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Insecticidal potential of entomopathogenic nematodes of Northern Kazakhstan

The goal of this research experiment is to create a biological preparation based on predatory nematodes to control potato pests. 15 isolates of entomopathogenic nematodes were isolated: 13 were isolated from coniferous and mixed forests; 2 from soils of agricultural fields: 1 from a potato field, 1 from a field sown with perennial grasses. Nematodes were identified as *Steinernematids* based on the color of the corpses of larvae of *Galleria mellonella* and the morphology of males. Isolates of entomopathogenic nematodes from various habitats (forests, agricultural fields, grasslands) of Akmola and Pavlodar regions were isolated. Screening of isolates on the larvae of the seed nutcracker (*Agriotes obscurus*) was carried out. It is established that the wireworm is sensitive to all isolates. Of the 15 isolates tested, 12 of these isolates showed high mortality ability in relation to wireworm: about 50–70 % mortality in laboratory biotests; 3 isolates (AF 15, AF 22, and AS 36) could not cause more than 40 % of the average mortality of larvae. The screening results showed that the isolates AF 29, AF57, and KP76 are good isolates for further studies as a possible bioinsecticide agent against *A. obscurus*.

Keywords: entomopathogenic nematodes, *Steinernematids, Agriotes obscurus, Galleria mellonella,* biological control.

Introduction

Insect pests are a dangerous threat to agricultural productivity. As a result of their activity, farms lose a significant part of their products every year. In addition to destroying crops due to their direct activities, insect pests are also carriers of plant and animal diseases. In recent years, an increase in insect activity is expected and, thus, yield losses due to climate change [1]. The most dangerous pests of agriculture in Kazakhstan are herd locusts, gray grain and cotton scoops, harmful turtle, Colorado potato beetle, bread beetles, spider mite, cabbage moth [2].

Chemical pesticides are widely used to control pests. However, the use of pesticides has significant drawbacks in the form of the toxicity of the pesticides themselves, as well as the non-selectivity of their action, which can lead to the death of some pollinating insects [3]. In addition, over time, insects can acquire resistance to pesticides, which significantly reduces the economic efficiency of pesticides.

One of the promising areas in the fight against insect pests is biopesticides. These are biological products based on bacteria, fungi, plants, and nematodes that fight pests without harming agriculture and humans. These biological products are obtained from extracts of vegetable oils or by breeding specialized bacteria, entomopathogenic fungi, viruses, as well as ethnomopathogenic nematodes.

Entomopathogenic nematodes (EPN) are roundworms, obligate parasites of insects. They are capable of infecting more than a thousand species of insects. These nematodes spread over all continents except Antarctica. Soil composition, temperature, and availability of suitable carriers are considered important factors influencing their distribution [4].

A distinctive feature of this group of worms is their mutualistic symbiosis with *Photorhabdus* and *Xenorhabdus* bacteria. EPN affects both larval and adult stages of insects. The life cycle of this nematode begins with the penetration of a young infecting larva into the body cavity of the insect through the natural openings of the body: mouth, anus, spiracles or scrawny areas of the cuticle. After the immersing them into an insect body, the nematode penetrates into the hemocele, where it releases symbiotic bacteria that live in the intestines of the worm. These bacteria multiply at a high rate, releasing various toxins and hydrolytic exoenzymes, which cause the transient death of the carrier after two to three days. After that, nematodes continue their development already in the corpse for several generations. The key feature of nematodes is that even when single individuals of nematodes enter the insect body, after 10–20 days, hundreds of thousands of young individuals enter the environment, where they are able to exist for long periods of time without nutrition while waiting for a new host [5].

The most studied representatives are nematodes of the families Steinernematidae and Heterorhabditidae due to their presence of the most promising biocontrol agents for use. Previous results have shown that the use of two families of Steinernematids and Heterorhabditids in combination to control the fruit weevil population can reduce their number to 70–90 %. In addition, a number of studies have recorded positive results on populations of scoop larvae [6]. A potential source of infection in the spring may be wintering larvae experiencing unfavorable conditions under the bark of trees, leaf piles or in wooden containers for apples. EPN have a high level of effectiveness in combating this threat. Thus, in the study of Unruh and Lacey, 2001 and Lacey et al. 2006a, it was possible to decrease the population of wintering larvae of the apple moth to 90 % [7].

