

Review

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on dissertation work for obtaining an educational and scientific degree "doctor" in: field of higher education 6. «Agrarian sciences and veterinary medicine», professional field 6.1. "Plant production", scientific field "Agrochemistry".

Author of the dissertation work: Wissam Hassan Hourani, distance doctoral student at the Department of Agronomy at the University of Forestry, Sofia.

Dissertation topic: „New approaches for saffron (*Crocus sativus L.*) fertilization in Lebanon”

Member of the scientific jury: Prof. Dr. Ivanka Georgieva Mitova, ISSAPP "N. Pushkarov", Sofia, professional field 6.1. Plant production, scientific field "Agrochemistry", designated as a member of the scientific jury by order ZPS No. 641/05.12.2022 by the Rector of The University of Forestry.

1. Brief introduction of the candidate.

Distance doctoral student Wissam Hourani was born on 02.04.1994, in Lebanon. During the period 2012-2017, he graduated from the Faculty of Agronomy of the Lebanese University in the city of Beirut with a master's degree in agricultural engineering, majoring in "Plant production". In 2017/2018, he received a master's degree in Mediterranean organic agriculture at the Mediterranean Agronomic Institute in Bari, Italy. Since 2019, Wissam Hourani has been a distance doctoral student at the Faculty of Agronomy of the Forestry University, Sofia.

His professional practice began in 2014 in Hazmieh, on an afforestation initiative with an internship and a visit to LRI afforestation sites throughout the country. He was working on a mapping component using ArcGIS. He participated in the preparation of the final report on afforestation. In 2015, he completed an internship in Zahrani at the private agricultural company called Debbane Saikali Group. In 2016, Hourani's internship was in Bint Jbeil at the Government Agricultural Center of the Ministry of Agriculture of Lebanon, where he was engaged in training activities on plant protection for farmers. In the town of Zalka (Lebanon) in 2016/2017, he worked as an agricultural engineer in the Department of Public Health. From June 2022 until now, Mr. Hourani has been working in Saadnayel as a coordinator of farmer networks, and his activities include developing, presenting, animating up-to-date information on sustainable agriculture, as well as knowledge transfer and technical assistance to farmers.

2. Relevance of the problem.

The knowledge and use of saffron for various purposes dates back thousands of years. Historical records indicate that saffron was used as far back as 4,000 years ago as food, medicine, fragrance and dye. Lebanese saffron was well known in France as early as the beginning of the 17th century. After a long period of underappreciated treatment in Lebanon, it was only at the beginning of the 21st century that interest in this exotic plant returned, not without good reason. The interest in the culture is based on the good balance between the initial investment and the high price of the product. Saffron is the world's most expensive spice for price by weight. Saffron cultivation can serve as a potential means of promoting socio-economic development in the underdeveloped areas of Lebanon, with available workforce, as

an alternative to some illegally grown crops (poppy, cannabis). So far, this plant has been grown mostly in the Baalbeck-Hermel region, the Aakkar plain and southern Lebanon. The study provides evidence that this type of cultivation has the potential to succeed in other Lebanese regions if appropriate agricultural practices (fertilization, irrigation, selection of planting material, etc.) are adopted.

The presented work proposes a technological solution for the production of saffron under the climatic conditions of Lebanon, in which nano- and organic fertilizers are applied in combination with superabsorbent polymers (SAP), saving fertilizers and irrigation water, and the same technology can also offer the production of propagating material from daughter corms. In terms of the information presented regarding the importance of culture and the factors providing a perspective for competitive and production comparable to that of world leaders in the industry, I believe that the topic is current and very well grounded.

3. Degree of knowledge of the state of the problem and creative interpretation of the literature review.

The degree of awareness and scientific preparation of the doctoral student can be assessed as excellent. The literature review covers 20.97% of the entire work. The overview is purposeful, properly structured, providing the necessary information related to the origin, morphology, economic and business importance of the species, the requirements for growing conditions, as well as the achievements of scientific research in the field of agrotechnics and the phenotypic, cytological, biochemical and molecular characteristics of the *Crocus* genus. The cited literature is correctly and intelligently commented on. Proof of the solid scientific preparation is also the bibliographic reference in the literature review, which is based on 226 literary sources, and the fact that 60.18% of the cited authors are dated after 2011 shows Wissam Hourani's extremely focused interest in modern and innovative developments in the field of breeding of saffron.

4. Purpose, tasks, hypotheses and research methods. Correspondence of the chosen research methodology with the set goal and tasks of the dissertation work.

