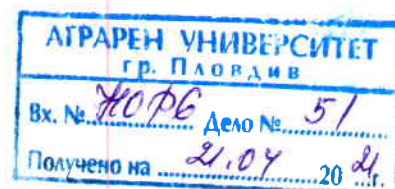


REVIEW



on a dissertation for obtaining the educational and scientific degree " Philosophy 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*

Review prepared by: Prof. RADOSLAV ANDREEV ANDREEV, PhD – Agricultural University of - 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. Brief information about the candidate.

Kostadin Kirilov Trayanov was born on 22.08.1990 in Petrich, where in 2009 he completed his secondary education at a humanities high school „Peyo Yavorov” in a profiled class Biology and Chemistry. During the period 2009 - 2013 he was a full-time student in the bachelor's course in Plant Protection at the Agricultural University - Plovdiv. After graduating as an Agronomist in plant protection, in 2014 he continued his studies in the master's course "Plant medicine" at AU-Plovdiv. and graduated with a master's degree in 2015. In 2016 he was accepted as a full-time doctoral student in the Department of Entomology at Agricultural university - Plovdiv, with scientific supervisor Prof. Hari Samaliev.

In 2015 he held the position of Junior Expert in the Plant Protection Department at the Regional Directorate of the Bulgarian Food and Safety Agency in Plovdiv. He has worked as an agronomist in plant protection at the companies PABI-FRUT Petko Ilinov ET and Rubus SL OOD to support himself during his doctoral studies.

2. Relevance of the problem.

The theme of the thesis is highly relevant as it considered economically dangerous pests on potatoes, which are the main agricultural crop in many parts of the country. Potato cyst nematodes (PCNs) are endoparasites on potato roots and are difficult to observe due to their specific subterranean lifestyle, and for control, because they form cysts containing eggs and larvae that remain viable in the soil for more than 15 years. In the thesis, besides valuable studies to establish the species composition, distribution and some biological features of these dangerous pests a successful attempt has been made to find an alternative to the chemical control method, in accordance with current trends in plant protection to reduce the harmful effects of mass application of pesticides.

3. Purpose, tasks, hypotheses and research methods.

The purpose and objectives of the thesis are focused mainly in two directions:

- to establish the species composition and distribution of cyst-forming nematodes of the genus *Globodera* in potato plantations of the main productive areas of the territory of Bulgaria, by making morphological and molecular characteristics of the identified species;
- to explore the possibilities for alternative means of controlling these parasites establishing the reaction (resistance / sensitivity) of potato varieties / lines to them, as well as by investigating possible biological control agents by screening plant extracts and rhizobacteria.

Adequate classical and modern research methods have been used to perform these tasks.

4. Visualization and presentation of the obtained results.

Kostadin Trayanov presents a 128-page PhD thesis, 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 results obtained are presented correctly and are illustrated in an appropriate way. The main text contains 24 tables and 16 figures, some of which are original color photographs. 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.

5. Discussion of the results and used literature.

The literature review is comprehensive and is made according to the set goals and objectives. It includes information on the species composition and distribution of the PCNs, as well as biological methods and controls, such as the use of resistant varieties, plant extracts and nematophage bacteria.

To establish the distribution of the PCNs from genus *Globodera* are investigated potato plantations in key regions of Bulgaria in Sofia, Pazardzhik, Smolyan and Burgas districts. It was found that the two species (*Globodera rostochiensis* and *Globodera pallida*) are distributed widely, and their population density. It was proved that the predominant species is *G. pallida*, which occurs in 86.6% of the studied areas.

Species have been identified both morphologically and molecularly.

The response of 13 potato varieties and lines to the attack by PCNs was studied. Four varieties (Cronos, Cekin, Gawin, Ovacij) and seven lines (E 1789, E 606, E 1096, E 1809, D 344, D497, D 348) were found to be resistant to *G. rostochiensis* (resistance index 6-8), and two varieties (Gandawa and Ivetta) show strong

resistance (resistance index 9). Two of the tested varieties (Cronoss and Ivetta), as well as three lines (E 1096, E 1809 and E 606) showed tolerance to *G. pallida* (resistance index 4-5).

The nematicidal action of 8 plant extracts against L₂ of both species of PCNs was studied. They have all been found to have a nematicidal effect, with the highest efficiency are *Juglans regia*, *Ruta graveolens* and *Plantago major*.

By gas chromatographic analysis of these extracts is defined composition of the compounds in them.

“In vitro” experiments have been performed to determine the effect of different temperatures on the inhibitory and the toxic effect of plant extracts on L₂ of *G. pallida*.