One of the most important agricultural crops affected by insect pests in Kazakhstan is potatoes. Thus, in 2021, the potato harvest amounted to 1 million 31 thousand 165 tons on the sown territory with a total area of 35.6 thousand hectares. Thus, the average yield is estimated at 29 t/ha [8]. For this reason, there is a need for methods of controlling potato pests — wireworms.

Wireworms are the larvae of click beetles. Imagos are usually buried in the soil for the winter and lay eggs in late spring-early summer. After that, the hatched larvae live in the soil for few years before pupating. During the larval cycle, wireworms are capable of causing enormous damage to the crop, which can be avoided by timely use of biological preparations based on EPN. In Russia, similar studies were conducted on wireworms with a noted high biological activity of nematodes against this pest using several different drugs based on EPN [9].

The goal of this study is to explore aboriginal entomopathogenic nematodes efficacy against common potato and vegetable pest larvae of *Agriotes obscurus* from different sides of northern Kazakhstan.

Experimental

Study sites. Experiments were conducted in the laboratory of LLP "Scientific-Analytical Center "Biomedpreparat" in Stepnogorsk, Akmola region in 2020. To isolate entomopathogenic nematodes, soil samples were collected in various habitats (forests, agricultural fields, grasslands) in Akmola and Pavlodar regions in May–June 2020. Samples were taken within 200 m from the road. The area of each sampling site was 2–4 m^2, 800 g of soil was collected from each site, which was placed in a plastic bag and stored at a temperature of 10 to 15 °C.

Entomopathogenic nematodes extraction method. 250 g of the sample from the soil thoroughly mixed in the bag was placed in a 300-milliliter plastic container. The remaining soil was stored at 4 °C, and a second isolation of nematodes was carried out after 2–4 weeks. Six *Galleria mellonella* larvae of the last age stage were placed in each container, and all containers were stored at room temperature 25 ± 2 °C. After 5 days, each container was inspected and dead or pupating larvae were removed, and live larvae were left in the soil. An equivalent number of larvae were added to replace the removed ones. Each dead larva was washed with distilled water, sterilized by immersion in 70 % alcohol and placed on filter paper in a Petri dish.

Corpses of *G. mellonella* larvae that showed symptoms of potential nematode infection, such as discoloration, were placed in the modified White traps, on which they were kept at room temperature. White traps were monitored daily for the appearance of nematodes. Then, 300 ml of distilled water was added to each cup to provide moisture to emerging nematodes. Each Petri dish was covered with a film and stored at 25 ± 2 °C [10]. Nematodes isolated from White traps were used to infect a group of *G. mellonella* larvae to check their pathogenicity.

Wireworm collection method. Larvae of the seed nutcracker (*A. obscurus*) for experiments were collected from May to August 2020 in potato fields of Pavlodar and Akmola regions. Larvae were collected using traps for stocking. Nylon stockings were filled with half a cup of wheat grain or a mixture of wheat grain and barley. These baits were soaked in water for 24 hours and buried 0.15–0.2 m below the soil surface in fields infected with wireworm. After 2 weeks, the traps were collected from the field in plastic buckets and brought back to the laboratory for extraction. The larvae were sorted from the traps using Berlese funnels. The collected wireworm larvae were stored in an incubator at 10 °C in plastic cups with sterilized sandy loam soil, soaked wheat seeds were used as feed. The cups were sprayed once a week to keep soil moist. The collected larvae were of almost all larval stages. The length of wireworm larvae ranged from 0.5 to 2.2 cm. In the experiments, medium–sized wireworm larvae (0.8–1.5 cm, more than one year old) were used.

