

# STATEMENT

УЧИЛИЩЕН УЧИТЕЛСКИ ЦЕНТЪР ГР. ПЛОВДИВ	
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on a dissertation for obtaining the educational and scientific degree "Doctor" in:  
Field of higher education Agricultural sciences and veterinary medicine,  
Professional field: 6.2. Plant protection, Scientific specialty: Plant protection

**Author of the dissertation:** KOSTADIN KIRILOV TRAYANOV, full-time PhD student at the Department of Entomology at the Agricultural University, Plovdiv

**Topic of the PhD thesis:** VEGETABLE PARASITE NEMATODES OF THE GENUS *GLOBODERA* SKARBILOVICH, 1959 ON POTATOES IN BULGARIA

**Statement prepared by:** Prof. MARIYANA YORDANOVA IVANOVA, PhD - University of Agribusiness and Rural Development (UARD) - Plovdiv, Professional field: 6.2. Plant protection, Scientific specialty: Plant protection (Entomology), appointed as a member of the Scientific jury by order № RD 16 - 211 / 05.03.2021 by the Rector of AU.

## 1. Relevance of the problem.

The PhD thesis developed by the PhD student KOSTADIN KIRILOV TRAYANOV is dedicated to a significant topic. Potatoes are one of the most common agricultural crops, most widely grown among tuberous plants in the world and in our country. They are a traditional food crop called second bread. They are also valuable as a technical raw material in the industry. In addition, the areas where potatoes are grown are not very suitable for other crops. However, potato cyst nematodes (PCNs) are one of the limiting factors for obtaining quality potato production. Therefore, the search for solutions for the control of PCN through the application of alternative means and approaches with minimal application of pesticides, in order to obtain clean production and reduce the risk of environmental pollution, is a topical issue. In this context, Kostadin Trayanov's dissertation is relevant and can be assessed as a contribution to agricultural science.

## 2. Purpose, tasks, hypotheses and research methods.

The aim of the PhD thesis is to establish the species composition and distribution of cyst-forming nematodes of the genus *Globodera* in the potato plantations of the main productive areas in Bulgaria, as well as to study the possibilities for alternative means to control these parasites.

The purpose of the dissertation is clearly and precisely formulated, and the following tasks are defined for the implementation:

- Study of the spread of cyst-forming nematodes of the genus *Globodera* on potatoes;
- Morphological and molecular characteristics of the identified species;
- Establishment of the reaction (resistance / sensitivity) of potato varieties / lines to *Globodera pallida* and *Globodera rostochiensis*;
- Selection of effective biological control agents with PCN by screening of plant extracts and rhizobacteria.

The methodology of the conducted experiments is correctly set, as methods have been selected that guarantee the receipt of reliable scientific results. Standard methods for morphological and molecular identification of *G. rostochiensis* and *G. pallida*, as well as for establishing the reaction of potato varieties and lines to *Globodera* spp. In the identification of *G. rostochiensis* and *G. pallida* for the first time in our country the method of polymerase chain reaction (PCR) was applied, using gene-specific primers, which can definitely be considered a contribution to agricultural science. Statistical data processing was performed, which allows for better interpretation of the obtained results.

### 3. Visualization and presentation of the obtained results.

The PhD thesis presented by Kostadin Trayanov has a total volume of 128 pages, which structurally contain the following sections - introduction, literature review, purpose and tasks, materials and methods, results and discussion, conclusions, list of publications in relation with the dissertation, and literature. The main text contains 24 tables and 16 figures. The experimental and laboratory work was performed in the Department of Entomology and the Department of Genetics and Selection of the Agricultural University - Plovdiv, the Institute of Vegetable Crops "Maritsa" - Plovdiv, the Institute of Plant Genetic Resources "K. Malkov" - Sadovo, the Central Laboratory for Plant Quarantine - Sofia, the Institute of Soil Science, Agrotechnology and Plant Protection "Nikola Pushkarov" - Sofia, during the period of 2016 - 2019. The studies for the establishment of potato cyst nematodes in the agroecosystem of potatoes cover four main potato production areas in our country - Pazardzhik, Sofia, Smolyan and Burgas, which include 15 production areas. 15 conclusions have been formulated.