The aim of the presented research is to optimize saffron production under Lebanese climatic conditions by providing a strategy where nano- and organic fertilizers are applied in combination with superabsorbent polymers (SAP). In fulfillment of the set goal, the tasks that the doctoral student solves in the individual stages of the experimental work are: 1. To investigate the effect of the climatic conditions of Northern Lebanon (mainly Douma) on the yield and quality of saffron compared to those obtained in Iran (Mahallat). 2. To identify the saffron species grown in Douma (Northern Lebanon). 3. Investigation of the effects of different corm weights, fertilizers and SAP (hydrogel) application on production and quality of saffron cultivated in Lebanon.

The working hypothesis is based on the statement that the area of Douma (Lebanon) offers suitable conditions for growing saffron with good production and quality characteristics with appropriate fertilization and planting material.

In the section "Materials and methods", which occupies 10.5% of the volume of the dissertation work, depending on the stage (phase) of the experimental work, detailed

information is presented regarding the soil and climatic characteristics of the experimental sites, scheme and location of the experimental options and detailed methodological instructions on the studied indicators. The research program includes a large number of indicators - soil-agrochemical, biometric and phenological measurements and reports, biochemical indicators related to the quality of saffron. Modern and adequate methods of analysis were used, as well as a suitable mathematical apparatus to establish statistically proven connections and dependencies between the investigated indicators. The experimental setups in the three research phases are presented in great detail and are well reasoned. The scheme of the outdoor experiment in phase -3 is impressive - a three-factor, precisely laid out according to a randomized block scheme including 16 variants with 5 repetitions of each variant. The individual and combined effects of three fertilizers were investigated: Seaumic, Super Plus ZFM++ (ZFM++) and LITHOvit FORTE (LIForte); and SAP, and the effect of planting stock with two corm sizes of 4-6 g (CW1) and 6-8 g (CW2) on saffron development, yield and quality.

During the doctoral studies in the implementation of the methodological plan, the doctoral student became familiar with the methods of setting up and conducting field trials, taking and preparing soil and plant samples for analysis, biometric measurements, agrochemical and biochemical methods of analysis, mathematical and statistical analysis of the obtained data and interpretation of the scientific results.

5. Transparency and presentation of the obtained results.

The presented dissertation contains 14 tables, 28 figures and 5 appendices. Designations of figures and tables, as well as basic symbols and abbreviations, are given in separate lists. The tables and figures are made in accordance with the requirements of the Department of Agronomy of the University of Forestry for the structure and design of the dissertation works. The graphical and tabular presentation of the results in the "Results and Discussion" section is done in an appropriate way to visualize and comment on the investigated factors and indicators. The way in which the dependencies between the investigated indicators in the multifactorial and correlation analyzes are presented in a table is original and facilitates the perception of the huge amount of information.

To assess the influence of the tested factors on the studied indicators, the obtained experimental data were subjected to three-factor dispersion and correlation analyses, which enriches the work with evidentiary material.

6. Discussion of results and used literature.

The "Results and Discussion" section represents 41.9% of the dissertation and includes three consecutively connected parts. In the first part of the research, the doctoral student makes an in-depth comparative analysis of the soil-climatic conditions, development, yield and quality of saffron grown according to a uniform scheme and methodology in Northern Lebanon (mainly Douma) and Iran (Mahallat) with the aim of expanding and optimizing the area of its cultivation. The precision with which the biometric measurements and phenological readings of the indicators were made is impressive, and it is known that the phenological observations and readings are an extremely important criterion for the degree of professional training of every agrarian specialist. All recorded phenological dates were earlier in Mahalat-Iran than in Duma Lebanon. Leaf and flower cataphylls appearance as well as harvest dates were earlier by around

30 days in Mahallat-Iran. The yield of fresh and dry stigmas per 1000 flowers and also the leaf lengths were higher in Duma-Lebanon than in Mahallat-Iran.

The average number of saffron leaves, as well as the weight of dry and fresh stigmas in grams per 1000 bulbs, was higher in Mahalat-Iran. Based on the results of the soil and climate analyzes in the two experimental sites, also citing supporting literature, the doctoral student successfully explains the higher yields with the altitude and the lighter mechanical composition of the soil in Mahallat. The favorable environmental factors improve the conditions for development, resulting in higher saffron yields. The explanation for the higher values of the quality indicators measured in Mahallat sounds well-reasoned. The content of crocin and safranal in the Iranian samples was of higher quality compared to that from Duma. The samples from both points meet the minimum requirements for picrocrocin content. The lower average temperature during the May to October period in Douma-Lebanon compared to Mahalat-Iran is a likely reason for the lower quality of stigmas in Douma. In addition, the author points out that mild water stress can, depending on the time, increase the amount of crocin and picrocrocin in the stigma by slightly slowing development, as a potential defense mechanism.