Successful studies have been performed to detect a potential nematicidal effect against *G. rostochiensis* and *G. pallida* of twelve isolates of rhizobacteria. They have all been found to have larvicidal action. *Serratia plymuthica*, isolate 72 showed the highest efficacy.

A number of experiments were performed to refine the parameters (concentration, duration of exposure, temperature and type of isolate – bacterial suspension or cell-free filtrate) in which *S. plymuthica* exhibits its maximum nematicidal action expressed both by direct larvicidal efficacy and by inhibition on larvae hatching of the cysts.

It was found that the bacterium suppresses the development of the females in the roots and reduce the fertility of nematodes, and invasive larvae 2nd instar and larvae 3rd instar are the most susceptible.

Successful observations have been made to determine the duration of nematicidal and nematostatic action of *S. plymuthica*, isolate 72.

The statistical treatment is adequate and kept to the minimum necessary.

6. Contributions to the dissertation.

Most of the contributions to the dissertation are scientific-applied, as this is the nature of the development.

Scientific contributions

- The only scientific contribution can be made to the determination of the metabolic profiles of plant extracts from *Juglans regia*, *Ruta graveolens* and *Plantago major* analyzed by gas chromatography - mass spectrometry (GCh-MS).

Scientific-applied contributions

- The distribution of the potato cyst-forming nematodes (PCNs) of the genus *Globodera* in the Sofia, Pazardzhik, Smolyan and Burgas potato-producing

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.

- A genetic bank of the two species of PCNs distributed in the potato-producing region of the country has been created: *Globodera rostochiensis* and *Globodera pallida*.
- 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.
- Plant extracts and isolates of rhizobacteria as biological control agents against *Globodera* have been established.
- 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*.
- 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*.
- 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.
- The effect of the rhizobacterium *S. plymuthica* on the development and reproduction of *G. pallida* in plant roots was determined.
- Important recommendations have been made for potato growers, namely that the application of *S. plymuthica*, for the control of potato cyst nematodes must take place during the period of active vegetation, in accordance with the development of J₂ - not later than the third-stage juveniles (J₃) of the parasite.

7. Critical remarks and questions.

I accept that Kostadin Trayanov has complied with the recommendations and remarks made during the discussion in the Department. I have the following questions, critical remarks and recommendations:

- Why not surveyed Plovdiv region, which also growing potatoes - the villages of Sitovo, Lilkovo and other regions have long been known for the production of quality potatoes?
- I believe that a more detailed discussion could have been held in the sections:
5.4.1. (4.4.1. in the Autoreferat) „Influence of bacterial suspension (BS)/cell-free filtrate (CFF) of *S. plymuthica* isolate 72 at different temperatures on the

invasion of *G. pallida* J2s in plant roots" and 5.4.2. (4.4.2. in the Autoreferat) "Influence of bacterial suspension (BS)/cell-free filtrate (CFF) of *S. plymuthica* isolate 72 on the development of *G. pallida* in plant roots". Are there similar studies in the world with which these results can be compared?

- Photos of an experiment in a thermostat (Fig. 13, p. 95) should be placed in the "Material and methods" section.

These critical remarks in no way diminish the merits of the dissertation.

8. List of publications on the dissertation and citations.

Kostadin Kirilov Trayanov has published 4 scientific publications related to the topic of the PhD thesis:

1. **Kostadin Trayanov**, Harry Samaliev, Silvia Valcheva, Strahil Berkov, Milena Nikolova, (2018). The effect of plant extracts on egg hatching and second-stage juvenile motility of potato cyst nematode *Globodera pallida*. *Journal of Mountain Agriculture on the Balkans*, 2018, 21 (2), 257-273
2. **Kostadin Trayanov**, Dima Markova, Ivanka Tringovska, Harry Samaliev, (2019). Influence of the Temperature and the Time of Exposure on the Inhibitory Effect of *Serratia plymuthica* on the Potato Cyst Nematode *Globodera pallida*. *Journal of Mountain Agriculture on the Balkans*, 2019, 22 (1), 348-357
3. **Trayanov, K.**, Samaliev, H., Kostova, M., Bojinov, B. and Besheva, A. (2020). Morphological and molecular identification of potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida* in Bulgaria. *Bulgarian Journal of Agricultural Science*, 26 (No 2) 2020, 416-422
4. **Kostadin Trayanov** and Milena Kostova, (2020). ISSR molecular markers for the study of the genetic diversity in bulgarian populations of PCN from genus *Globodera*. *Agricultural Sciences*, Volume 12 Issue 27 2020, 25-28

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 "**Philosophy Doctor**" in the scientific specialty: Plant Protection.

15.04.2021.

Plovdiv

REVIEWER:



(prof. Radoslav Andreev Andreev)