### 4. Discussion of the results and used literature.

The "Results and Discussion" section is with a volume of 51 pages. In 2017 - 2019, areas with potatoes in Sofia, Pazardzhik, Smolyan and Burgas regions were surveyed, where the spread of potato cyst nematodes of the genus *Globodera* was found, and the highest population density is in the area of the village of Ravnogor. Morphologically established and molecularly proven are two species of nematodes of the genus *Globodera* - *Globodera rostochiensis* and *Globodera pallida*, with the predominant species being *G. pallida*, which occurs in 86.6% of the studied potato production areas.

In "in vitro" laboratory experiments, screening of bacterial isolates and plant extracts was performed for their efficacy against *Globodera* spp. three of them - *Juglans regia*, *Ruta graveolens* and *Plantago major*. Twelve isolates of rhizobacteria were found to have larvicidal action against *G. rostochiensis* and *G. pallida*, and the isolate of *Serratia plymuthica* showed the greatest efficacy. Of importance for the practice is the established conclusion that the application of *S. plymuthica* should take place during the period of active vegetation of the plants, as the moment of application takes into account the development of PCN. The best results can be expected if *S. plymuthica* is applied no later than the 3<sup>rd</sup> larval age.

The literature review addresses issues related to the history and systematic classification of potato cyst nematodes, species composition and geographical distribution, identification of species of the genus *Globodera* and pathotype composition, as well as methods for control of PCN – including the use of resistant and tolerant varieties of potatoes, various means of biological control (plant extracts, nematophagous bacteria, etc.). The bibliographic reference contains 248 literary sources, of which 5 in Cyrillic and 243 in Latin.

### 5. Contributions to the dissertation.

I accept the contributions as actually proven in the course of the research and in the publications related to the PhD thesis. I consider them to be the personal work of the PhD student. Among them, I would like to highlight the following more important contributions of an original nature:

- A genetic bank of the two species of PCNs distributed in potato-producing regions of the country has been established: *Globodera rostochiensis* and *Globodera pallida*, and plant extracts and isolates of rhizobacteria have been studied as agents for biological control of PCNs of the *Globodera* genus.
- For the first time in the country, the optimal concentrations and temperatures have been established, at which the plant extracts *Juglans regia*, *Ruta graveolens* and *Plantago*

- major* show the highest nematicidal activity against *G. pallida*.
- The metabolic profiles of *J. regia*, *R. graveolens* and *P. major* were determined by gas chromatography - mass spectrometry (GC-MS).
  - For the first time, the optimal parameters (concentration and temperature) at which the rhizobacterium *Serratia plymuthica* shows the highest efficiency against *G. pallida* were established.
  - The period of nematicidal and preventive action of *S. plymuthica* against the invasion of larvae 2<sup>nd</sup> age of *G. pallida* on the roots of potato plants was established and the effect of *S. plymuthica* rhizobacter on the development and reproduction of *G. pallida* in the roots of plants.
  - The application of *S. plymuthica*, for the control of PCN on vegetable crops - potatoes, to be done during the period of active vegetation, in accordance with the development of larvae 2<sup>nd</sup> age - not later than 3<sup>rd</sup> larval age of the parasite.

#### 6. Critical remarks and questions - none.

#### 7. Published articles and citations.

Kostadin Kirilov Trayanov has published 4 scientific publications related to the topic of the PhD thesis. They summarize and publish the results of the research. The presented abstract (with a volume of 43 pages) objectively reflects the structure and content of the PhD thesis.

#### CONCLUSION:

Based on the learned and applied by the PhD student **Kostadin Kirilov Trayanov** different research methods, correctly performed experiments, summaries and conclusions, I believe that the presented PhD thesis meets the requirements of the Law for development of the academic staff in the Republic of Bulgaria and the Regulations of the Agricultural University for its application, which gives me grounds to evaluate it **POSITIVELY**. The author demonstrates with this the acquired new knowledge, as well as the ability to perform independent scientific work, to interpret the results and to formulate conclusions, which fulfills the main educational and scientific goal of the PhD studies. I allow myself to suggest to the esteemed Scientific Jury also to vote positively and to award **Kostadin Kirilov Trayanov** the educational and scientific degree "**Doctor**" in the scientific specialty: Plant Protection.

08.04.2021  
Plovdiv

PREPARED BY:

  
(Prof. M. Ivanova, PhD)