ABSTRACT

of dissertation for the degree of Doctor of Philosophy (PhD)
of Kudasova Darikha Yeradilovna
in the specialty 6D070100 – Biotechnology
on the topic:
"Development of technology for microencapsulation of bioactive ingredients to increase the efficiency of their usage"

General description of the work. The dissertation work is devoted to the development of technology for microencapsulation of biologically active ingredients to improve the efficiency of their use in crop growing during sowing.

Relevance of the research topic. Agriculture is one of the leading sectors of the economy of Kazakhstan. The main objectives of the implementing reforms in agriculture in Kazakhstan are to maintain the number of sown areas and their fertility, increase the production of high-quality seeds, increase productivity, improve product quality, efficient use of water resources, reduce the impact of the agro-industrial complex on the environment, ensure food safety.

It is well known that plant protection products and plant growth regulators are widely used in agricultural practice to increase crop yield, enhance plant resistance to adverse factors, and reduce crop losses. These include phytohormones and their synthetic analogues, microelements, bactericides, fungicides, acaricides and other bioactive compounds. Such chemicals are used in the treatment of plant seeds at different stages of ontogeny.

Agriculture is now seeing real impacts from the use of agrochemicals on the environment, food safety and human health, some of which are persistent organic pollutants. In recent years, their use has been limited worldwide and balanced agrotechnical measures have been proposed for environmentally and economically safe products that combine, along with improved functional characteristics, the possibility of less use of agrochemicals.

In this regard, it is relevant to reduce the use of protective agents and increase the effectiveness of plant stimulants by encapsulation, their dosed and controlled release, which will positively affect the intensification of crop growing and the rural economy as a whole.

Traditionally, growth stimulants can be applied by direct introduction; however, this method can lead to an excessive overload of the active agent, which may reduce the formulation's efficacy and exert adverse effects on plants. Furthermore, the direct delivery of plant growth stimulants can result in their inactivation or degradation depending on the environmental conditions.

The development of active materials with the property of increasing the shelf life is currently one of the urgent problems. The use of a shell made of biodegradable and non-toxic materials is a possible solution to control the stability of active agents during shelf life, as well as to improve their safety and longevity when used on various crops. The use of the microencapsulated form of the active agents may be effective during the period of storage, processing or transport, and once the microcapsules have been applied, the active agents will gradually be

released and retain their activity for a longer time compared to non-encapsulated forms. In addition, in contrast to the usual introduction of the active substance and its distribution throughout the plant body, the use of nanocontainers allows to reduce the dose of the agent used and minimize its side effects. Additionally, it becomes possible to control the release of the substance from the container.

Thus, both plant stimulants and plant protection agents as active agents can be used in the form of microcapsules, since the latter with biodegradable matrices have been found to be an effective method of controlled and sustained release of the active substance used for plant protection, growth or nutrition. The benefits of encapsulation include more efficient operation, greater user safety, and better environmental protection. The key functionality that can be provided by encapsulation is the controlled release of the bioactive agent at the right place at the right time.

Moreover, the control of molecules at this level requires a comprehensive understanding of the structure and dynamic properties of the system to justify the use of active agents in microencapsulated form. The main hypothesis of the work is that an improved understanding of the molecular structure/reactivity relationship in complex microcapsules loaded with bioactive agents can help develop a new composition of microcapsules with specially selected properties for plants, as well as adjust their optimal composition.

In this regard, the research work on the development of technology for microencapsulation of bioactive ingredients in order to increase the efficiency of their application is relevant.

The research purpose: Development of technology for microencapsulation of bioactive ingredients to improve the efficiency of their application in crop growing during sowing.

To achieve the research purpose, the following objectives were set:

- selection of plant growth stimulants based on the screening of their biological activity in plants;
- selection of a method for the microencapsulation of plant growth stimulants;
- study of the stability, activity, and prolonged-release properties of encapsulated plant growth stimulants, as well as release kinetics of the active agent;
- determination of the morphological characteristics of encapsulated plant growth stimulants;
- optimization of the composition of microcapsule shells containing plant growth stimulants using a biodegradable system based on sodium alginate and chitosan, with calcium chloride as a cross-linking agent, followed by screening tests of microencapsulated and non- encapsulated stimulants on seeds and vegetative plants;
- development of a scientifically grounded technology for the microencapsulation of plant growth stimulants.

