# OPINION

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on a dissertation for obtaining 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"

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## Topic of the dissertation: "PLANT PARASITIC NEMATODES OF THE GENUS GLOBODERA SKARBILOVICH, 1959 ON POTATOES IN BULGARIA"

**Reviewer:** Prof. Dr. Vili Borisova Harizanova, Agricultural University-Plovdiv, field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.2. Plant protection, scientific specialty "Entomology", 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. Relevance of the problem

Cyst-forming nematodes are among the pests of agricultural crops, which are particularly difficult to identify and control. Potatoes are an important crop in many European countries, as well as in many parts of the country. I consider the search for alternative means and methods to control these pests on potatoes to be an extremely topical issue.

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

The goal, as well as the tasks for its achievement, are well formulated. The working hypothesis is: after establishing the species composition and distribution of cyst-forming nematodes of the genus Globodera in the main production areas in our country through morphological and molecular characteristics, to study the possibilities for alternative means of control through resistant varieties / lines, plant extracts and rhizobacteria.

Appropriate research methods (classical and molecular) have been used to perform these tasks. The four-year experiments were performed with a sufficient number of replicates, and an appropriate software product was used for statistical processing and analysis of the data.

## 3. Visualization and presentation of the obtained results

The obtained results are presented in a volume of 53 pages, which makes up about 40% of the entire dissertation. They are illustrated with 24 tables and 16 figures,

correctly marked and titled. Statistical processing of the obtained data is suitable for this type of research.

# 4. Discussion of the results and used literature

A total of 248 sources were used in the literature review and in the commentary on the results, 5 of which were in Cyrillic. The results are divided into several subsections: establishing the species composition of cyst-forming nematodes in potato-producing regions in the country; establishment of varietal sensitivity; establishing the effect of plant extracts and species of rhizobacteria on different characteristics of the main species of

As a result of the surveys in Sofia, Pazardzhik, Smolyan and Burgas regions in the period 2017-2019, the ubiquity of potato cyst nematodes of the genus Globodera is confirmed. At the time of the survey, the highest population density was found in the village of Ravnogor (610 cysts / 100 g of soil) and the lowest in the village of Vezenkovo (3.5 cysts / 100 g of 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), of which the second is predominant. The susceptibility of different potato varieties and lines was studied, with 4 varieties (Cronos, Cekin, Gawin, Ovacij) and I lines showing resistance, and two varieties (Gandawa and Ivetta) being highly resistant to *G. rostochiensis*. The Cronoss and Ivetta varieties, as well as line E 1096, E 1809 and E 606, are tolerant to *G. pallida*.

Of the 8 plant extracts tested, all showed nematicidal action against L2 of *G. rostochiensis* and *G. pallida*, with three of them having the highest efficacy - *Juglans* regia, Ruta graveolens and Plantago major.

Of the 12 isolates of rhizobacteria tested, the highest larvicidal effect against *G. rostochiensis* and *G. pallida* was found in *Serratia plymuthica* isolate 72, with the most susceptible being invasive larvae at 2<sup>nd</sup> instar and larvae 3-rd instar. The main findings of the studies (15 in total) are derived from the results obtained.

A valuable recommendation has been made for the practice: the application of the bacterium should take place during the period of active vegetation of the plants, as the moment of application takes into account the development of the nematode.

It was found that a single application of *S. plymuthica* (x108 cells / ml) - BS at a dose of 20 ml / plant / BF (5% solution with 20 ml / plant), protects the roots from the invasion of cyst nematodes during the first 24 days and increases the yield by about 1.9 times compared to untreated plants. At temperatures below 14oC as an alternative, *S. plymuthica* BF can be applied.

#### 5. Contributions of the dissertation

#### Scientific contributions

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

• The metabolic profiles of plant extracts from Juglans regia, Ruta graveolens and Plantago major were determined by gas chromatography - mass spectrometry (GC-MS).

# Contributions of applied character

- The distribution of potato cyst nematodes of the genus Globodera in Sofia, Pazardzhik, Smolyan and Burgas regions was studied, and for the period 2017-2019, in each of the 15 identified areas infected with these parasites, the
- species composition and population density were determined. A genetic bank of the two types of potato cyst-forming nematodes common in • the potato-producing regions of the country has been created: Globodera rostochiensis and Globodera pallida.
- For the first time in the country, the optimal concentrations and temperatures at which the plant extracts Juglans regia, Ruta graveolens and Plantago major show the highest nematicidal activity against G. pallida have been
- The effect of the rhizobacterium S. plymuthica on the development and reproduction of G. pallida in the roots of plants, as well as the period of nematicidal and preventive action against the invasion of larvae 2<sup>nd</sup> instar of G. pallida was determined.
- Important recommendations have been made for potato growers, namely that the application of S. plymuthica to control potato cyst nematodes on potatoes should take place during the period of active vegetation, in accordance with the development of larvae 2<sup>nd</sup> instar - no later than occurrence of the larva 3<sup>rd</sup>
- instar of the parasite. •

# 6. Critical remarks and questions

The critical remarks made during the examination of the draft dissertation, as well as the questions asked were taken into account and received an answer in the current version of the dissertation.

# 7. Published articles and citations

The doctoral student has indicated 4 publications related to the dissertation, in which he is the first author. Two of them are published in the Journal of Mountain Agriculture on the Balkans and one in the Bulgarian Journal of Agricultural Science and one in the Agricultural Sciences. For his participation in these publications, the doctoral student receives 33.25 points, if necessary 30.

The presented abstract objectively reflects the structure and content of the

# CONCLUSION:

Based on the different research methods learned and applied by the doctoral student, the correctly performed experiments, the summaries and conclusions made, I believe that the presented dissertation meets the requirements of ZRASRB and Agricultural University's Regulations for its application, which gives me reason to

I allow myself to propose 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.

Date: 19.09.1021 Plovdiv

PREPARED THE OPINION: .

(prof. Vili Harizanova)