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## CAN ENTOMOPATHOGENIC NEMATODES BE THE BEST MEASURE FOR GROWING ECO-FRIENDLY AGRICULTURAL PRODUCTS?

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### ABSTRACT

Nematodes, which are the subject of this study, are the 3rd most widely used bioagent after *Trichogramma* and *Bacillus thuringiensis* for biological control purposes. To collect entomopathogenic nematodes, they are placed in a "White trap". The research was conducted on a potato farm in Surkhandarya, southern region of Uzbekistan. The main tasks were defined as: potato agrobiocenosis of entomopathogenic nematodes determine species composition; reveal the bioecology of entomopathogenic nematodes ; It is to carry out a molecular-genetic analysis of entomopathogenic nematode species identified for the first time in potato agrobiocenoses in the conditions of Uzbekistan.

### KEYWORDS

Uzbekistan, Surkhandarya, entomopathogenic nematodes, *Steinernema* spp, *Heterorhabditis* spp, in vivo, potato.

### INTRODUCTION

It is known that the global bioproducts market will reach \$4,852 million in 2021 and reach \$13,634 million by 2030, with a CAGR of 12.2% during 2021-2030 is expected to grow. In this case, the increase in the use of biological products in relation to chemical plant protection means has ensured the growth of the corresponding bioproducts market [26]. Therefore, biological control agents are environmentally friendly, do not have a harmful effect on the human body, and do not cause genetic resistance in pests.

Currently, the major drivers of the biocontrol agents market are increasing global demand for organic farming, stringent regulations against chemicals, and positive consumer attitudes toward biocontrol agents. The reason is that Europe aims to reduce the use of dangerous pesticides by 50% by 2030 [3].

According to the FAO, potatoes are grown in 150 countries of the world on more than 20 million hectares. World production is 359 million tons. This

indicator may increase to 500 million tons in 2025, and to 750 million tons in 2030 [26].

While resistant cultivars are considered a good method of disease management, this trait declines over the years, and there are no cultivars that are completely resistant to some pests. And chemicals can cause environmental pollution and food poisoning.

Therefore, the use of bioagents for biological control of potato crop pests is an urgent research area. In particular, entomopathogenic nematodes, which are the object of this research work, are the 3 most widely used bioagents after *Trichogramma* and *Bacillus thuringiensis* [24].

Based on these tasks, including our country determining the fauna and species composition of entomopathogenic nematodes distributed in potato agrobiocenoses, systematic analysis, their morphology, ecology, economic importance, development of measures for sustainable use for the purpose of biological pest control is of great scientific and practical importance.

In this regard, recommendations on the optimal methods and norms of using entomopathogenic nematodes for the purpose of biological control against harmful insects are the development of practical experimental materials for farms, clusters and all such agricultural crops growers in our republic is an urgent task for the scientists of the field.

In Uzbekistan, no research has been carried out to study the species composition, morpho-biology and economic importance of entomopathogenic nematodes, and the fauna, biology and systematics of entomopathogenic nematodes of Uzbekistan have not been studied to date. Therefore, it is important to study the species composition, distribution, morpho-

biologic characteristics of entomopathogenic nematodes, and to develop and implement methods of biological control of agricultural crop pests.

## MATERIALS AND METHODS

For in vivo propagation of entomopathogenic nematodes, all debris is removed from soil samples collected from the field. If necessary, sprinkle water and improve soil moisture. Prepared soil samples are placed in plastic containers with lids and ten instar larvae of *G. mellonella* are placed on the surface of each soil sample. Finally, cover the plastic containers with lids and incubate upside down in a dark place at 55% relative humidity (RH) and 25°C [12].

After 7 days, dead larvae with specific signs of infection with entomopathogenic nematodes are removed from the soil and placed in a "White trap" [6] to collect entomopathogenic nematodes in the third stage of infection. Identification of entomopathogenic nematodes is determined by the color characteristics of dead larvae. In this case, the larvae of the wax moth turn gray if they are infected with entomopathogenic nematodes of the *Steinernema* genus - the third infection stage, and red-black if they are infected with entomopathogenic nematodes of the third infection stage – *Heterorhabditis* [15,16].

The "White trap" method helps to collect entomopathogenic nematodes from infected wax moth carcasses. In this case, a Petri dish is placed upside down inside a large plastic container. Then 200 ml of sterile distilled water is poured into a plastic container. A circular Whatman filter paper is placed in a Petri dish. It is important to ensure that the perimeter of the filter paper touches the water in the plastic

container. Approximately 2-4 to 10 wax moth larvae per trap are then placed on filter paper in a Petri dish. The plastic container is closed with a lid and the container is incubated at a temperature of 20-27°C. After 8 days, new entomopathogenic nematodes develop from dead wax moth larvae and are collected from the water near the filter paper in the trap for 8-20 days. Then put the collected water containing nematodes in a 100 ml glass and let it sink to the bottom of the container for 30 minutes. After the required time, nematodes are washed by filling the container with sterilized distilled water, this process is repeated 3-4 times until the suspension looks clear. At the end of the procedure, the suspension is collected in a clean, transparent tissue culture flask. The time and date of collection and the type of nematode are recorded on the label on the special tube. A special flask containing a suspension of entomopathogenic nematodes is stored in an incubator at a temperature of 10-15 °C [2,8,9].

