in Section 3 (Experimental Results) through 53 tables and 63 figures. The section is differentiated into 8 identically structured subsections. My professional competence allows me to comment in more detail the results of tasks 2, 3 and 4.

The results in subsection 3.3. (Task 2) show high efficiency of the synthesized Zn-containing foliar product as a means of restoring the zinc status of maize plants. I accept that they are reliable, because were obtained by appropriate methodological approach, namely: (1) Zn-deficient maize plants, grown in controlled environment, were used as a model, (2) the newly synthesized Zn-containing product was tested in comparative study with competition product and (3) the evaluation is based on both increased tissue Zn accumulation as well as improved physiological status of the plants. Subsequently, the plants were grown in field conditions to full maturity, where the strong effect of foliar feeding with Zn on the productivity of maize plants was established.

The results in subsections 3.4., 3.5. and 3.6. (Task 3) reflect the influence of foliar application of the synthesized zinc hydroxy nitrates on the yield and quality of different maize hybrids depending on the following factors - dose, phase, frequency of treatment and composition of Zn product (alone or in combination with mineral and organic additives). One of the great merits of this largest section is the abundance of data on the dynamic changes in the mineral composition of the organs of maize plants. They allowed Nguyen Cao Nguyen to establish relationships between the dose and the frequency of the foliar application, the translocation of Zn in the maize organs and the productivity. He points out that foliar application of the synthesized Zn-containing nanofertilizers in the early stages of maize vegetation can increase grain yield by up to 25%, with the effect being highly genotype dependent. The situation with the Zn content in the grain is opposite. It is not significantly affected by foliar Zn nutrition and is not clearly correlated with the studied factors.

The information in the subsections 3.7. and 3.8. (Task 4) presents the effects of suspensions, containing Zn hydroxy nitrate on both mineral content and some useful substances in the organs of turmeric (*Curcuma longa* L) and phyllanthus (*Phyllanthus amarus* Schum). The experiments were conducted in Lam Dong Province, Vietnam. In brief, the experiments show

that the foliar application by Zn-containing products increases the content of curcumin in the productive parts of turmeric as well as the content of Zn in the organs of both plant species.

The results of the performed study are summarized in Section 4. Fourteen conclusions have been formulated, which I fully accept, as they correctly reflect the results obtained.

4. Research contributions of the dissertation

As a result of the conducted study, important results have been obtained, the main part of which have an innovative character and can be considered as scientific and scientific-applied contributions. In my opinion, the most important of them are the following:

- ***Original research contributions***

For the first time, the optimal conditions for controlled synthesis of zinc and mixed zinc-copper hydroxy nitrates were established and their physicochemical characteristics were performed. It has been found that they can be suitable sources for the production of nanosized foliar fertilizers with prolonged release of the necessary for plants micronutrients Zn and Cu.

- ***Original research contributions in applied aspect***

It has been established that the synthesized Zn-containing nanosized product, applied as foliar fertilizer, has high efficiency for restoration of Zn tissue content, photosynthetic activity and grain productivity of Zn-deficient maize plants.

It was found that preventive foliar treatment of maize with the synthesized Zn-containing product in the early phenophases of the vegetation period increases grain yield and quality, and the strength of the effect depends on the applied dose and the genotype.

- It has been shown that the zinc hydroxy nitrate suspensions have a positive effect on Zn status of the plant *Phyllanthus amarus* Schum and the curcumin content in the organs of the curcuma plant (*Curcuma longa* L).

- ***Research contributions with confirmatory character***

Studies have confirmed that Zn-containing hydroxy nitrates can be used as long-acting foliar fertilizers.

5. Critical remarks and recommendations

Some critical remarks and recommendations can be addressed to the dissertation, the main ones of which are the following:

- ***Critical remarks:***

The dissertation is written precisely, but still some omissions and errors of a technical nature can be found. One of them is annoying, that is why I point it out. On page 25 in the dissertation (and on page 6 in the abstract) it is written incorrectly that the experiments were performed with sunflower hybrids and not with maize hybrids.

- **Recommendations:**

The statistical data treatment would be more appropriate if multiple comparison tests (post hoc tests, e.g. Tukey, Duncan, etc.) were used to a greater extent, as it was done with physiological data (subsection 3.3.). This will describe the provability of the differences between the compared variants, which would deepen the discussion of the results obtained.

Newly synthesized Zn-containing foliar fertilizers have been found to increase maize yields. I believe that the results would be even more convincing if were accompanied by a structural analysis of the productivity (number of rows per cob, number of grains per row, weight per 1000 seeds, etc.).

The information about the synthesis and the technical characteristics of the synthesized Zn-containing fertilizers is clearly enough presented in the first two experimental parts of the dissertation (3.1. and 3.2.), so it may be completely eliminated in the following sections (3.3., 3.4., 3.5 and 3.6.).

The mentioned critical remarks do not reduce the qualities of the presented dissertation work. Technical shortcomings are inevitable in writing this significant volume of work, and the recommendations made are intended to improve the future scientific activity of the doctoral student.

6. Evaluation of the abstract

The presented abstract objectively reflects the structure and content of the dissertation.

7. Evaluation of the quality of scientific publications

Nguyen Cao Nguyen has presented 5 scientific publications on the topic of his dissertation. Four of them have been published, and one was recently accepted in *Italian Journal of Agronomy*. He is the first author in 2 of the publications. Three of the presented publications are in renowned journals having impact factor (*Bulgarian Chemistry Communications*, IF = 0.242; *Italian Journal of Agronomy*, IF = 1.5; *Emirates Journal of Food and Agriculture*, IF = 0.921). The information in the mentioned articles completely corresponds to the data presented in the dissertation.

According to the Regulations for application of LDASRB, the presented publications form 40.67 points, which exceeds the minimum requirements (30 points) for admission to the defence of a dissertation for the educational and scientific degree "Doctor".

Nguyen Cao Nguyen has participated in 3 international and 1 national scientific conferences. The indicated information about the publishing and presentation activity of the doctoral student and his supervisor shows that they have presented in detail the results of the research to the international and national scientific community. The high significance of the obtained results is evidenced by the fact that 7 citations of 2 of the scientific publications were quickly obtained.

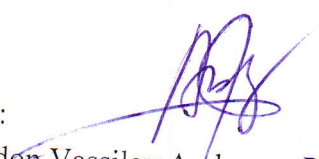
8. Conclusions

Nguyen Cao Nguyen's dissertation shows that he has in-depth knowledge and professional skills in several scientific fields related to the professional area 4.4. Earth sciences, which allow him to conduct research on issues important to society and science. Such a problem of high public importance is the zinc (Zn) deficiency in the crop production of major crops. He proves that one part of the problem - the zinc deficiency-induced yield reduction - can be overcome by developing and applying specific foliar fertilizers developed on the basis of nanosized Zn-containing hydroxy nitrates. The presented work contains significant scientific results, representing an original contribution to science and a real interest for practice. It fully meets all the requirements of the Law for the development of the academic staff in the Republic of Bulgaria and the Regulations for its application in the Agrarian University - Plovdiv.

The above gives me reason as a reviewer to evaluate positively the dissertation of Nguyen Cao Nguyen and to recommend to the esteemed members of the Scientific Jury to award him the educational and scientific degree "Doctor" in the scientific specialty "Ecology and Environmental Protection" in the professional field 4.4. Earth sciences and the Higher education area 4. Natural sciences, mathematics and informatics.

15.02.2021

Reviewer:


/Prof. Andon Vassilev Andonov, PhD/