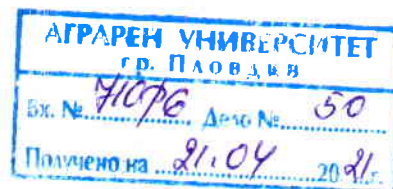


## REVIEW



on a dissertation for awarding the educational and scientific degree "Doctor" in: field of higher education **6. Agricultural sciences and veterinary medicine**, professional field **6.2 Plant protection**, scientific specialty **Plant protection**

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

**Topic of the dissertation:** Plant parasitic nematodes of the genus *Globodera* Skarbilovich, 1959 on potatoes in Bulgaria

**Reviewer:** Assoc. Prof. Vinelina Panayotova Yankova-Mihaylova PhD, Maritsa Vegetable Corps Research Institute - Plovdiv, field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.2 Plant protection, scientific specialty Plant protection

appointed as a member of the scientific jury by order № RD 16-211/05.03.2021 of the Rector of the Agricultural University - Plovdiv

### 1. Brief introduction of the candidate.

Doctoral student Kostadin Kirilov Trayanov was born on August 22, 1990 in the town of Petrich. In 2009 he has graduated "Peyo Kracholov Yavorov" secondary school – Petrich and in 2013 he has graduated the Agricultural University - Plovdiv with a bachelor's degree in Plant Protection. He has obtained a master's degree in 2015 at the Agricultural University of Plovdiv as an Agronomist - Plant medicine (international course in Plant Protection). In 2015 he held the position of junior expert in the Plant Protection Department at the Regional Board of Food Safety - Plovdiv, Bulgarian Food Safety Agency (BFSA). From 2016 to 2020 he was a full-time doctoral student in the Department of Entomology at the Agricultural University - Plovdiv. Since 2016 he has been working as an agronomist in plant protection (sales consultant) in an agro-pharmacy at the company "PABI-FRUT Petko Ilinov" in the village of Kalekovets. From 2018 until now he has been working as an agronomist in plant protection at "Rubus SL" Ltd. Fluent in English, has a basic level of Russian.

### 2. Actuality of the problem.

The problem studied in the dissertation of Kostadin Trayanov is related to establishing the species composition and distribution of cyst nematodes of the genus *Globodera* in the potato of the main productive areas of Bulgaria, as well as to study the possibilities for alternative means to control these parasites. Potatoes are a valuable food crop. Their nutritional value is determined by their rich starch content, as well as the presence of many other valuable substances, such as proteins, amino acids, sugars, vitamins etc. Potatoes dominate the world as the fourth largest crop in yield (after wheat, rice and corn). The problem with cyst

nematodes on potato has been known to science and growers for many years, but still remains unresolved. Potato cyst nematodes are endoparasites on potato roots that are difficult to be controlled because they form cysts containing eggs and larvae that remain viable in the soil for more than 15 years. The main method of controlling nematodes is still chemical, but it is not always effective enough, and at the same time it carries risks of residues and environmental pollution.

I believe that the problem researched in the dissertation is interesting and relevant in finding of alternative methods for control of cyst nematodes on potato.

### **3. Purpose, tasks, hypotheses and methods of study.**

The doctoral student Kostadin Trayanov has clearly formulated the purpose and tasks of the dissertation. They are well defined and directed on the specific experimental activity.

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

To achieve this purpose, four tasks have been set. They reflect the main stages of the study:

1. Study of the distribution of cyst nematodes (potato cyst nematodes) of the genus *Globodera* (PCN).
  2. Morphological and molecular characteristics of *Globodera* spp.
  3. Establishment of the response (resistance/susceptibility) of potato varieties/lines to *Globodera* spp.
  4. Selection of effective agents for biological control with PCN
- a) Screening of plant extracts as possible biological control agents for *Globodera*
- Identification of the factors determining the efficacy of the selected plant extracts (*Juglans regia*, *Ruta graveolens* and *Plantago major*) against eggs and second stage larvae (J2) of *Globodera rostochiensis* and *Globodera pallida* - *in vitro*;
  - Establishment of their metabolic profiles (available substances) of *J. regia*, *R. graveolens* and *P. major*, by gas chromatography - mass spectrometry (GC-MS).
- б) Screening of rhizobacteria as possible control agents with *Globodera* spp.
- Study of the factors determining the efficacy of the selectin rhizobacterium *S. plymuthica* isolate 72 against eggs and J2 of *G. pallida* - *in vitro*;
  - *In vivo*, establishing the efficacy of *S. plymuthica* isolate 72 against the invasion, development and reproduction of *G. pallida* on potato.