All insects suspected of having nematodes should be dissected, requiring the use of saline (Ringer's solution or 0.9% NaCl) to prevent nematode rupture as a result of osmotic imbalance. It is necessary to examine various tissues of the observed insect, including hemolymph, digestive system, salivary ducts, muscles, adipose tissue, glands, reproductive system.

Examination samples of entomopathogenic nematodes with caution should be prepared. Live specimens should be heat-killed and placed directly into the fixative to avoid damage to the body structure. Physiological solution at a temperature of 60 °C is best for living specimens. As fixatives, it is optimal to use TAF fixative (97 ml 40% formalin, 2 ml of triethanolamine and 91 ml of distilled water). If this fixative is not available, 3% formalin solution can be used instead. If formalin solution is not found, alcohol

solution (70%) can be used. After one week, the samples placed in a small petri dish are free from the fixative in order to do this, an equivalent container with 70 ml of 95% ethanol solution and a mixture of 5 ml of glycerin and 25 ml of water is used. In this case, the container is partially filled with this liquid and left for one to two weeks depending on the room temperature. This simple method is due to nematodes removing water from the sample and evaporating it helps to create quality material [2].

So,

1. Nematodes are transferred under a microscope to a slide with a drop of glycerin (pereparoval nina).
2. Put three pieces of glass cotton fiber on the product window and cover the window, then the windows are sealed with nail polish or wax.
5. After 48 hours, the quality is checked and resealed if necessary [22].

The following diagnostic key can be used to identify types of entomopathogenic nematodes.

Dianostics of the family Steinernematidae

### The female

1. The body is large, variable in size. They have smooth skin or lateral ring.
2. Separating holes are clearly visible.
3. The head is round, rarely flat. They have six labia that are fully fused or sometimes separated, with one labial papilla per lip, and occasionally additional papilla-like structures appear next to the labial papillae.
4. The number of head papillae is 4. Has small amphids. The stoma is usually short and in

lateral view forms a ring resembling two large sclerotized dots.

5. Additional elements of the stoma asymmetrically form a funnel with a thick anterior end. Esophagus with metacarpus is slightly swollen, short isthmus is surrounded by nerve ring and large basal bulb with valve is clearly visible.
6. The esophageal valve is usually simple in structure.
7. The reproductive system is usually didelphous, amphiphilous and reflexive. Vulva in the middle of the body, with or without an epiptigma.

#### Male

1. The body is smaller than the female.
2. The anterior end usually consists of six labial papillae, four large cephalic papillae, and usually a perioral disc.
3. The esophagus is similar to that of the female.
4. Testicle is single, reflexed; spicules paired; gubernaculum sometimes as long as spicule; bursa is not available.
5. The tip of the tail is rounded, with a muzzle.

There are 10-14 pairs of genital papillae.

#### Infectious larvae

1. They usually do not have a stoma.
2. Their bodies have extra skin (in exchange for their second-stage larvae shedding their skin).
3. The esophagus and intestine are shortened. There are separate outlets.
4. The tail is usually cone-shaped.
5. Phasmid is not visible.

Key to identification of Steinernema species

Steinernema species can be recognized using the following key, but identification can be confirmed by analyzing its morphometry in parallel with data from the original description.

YL = 3rd instar larva of infective juvenile nematode.

EP = distance from anterior end to excretory orifice.

ES = length of esophagus.

SP = spicule length.

T = tail length.

D% =  $EP/ES \times 100$ .

E% =  $EP/T \times 100$ .

SW = spicule length divided by anal body width [28].

#### RESULTS

The structure of these entomopathogenic nematodes belonging to the family Rhabditida (Oerley, 1880) [23] is unique and is based on the following diagnostic features: the number of lips varies from one to six, the stoma is tubular, and the walls of the stoma are covered with rhabdions. Esophagus consists of parts such as procorpus, metacarpus and isthmus and is in the form of a basal bulb with valves. The separation system consists of lateral channels, and the terminal channel is the cuticle.

Females have one or two ovaries. The vulva is located further back. There is a bursa [10,20].

This family is divided into two subfamilies: Rhabditina and Cephalobina. All species belonging to the family Steinernematidae and Heterorhabditidae of the suborder Rhabditina are pathogenic species.

Steinernema carpocapsae (Weiser, 1955)





## Figure 2 . *Steinernema carpocapsae* ( Weiser,1955) based on sequence material nucleotides of the ITS region of pDNA of species belonging to the genus sequence comparison .

### CONCLUSION

It is necessary to carry out many new studies on the isolation and identification of these entomopathogenic nematodes from the territory of Uzbekistan and their biocontrol potential against agricultural crops. In particular, the above-mentioned species *Steinernema carpocapsae* (Weiser, 1955) is used today against the main pests of many countries [4,5,7,11], and good results are obtained. If the researchers master the methods of reproduction of such new species in vivo and test them against the pests of potatoes, cotton, grain and orchards, in the future, the environmentally friendly, inexpensive local bioagent will be widely used [13,17,21] would have contributed to it.

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