In the second phase of his research work, Wissam Hourani performed a phylogenetic analysis using the nucleotide database in the Gene Bank of the National Center for Biotechnology Information. The analysis shows that saffron (*Crocus sativus*) samples share a 99-100% level of similarity with several single nucleotide polymorphisms (SNPs). A partial plastid genome sequence in the saffron samples shared 100% identity with the available plastid genome in GenBank. Overall, the phylogentic tree classified all used sequences into two main clusters. The first one contains 2_ *Crocus* sp._Y and the second cluster is divided into three sub-clusters; the first containing 1_ *Crocus sativus*_Y and AB699586.1:228-682_ *Crocus sativus*, the second containing HE801161.1_ *Crocus oreoreticus*, and the third containing 3_ *Crocus* sp.

The phase 3 study was conducted using the species *Crocus oreoreticus*. DNA testing of samples of saffron cultivated in northern Lebanon in Douma proved it to be *Crocus oreoreticus*. In the third part of the dissertation, Wissam Hourani makes a detailed analysis and interpretation of the results obtained from the influence of the tested fertilizers, SAP and the size of the planting material on a large number of indicators related to phenological observations and measurements, biometrics, yields, and quality indicators. The interpretation of the results is greatly facilitated by the extremely concise and comprehensive way in which the data from the three-factor statistical analysis and the correlation dependences between the tested indicators are presented, as well as by the correct and convincing citation of many authors related to the topic of the dissertation work. The multivariate analysis clearly shows the relationship and evidence (significant at $P \leq 0.05$) of the studied factors (corms weight, fertilization and SAP) on the studied biometric and phenological indicators, yields and quality indicators. The effect of corm weight was significant for all indicators except for picrocrocin and saffron. The individual effect of fertilizers was significant in all parameters and that of SAP was significant in all parameters except number of leaves, saffron and number of daughter corms. The joint effect of corm weight and fertilizer was significant only for phenological dates (DLA, DFCA and DFH), dry stigma yield per flower, flower number, fresh and dry stigma yields per m² and daughter corms yield. The combined effect of corm weight and SAP was significant on first leaf emergence, first flower bud, leaf length, yield of fresh mass of stigmas

per flower, number of flowers, yield of fresh and dry stigmas per m² and yield of daughter corms. The combined effect of fertilizers and SAP was significant on yields of fresh and dry stigmas per flower, number of flowers, yields of fresh and dry stigmas per m², picrocrocin, crocin, yield of daughter corms and number of daughter corms.

The correlation analysis made proves positive dependences of the date of leaf appearance on the dates of the appearance of the first flower buds and the date of the first flower harvest and is negatively correlated with the number of leaves, the length of the leaves, the number of flowers, the fresh and dry yield of stigmas, as well as with the yield and number of replacement corms. The dates of the appearance of the first flower buds and the date of the first flower harvest are positively correlated with the date of the appearance of leaves or planting and negatively correlated with all other investigated indicators. Leaf number is positively related to leaf length, as well as yield and number of replacement corms. On the other hand, leaf length was positively correlated with all the parameters tested, except the date of appearance of the first leaves and the date of the first harvest of flowers. Flower number was positively and strongly correlated with stigma fresh and dry yield and replacement corm yield and number. In addition, positive correlations were found between yield and number of replacement corms and fresh and dry stigma yield. Finally, as the number of replacement corms increases, the replacement corm yield also increases.

The specified correlation dependencies could also have a serious practical application, as an indication of interrelationships and dependencies between the studied indicators.

Thanks to his excellent statistical skills and scientific training on the subject, Wissam Hourani presents not only the results of the statistical analysis of the independent and combined influence of the tested fertilizers Seaumic, Super plus ZFM++ and LITHOvit FORTE and SAP in two sizes of planting material on the indicators: date of emergence on the leaf; date of appearance of the flower buds; leaf numbers; leaf length; the number of flowers; fresh and dry yield of stigma; the quality indicators (picrocrocin, crocin and safranal), but he also makes comments and explanations of the results that are impressive with logic and competence.

Keeping in mind the logical connection and sequence in the implementation of experimental phases 1 and 3, I would present them one after the other. I would place the part related to the genetic characterization based on the molecular study of cultivated crocus at the end of the "Results and discussion" section, not because of lack of importance, but because I think that it thematically does not fully correspond to the thesis work and the defended scientific specialty.

7. Contributions of the dissertation work.

The formulated conclusions and contributions are a logical consequence and for the most part reflect objectively the obtained results. I do not accept conclusion-VII, since the study does not include an option with classical mineral fertilization, so there is no experimental comparison between applied nano- and organic fertilizers on the one hand and conventional fertilization on the other.