The aim of the study is based on a **hypothesis** that suggests that alternative control methods can be explored, such as the establishment of resistant/tolerant potato lines and the use of microbial products and plant extracts against cyst nematodes.

## **Material and methods**

The part Materials and methods includes a clearly defined and consistently described methods of the conducted experiments, which allows the set tasks to be performed correctly and in detail.

The experimental and laboratory work was carried out in the Department of Entomology and the Department of Genetics and Selection at the Agricultural University - Plovdiv, "Maritsa" Vegetable Crops Research Institute - Plovdiv, Institute of Plant Genetic Resources „K. Malkov"- Sadovo, Central Laboratory for Plant Quarantine - Sofia, Institute of Soil Science, Agrotechnology and Plant Protection „Nikola Poushkarov", during the period 2017-2019.

The studies for the establishment of potato cyst nematodes in the agroecosis of potatoes cover four main potato production areas in our country - Pazardzhik, Sofia, Smolyan and Burgas, which include 15 production areas. In identifying species of the genus *Globodera* cysts and second stage juveniles were examined. Molecular characterization of populations was performed by the polymerase chain reaction method. Thirteen *S. tuberosum* lines - E 1809, E 606, E 1789, E 1096, E 1811, E 1210, E 68, E 292, E 330, D 112, D 348, D 497, D 344- were screened to establish their reaction to PCN and seven commercial varieties - Cronos, Cekin, Gandawa, Gawin, Owacij, Ivetta and Desiree (control). Standard nematological methods were used to determine the efficacy of bacterial isolates and plant extracts against larvae and cysts of *Globodera* spp. Test bacteria: *Pseudomonas chlororaphis* isolate 109A, *Pseudomonas chlororaphis* isolates Po4, *Bacillus amyloliquefaciens* isolate 162, *Bacillus pumilus* isolate 109, *Bacillus amyloliquefaciens* isolate 185, *Bacillus amyloliquefaciens* isolate 186, *Bacillus megaterium* isolate 95, *Bacillus megaterium* isolate 174, *Bacillus subtilis* isolate 132, *Bacillus subtilis* isolate 164, *Serratia plymuthica* isolate 201 and *Serratia plymuthica* isolate 72. Test plant extracts from fully developed plants of: *Tanacetum vulgare*, *Artemisia vulgaris*, *Allium ursinum*, *Tagetes patula*, *Juglans regia*, *Salvia officinalis*, *Ruta majorolens*, *Plantago major* representative of the Bulgarian flora were used. *In vitro* and *in vivo* laboratory experiments were performed. The specific experiments are described in detail in the dissertation.

Software products such as Microsoft Excel and SPSS 13.0 for Windows were used for statistical data processing.

## **4. Visualization and presentation of the results obtained.**

The dissertation is well structured and clearly presented. The dissertation is written on 128 pages and contains 24 tables and 16 figures. The list of cited literature contains 248 sources, of which 5 are in Cyrillic and 243 in Latin. The dissertation includes 8 main parts: Introduction - 2 pages; Literary review - 24 pages; Purpose and tasks - 2 pages; Materials and methods -16 pages; Results and discussion - 52 pages; Conclusions - 3 pages; List of publications on the dissertation - 1 page; Reference - 23 pages. The dissertation is written concisely, in a good scientific style, well designed and containing all the necessary sections.

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

The Literary Review presented in the dissertation is up-to-date and it is related to the topic of the thesis. The doctoral student presented the state of the problem and the reasoned need for the current study, which shows his good information and is evidence of the acquired theoretical knowledge in the field of nematology on the research topic.

In the part Results and discussion, which is 41% of the total volume of the dissertation, the experimental data are summarized and interpreted and they are formed in four subsections, corresponding to the tasks.

The results presented in subsection 5.1. show the distribution of the potato cyst nematodes in the agroecosystem of potato in our country. Observations of potato growing areas in the districts - Sofia, Pazardzhik, Smolyan and Burgas, in the period 2017-2019 confirm the total distribution of potato cyst nematodes of the genus *Globodera*. The highest population density of the studied areas was found in the village of Ravnogor (610 cysts/100 g soil), and the lowest in the village of Vezenkovo (3.5 cysts/100 g soil). Morphologically established and molecularly proven are two species of nematodes of the genus *Globodera* - *Globodera rostochiensis* (golden potato cyst nematode) and *Globodera pallida* (pale potato cyst nematode). The predominant species is *G. pallida*. The species is found in 86.6% in the studied potato production areas.