The larvae were identified based on their distinctive morphological features, and the age was determined by measuring the width of the head capsule [11]. Wireworms were kept in plastic containers with sterile soil at 10 °C and fed slices of fresh potatoes for 1 week to remove unhealthy larvae before use in experiments.

Efficacy of EPNs against *A. obscurus* larvae. 25 g of soil was placed in a Petri dish with a diameter of 5 cm. Before use, the soil was sterilized in an autoclave at 121 °C and 1.2 atm for 1 hour and left at room temperature for at least two weeks for acclimatization. One wireworm larva with a piece of potato (1 cm^3) as food was placed in each cup.

The number of nematodes in the biotest for all 15 isolates was 200 EPN/cm^2. 1 ml of EPN suspension was pipetted onto the soil surface, and 1 ml of tap water without nematodes was introduced into the control cups. The final soil moisture was brought to 15 % (by volume/wt). There were 10 repeats for all 15 EPN isolates. The biotest was repeated five times on different dates from August to November 2020. The cups were placed in trays with holes in the lids for aeration, and then placed in an incubator at 22 °C and 75 % relative humidity in the dark.

The mortality of larvae was monitored daily for a month. During the laboratory bioanalysis described above, the dead larvae found during daily observations were collected and washed with water, and then transferred to separate Petri dishes (5 cm in diameter) lined with wet filter paper for 2–3 days to allow EPN to multiply. The number of penetrated EPNs was defined as the number of invasive juveniles found inside each wireworm larva. To do this, the dead larvae were opened with a scalpel in a mixture of water and Ringer's solution (50:50) in a Petri dish (diameter 9 cm). The contents were poured onto a slide for counting nematodes and the number of adult nematodes was counted. In total, 20 dead wireworm larvae were examined for all EPN strains.

Results and Discussion

Of 80 soil samples, entomopathogenic nematodes were detected in 15 (18.75 %): 13 were isolated from coniferous and mixed forests; 2 from soils of agricultural fields: 1 from a potato field, 1 from a field sown with perennial grasses. Nematodes were identified as *Steinernematids* based on the color of the corpses (cream, ochre color) and the morphology of males.

It was believed that the larvae of *G. mellonella*, which contained entomopathogenic nematodes, were killed by these pathogens. No larvae killed by nematodes were found in the control group, which confirms that there was no previous contact between hosts and pathogens. All isolates caused 100 % death of larvae, demonstrating their pest control potential.

Next, EPN screening was carried out using the larvae of the nutcracker beetle (Coleoptera: *Elateridae*) — wireworm. Wireworms are economically important soil-dwelling pests that infect the underground parts of many field crops around the world. Wireworms are omnivorous pests that feed on seeds, roots, stems, tubers, and underground parts of plants, inhibiting plant growth, causing wilting and death of plants and reducing crop yields. Crop losses from wireworms living in the soil can reach 25 % [12].

Figure 1 illustrates a Petri dish with larvae infected with EPN. Figure 2 illustrates a snapshot of EPN observed under a stereomicroscope in an autopsied deceased *G. mellonella* larva.

Figure 1. Petri dish with *G. mellonella* larvae infected with EPN

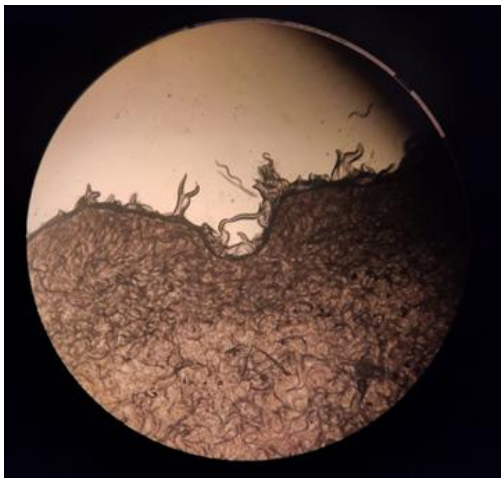

Figure 2. A snapshot of EPN observed under a stereomicroscope in an autopsied deceased *G. mellonella* larva

According to Traugott et al. [13], high species diversity, soil habitat, long life expectancy of larvae underground, poorly studied taxonomy and life history of wireworms make it difficult to control them. Figure 3 demonstrates the results of wireworm mortality observed within 28 days after infection with isolated EPN.