To the contributions formulated by the doctoral student, I have allowed myself to add two more, in my opinion, essential contributions to his work. *I believe that the contributions formulated as a result of the research carried out have a high scientific and scientific-applied significance, and I would divide them, although rather tentatively, in the following way:*

Scientific contributions

1. It has been established, according to the results of the DNA test, that the Spanish Crocus cultivated in Northern Lebanon in Douma is *Crocus oreocreticus*.
2. Through the proven numerous dependencies between the studied factors and indicators with the help of the correlation and regression coefficients obtained in the research, in the future it will be possible to predict with great probability the changes of given indicators when the change of others is known.

Scientific and applied contributions

1. It has been proven that the soil-climatic conditions in Douma (Northern Lebanon) are suitable for growing saffron, which allows good production and stigma extraction.
2. It has also been proven that Nano-fertilizers and Seaumic applied alone, can improve all quantitative and qualitative characteristics of saffron compared to unfertilized plants.
3. It has been clearly demonstrated that the use of SAP can complement the effect of the tested fertilizers, improving overall plant development and providing higher yield and better quality.
4. The use of the tested fertilizers and SAP in combination can compensate for the smaller size of saffron corms, allowing the production of good yield and quality from 4-6 g corms.
5. It has been proven that nano-fertilizers and Seaumic can be successfully applied in organic farming conditions.

8. Evaluation of the degree of personal participation of the dissertation student in the contributions.

I do not know Wissam Hassan Hourani personally, but judging by the photographic material included in the dissertation, and also by the competent and intelligent way in which he presented the presentation of his dissertation work and answered the questions asked before the extended composition of the scientific unit of the Department of Agronomy, I think, that the dissertation and the contributions to it are his personal work.

9. Critical Notes and Questions.

I have some questions, remarks and recommendations regarding the presented dissertation work.

Question: In section IV.3.1. " Results of Factorial ANOVA". The three factors included in the study have interactive and non-interactive effects on which indicators?

Notes: The organic fertilizers used in the study (phase-3) have a rich content of nutritional macro- and predominantly microelements, organic acids, growth regulators. Applied Seaumic, Super plus ZFM++ and LITHOvit FORTE are very different not only in composition, but also in quantitative content of nutritional elements and substances. It would be impossible to "equalize" them according to the nutritional elements contained in them, as is the practice in classical agrochemical studies. Therefore, an indirect way to answer the question of how the individual nutrients in the applied fertilizers affect the nutrition of the crop is through possible soil and plant analyses, which are unfortunately missing in the study. Not only the soil, but also the plant analysis for basic macro- and microelements in certain key phases of saffron development would give a clear and very accurate idea of the effect of the application of the tested products.

In table-3, where the physico-chemical composition of the soils from the experimental sites is presented, the values of total nitrogen are shown. For the purposes of the study, it would be much more informative to determine and comment on mineral nitrogen.

I believe that the conclusions can be formed more precisely. Many more conclusions with specific content and cited value indicators can be "extracted" from this work, which is huge in terms of its results.

Recommendations: Continue the derivation of the three-factor experiment (phase-3) by "imposing" the variants, thereby accumulating more evidence about the impact of the test factors on the crop.

The presented remarks and recommendations do not discount the quality of the dissertation work. The remarks and recommendations made are recommendable and their sole purpose is to improve and enrich the quality of future scientific developments.

10. Published articles and citations.

The main results of the dissertation are presented in 3 scientific papers (1 with a journal note in the process of printing and 1 under print) in English in specialized scientific publications, referenced and indexed in the world database: 1 issue in Agronomy Research (SJR(2020) 0.369(Q2)); 1 issue of Fresenius Environmental Bulletin (SJR(2019) 0.607(Q2)); 1 issue in the Bulgarian Journal of Agricultural Science (SJR(2021) 0.25(Q3)); In one of the publications Wissam Hourani is the sole author and in one he is the first author. Hourani has been cited 3 times with one of his publications, one of the citations being in a journal with (SJR(2021) 0.31(Q3));

11. Evaluation of the publications on the dissertation work: number, nature of the editions in which they are printed. Reflections in Science - Use and Citation by Other Authors.

Hourani's publications are on the same subjects as the dissertation work and are at a high scientific level, as evidenced by their publication in globally prestigious scientific publications. The presented abstract reflects objectively the structure and content of the dissertation work.

CONCLUSION:

Based on the various research methods learned and applied by the doctoral student, the experiments correctly carried out, the generalizations and conclusions drawn, I believe that the presented dissertation meets the requirements of Law on the development of the academic staff in the Republic of Bulgaria and the Regulations of the University of Forestry for its application, which gives me reason to evaluate it **POSITIVELY**.

I take the liberty of proposing to the honorable Scientific Jury to also vote in favor and award *Wissam Hassan Hourani*, the educational and scientific degree "Doctor" in the scientific specialty "Agrochemistry".

Date: 12.12.2022
Sofia

Reviewer:
(Prof. Dr Iv. Mitova)