In subsection 5.2. the results of the screening tests of potato varieties and lines for establishing the relative resistance/susceptibility to *Globodera* spp. are discussed. Four varieties (Cronos, Cekin, Gawin, Ovacij) and seven lines (E 1789, E 606, E 1096, E 1809, D 344, D497, D 348) *Solanum tuberosum* are resistant to *G. rostochiensis* (resistance index 6-8). Two varieties (Gandawa and Ivetta) show strong resistance (resistance index 9) to *G. rostochiensis*. Two of the tested varieties Cronos and Ivetta, as well as line E 1096, E 1809 and E 606 have an established tolerance to *G. pallida* (resistance index 4-5).

The results in subsection 5.3. give an information concerning *in vitro* laboratory experiments. All 8 tested plant extracts showed nematicidal action against second stage juveniles (J2) of *G. rostochiensis* and *G. pallida*, with three of them having the highest efficacy - *Juglans regia* 80.00 and 78.00%, *Ruta graveolens* 69.00 and 67.00% and *Plantago major* 47.00 and 42.00%. *In vitro* experiments with plant extracts at different temperatures show that it does not have a significant effect on the inhibitory efficacy on cysts and their toxic efficacy on J2 of *G. pallida*. GC-MS analysis of plant extracts determined the composition of some of the compounds (mainly non-polar) in them. Twelve isolates of rhizobacteria showed larvicidal action against *G. rostochiensis* and *G. pallida*. *Serratia plymuthica* isolate 72 shows the highest efficacy. Highest degree of inhibition of hatching of J2 in *G. pallida* (94.50 - 95.75 and 100.00%), bacterial suspension (BS) and cell-free filtrate (CFF) of *S. plymuthica* isolate 72 caused in concentration, respectively  $10^8$  cells/ml and 10.0% at 3 and 6 weeks of exposure. At the same doses, *S. plymuthica* showed the best larvicidal efficacy at 72 hours of exposure - 86.75% and 86.50%, respectively. In the range of 19-24°C, the BS and CFF efficacy of *S. plymuthica* isolate 72 against J2 of *G. pallida*, at

concentrations of  $10^8$  cells/ml and 5.0% at exposures of 72 hours, respectively, caused the highest J2 mortality and best expressed inhibitory efficacy on the hatching of juveniles from cysts at exposure 3 and 6 weeks. At 14°C, the CFF efficacy of *S. plymuthica* on *G. pallida* is better expressed.

In the subsection 5.4. are given the results from the *in vivo* laboratory experiments. The nematicidal action of *S. plymuthica* isolate 72 against the invasion of J2 on the roots of potato plants lasts until the 14th day, and the nematostatic - until the 21st day. The bacterium inhibits the development of female specimens in the roots and reduces the fertility of nematodes. Invasive J2 and third-stage juveniles (J3) are most susceptible to *S. plymuthica* isolate 72. 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 PCNs. The best results can be expected if *S. plymuthica* is applied no later than the J3. Once application of *S. plymuthica* ( $\times 10^8$  cells/ml) - BS at a dose of 20 ml/plant/CFF (5% solution with 20 ml/plant), protects the roots from the invasion of PCNs during the first 24 days and increases the yield by about 1.9 times compared to untreated plants. The use of *S. plymuthica* BS should be limited to temperatures above 14°C. At lower temperatures as an alternative can be applying cell-free filtrate of the bacterium *S. plymuthica*.

At the end of the dissertation the doctoral student formulates 15 conclusions, which summarize the results of the experimental work. The dissertation contains scientific results that represent a contribution to science. The presented dissertation shows that the candidate has extensive theoretical knowledge. He has mastered various nematological methods, which allows him to conduct independent research.

## **6. Contributions to the dissertation**

On the basis of the investigation performed, results obtained their analyzing and according to the conclusions made in the dissertation, eleven contributions divided in two groups are made and I accept them completely.

### **➤ Original contributions**

1. The distribution of the potato cyst nematodes (PCN) of the genus *Globodera* in the Sofia, Pazardzhik, Smolyan and Burgas potato growing regions of Bulgaria has been studied. For the period 2017-2019, for each of the identified 15 areas infected with these parasites, the following have been identified: *Globodera* spp. and their population density.

2. A genetic bank of the two species of PCNs distributed in the potato growing region of the country has been created: *Globodera rostochiensis* and *Globodera pallida*.

3. In the identification of PCNs for the first time in our country the method of polymerase chain reaction (PCR) was applied, using gene-specific primers.