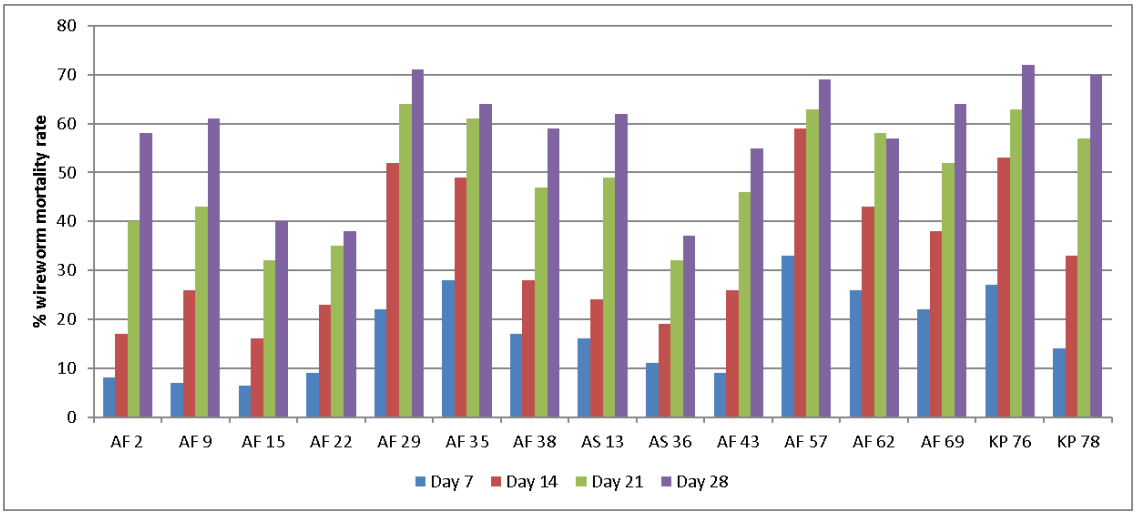

Figure 3. Wireworm mortality observed within 28 days after infection

We found that the wireworm is sensitive to all EPN. Of the 15 EPN isolates tested, 12 were the most virulent in relation to wireworm: about 50–70 % mortality in laboratory biotests; 3 EPN isolates (AF 15, AF 22 and AS 36) could not cause more than 40 % of the average mortality of larvae. Mortality during control treatment was absent and therefore was not indicated in the graph.

Wireworm mortality 7 days after infection was significantly lower than mortality at 14, 21, and 28. Significant differences were found between 14 and 21 and between 21 and 28 days in wireworm mortality. Mortality gradually increased over time, and even the most effective EPN needed at least 14 days after treatment to cause mortality of 50 % or more. Only isolates AF 29, AF 57, and KP 76 were able to cause 50 % mortality of larvae on day 14 after treatment. In variants with isolates AF 35, AF 62, AF 69, and KP 78, mortality reached 50 % for only 21 days after treatment. For AF 15, AF 22, and AS 36, mortality did not reach 50 % until 28 days after treatment.

The tendency for significant differences between EPN isolates on day 28 after treatment was similar to 21 days after treatment. The only difference was an increase in mortality for all variants of EPN isolates. In the variants with isolates AF 29, KP 76, and KP 78, the mortality of wireworms on the 28th day after treatment increased and reached 70–72 %.

The results showed that the isolates AF 29, AF 57, and KP 76 have the potential for biological control of the number of larvae of the seed nutcracker. The high infectivity of EPN tested in our study is consistent with the data of Ansari et al. [14] and Morton and Garcia-del-Pino [15], who reported 50 and 50–75 % mortality of *Agriotes spp.*, respectively.