The research object: bioactive ingredients: dimethyl ester of amino-fumaric acid - sample no. 1, Z-isomer of potassium salt of 2-amino-3-methoxycarbonyl

acrylic acid - sample No. 2, 1-methyl-3-methylaminomaleimide - sample no. 3, 1-amino-3-keto-5,5-dimethylcyclohex-1-ene - sample no. 4, trans-diaqua-bis{1-hydroxy-1,2-dimethoxycarbonylethenato} copper (II) - sample no. 5. Biopolymers: systems based on of chitosan, sodium alginate, as well as calcium chloride ($CaCl_2$) as a cross-linking agent. Seeds of cucumber, corn, wheat, barley were chosen as the main material for screening tests. The basis of the oil phase is soybean oil.

Research methods: the work utilized methods for the preparation of emulsion and emulsion-free systems, dynamic light scattering (DLS,) electrokinetic zeta-potential analysis, scanning and transmission electron microscopy (SEM/TEM), evaluation of the activity of bioactive compounds, screening assays, Fourier-transform infrared spectroscopy (FTIR), methods for determining microencapsulation efficiency, loading capacity, swelling degree, and release kinetics of the stimulant from microcapsules, as well as methods of statistical analysis.

Scientific novelty of the work:

-For the first time, a scientifically substantiated technology for the application of amino-fumaric acid derivatives in microencapsulated form as plant growth stimulants in agriculture has been developed;

-The morphological characteristics of microencapsulated forms of plant growth stimulants were determined;

-The growth-regulatory properties of novel microencapsulated stimulants derived from amino-fumaric acid have been determined, demonstrating enhanced functional performance and environmental safety;

-The practical effectiveness of the microencapsulation technology for plant growth stimulants in cultivated crops has been established;

-The microencapsulated stimulants are recommended for industrial-scale production and application as preparations for promoting plant growth and development.

Connection with the research plan.

The dissertation was carried out as part of the implementation of the grant of the Ministry of Education and Science of the Republic of Kazakhstan for 2018-2020 on the topic AP05132810 “Scientific and practical foundations of the technology of microencapsulation of bioactive substances and fundamentally new stimulants of plant development in order to intensify agricultural production” (2018-2020).

The degree of reasonableness and reliability of the dissertation:

The results obtained during the research were proven as a result of physicochemical, fluoroscopic, microscopic methods and statistical processing of experimental data. The equipment and materials used in the course of the research comply with the requirements of regulatory and technical documents.

The main provisions for the defense:

1. A scientifically substantiated selection of plant growth stimulants based on the results of screening studies of their biological activity in plants;

2. A technology for the microencapsulation of amino-fumaric acid derived stimulants using sodium alginate, chitosan, calcium chloride, in order to reduce the

environmental negative impact of plant protection products and decrease their application rates;

3. Optimized conditions for the production of micro- and nanocapsules containing plant growth and development stimulants, established on the basis of the studies of the stability, activity and prolonged-release properties of the encapsulated objects, and the release kinetics of the active agent;

4. Morphological characteristics of microencapsulated plant growth stimulants;

5. Growth-regulating properties of the microencapsulated stimulants with an optimized composition, recommended for promoting plant growth and development, in comparison with their non-encapsulated form.

6. The practical effectiveness of the application of microencapsulation technology for plant growth and development in cultivated crops;

Approval of the research work. The results of the research work were discussed at the following international conferences: "Topical issues of pharmacology: from drug development to their rational use", 28-29 May, I Republican Scientific Practical Conference of Pharmacologists with international participation (Bukhara, Uzbekistan, 2020), LXVI International Scientific Conference "Actual scientific research in the modern world", 26-27 October (Pereyaslav, Ukraine, 2020), scientific achievements. Collectied works of International Scientific Practical Conference, 25 September (Moscow, 2019).

Personal contribution of the author. Processing and analysis of the obtained data, development of the theoretical framework of the study, preparation of the research methodology, design of the technological scheme of microencapsulation and the research workflow chart, preparation of graphs, figures, and tables based on the research results, conducting experimental, pilot-controlled, and field studies, as well as the calculation of economic efficiency, were performed with the direct participation of the author.

Publications on the topic of the dissertation. On the topic of the dissertation, 4 scientific papers were published at international and republican scientific practical conferences, 1 article in a journal indexed in the Science Citation Index Expanded, ranked in the first quartile (Q1) by Impact Factor in the Web of Science database, and having a CiteScore percentile in the Scopus database, 2 articles in publications recommended by the Committee for Control in Education of the Ministry of Education and Science of the Republic of Kazakhstan, 2 articles in the journal included in the RSCI database, and 3 innovative patents were received on the research topic.

Structure and scope of the dissertation. The dissertation consists of introduction, 3 chapters, list of references and appendix, research results on 122 pages of text, 29 figures, 40 tables.