4. Plant extracts and isolates of rhizobacteria as biological control agents against *Globodera* have been studied.

5. For the first time in the country, the optimal concentrations and temperatures have been established, in which the plant extracts *Juglans regia*,

*Ruta graveolens* and *Plantago major* show the highest nematicidal activity against *G. pallida*.

6. The metabolic profiles of *J. regia*, *R. graveolens* and *P. major* were determined by gas chromatography - mass spectrometry (GC-MS).

7. For the first time were established the optimal parameters (concentration and temperature) in which the rhizobacterium *Serratia plymuthica* showed the highest efficacy against *G. pallida*.

8. The period of nematicidal and preventive action of *S. plymuthica* against the invasion of second-stage juveniles (J2) of *G. pallida* on the roots of potato plants was established.

9. The effect of the rhizobacterium *S. plymuthica* on the development and reproduction of *G. pallida* in plant roots was determined.

10. 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 J2 - not later than the third-stage juveniles (J3) of the parasite.

➤ **Contributions with a confirmatory nature**

1. The studies on the morphological and morphometric characteristics of the identified two species of PCN of the genus *Globodera* are of a confirmatory nature.

**7. Critical remarks and questions.**

Some critical remarks, questions and recommendations can be addressed to the dissertation.

**Critical notes:**

The dissertation is written precisely, but still omissions and errors of a technical nature can be found. In the Literary Review section, as well as further in the text, when writing the full name of the species (nematodes, plants) in Latin, it is necessary to indicate the author, after which an abbreviation may be used. In citation it is better to follow the spelling of the authors in the accepted way. There are some mistakes on pages 12 and 18. It is not necessary to cite materials that are in print or unpublished. Regarding the text, there are some ungrammatical sentences in the dissertation. I suppose that this is due to the translation of the cited literature. On pages 13-14, in item 2.3.2 Pathotype composition, a technical error was probably made, Kort et al. (1997), and the year is actually 1977. It is given that Nijboer and Parlevliet (1990) classify the pathotypes of *G. rostochiensis* into three groups, and only two are described. In the section Results and discussion, the last paragraph of item 5.1.1 regarding the distribution of the two species an error was made - *G. Pallida* was found in eight, not in nine of the potato growing areas. A technical error was made in the text in many places in the spelling of the species *Juglans regia*. The discussion could be expanded and bound to the research conducted. The results of the screening test for susceptibility of different potato lines to *G. rostochiensis* (Koprivshitsa population) and *G. pallida* (Ravnogor population) could be distinguished as scientific and applied contributions in view of the fact that they are useful information for future

breeding programs. In the References section, the indicated sources must be written in the same way in accordance with the accepted requirements, and the year of publication must be indicated.

**Questions:**

The creation of a genetic bank of the two PCNs species: *Globodera rostochiensis* and *Globodera pallida*, distributed in the potato-growing regions of the country is indicated as a contribution. What it includes and where it is stored?

**Recommendations:**

It would be better the future scientific work of the doctoral student to be with applied character in greater degree. It is well known that the fundamental researches are important, but they are the basis for solving problems of the farmers.

These critical remarks do not reduce the value of the presented dissertation. The recommendations are aimed at improving the future research work of the doctoral student.

**8. Published articles and citations.**

The presented four articles relevant to the dissertation are published in scientific journals, referenced and indexed in world-famous databases (*Scopus*, *Web of Science*). According to the minimum scientometric requirements specified in the Regulations for the Application of the Act on Development of the Academic Staff in the Republic of Bulgaria, these publications fully cover the required number of points (34.5). The doctoral student is the first author in all publications.

The presented author's summary of the thesis objectively reflects the structure and content of the dissertation. It presents in a synthesized form the researched and discussed problems and includes the conclusions and contributions made.

**CONCLUSION:**

Based on the learned and applied by the doctoral student, different research methods, correctly performed experiments, summaries and conclusions, I believe that the presented dissertation covers the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria and the Rules of the Agricultural University for its Application, which gives me reason to evaluate it **POSITIVE**.

I allow myself to propose to the honourable Scientific Jury also to vote positively and to award **KOSTADIN KIRILOV TRAYANOV** full-time doctoral student, at the Department of Entomology at the Agricultural University, Plovdiv, the educational and scientific degree "**Doctor**" in the scientific specialty **Plant Protection**.

**Date:** 16.04.2021  
**Plovdiv**

**REVIEWER:**   
(Assoc. Prof. Vinelina Yankova PhD)