The survival of the larvae of click beetles (*Elateridae*) to infection with EPN was associated with the existence of a thick brush of hair in the oral cavity, a muscular structure covering the anus, valvular spiracles, or heavily sclerotized integuments [15]. These structures may explain the difficulty of penetration of some EPN into host body. Thus, size may be important for the penetration of invasive larvae through insect barriers. The above-mentioned factors may be crucial for infection with wireworm nematodes, since some studies have proved that nematode strains that do not have virulence against live wireworms are, nevertheless, able to penetrate dead samples.

Throughout the analysis, high mobility of wireworms exposed to EPN was observed, and these larvae made fewer holes in potato slices than in control cups. Arrington et al. [16] also reported that EPN introduced by drip irrigation reduces the severity of damage to sweet potatoes caused by wireworms, compared with control. Avoiding infected areas is a common behavioral defense used by insects against EPN. Since wireworms are attracted to plants for feeding, applying EPN ambushes around the roots can help to reduce the damage to roots and tubers caused by these insects. Nevertheless, this assumption should be further considered in future studies.

The concentrations of EPN per larva used in our biotest were intentionally high to achieve high mortality of wireworms. The main reason for using relatively high concentrations of nematodes was the low mortality of wireworms when using lower concentrations in preliminary biotests. Some previous studies have reported high concentrations to achieve significant control over wireworm populations.

The results showed that both the concentration used, and the time required for nematodes to infect hosts is the main factors affecting the effectiveness of EPN against various insects. Some other factors affecting the effectiveness of EPN have already been reported, such as the stages of development and immaturity of insects, species, or strains of nematodes, as well as environmental variables such as temperature, aeration, humidity, soil type, food availability and exposure time. Soil characteristics such as texture, moisture, and pH play an important role in the effectiveness of EPN. Identifying factors, including nematode dose and application time, can improve efficiency in the field.

Conclusions

We found that the wireworm is sensitive to all isolates. Of the 15 isolates tested, 12 turned out to be the most virulent in relation to wireworm: about 50–70 % mortality in laboratory biotests; 3 isolates (AF 15, AF 22 and AS 36) could not cause more than 40 % of the average mortality of larvae. There was no mortality during the control treatment. The results showed that the isolates AF 29, AF 57, and KP 76 are good candidates for further studies as a possible biocontrol agent against *A. obscurus*. Identifying factors such as nematode dose and application time can improve efficiency in the field.

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Солтүстік Қазақстанның энтомопатогенді нематодтардың инсектицидтік потенциалы

Зерттеудің мақсаты — картоп зиянкестерімен күресу үшін жыртқыш нематодтар негізінде биологиялық өнімді жасау. Энтомопатогенді нематодтардың 15 изоляты анықталған: 13-і қылқан жапырақты және аралас ормандардан; 2-еуі ауыл шаруашылығы алқаптарының топырағынан, яғни 1-еуі картоп алқабынан, 1-еуі көп жылдық шөптер егілген алқаптан. Нематодтар *Galleria mellonella* дернәсілдерінің мәйіттерінің түсі мен аталықтарының морфологиясы негізінде *Steinernematids* ретінде сәйкестендірілген. Энтомопатогенді нематодтардың изоляттары Ақмола және Павлодар облыстарының әртүрлі мекендеу орындарынан (орман, егіншілік алқаптары, шабындықтар) табылған. Қоңыз тұқымының (*Agriotes obscurus*) дернәсілдерінің изоляттарына скрининг жүргізілді. Ызылдауық қоңыздың барлық изоляттарға сезімтал екені анықталған. Сынақталған 15 изоляттың 12-сі ызылдауық қоңызға қатысы бойынша ең вирулентті болып шықты: зертханалық биоталдауларда өлім-жітім шамамен 50-70%; 3 изолят (AF 15, AF 22 және AS 36) дернәсілдердің орташа өлімінің 40%-дан астамын тудыруы мүмкін емес. Сыналған 15 изоляттың 12-сі сым құртына қатысты вирулентті болып шықты: зертханалық биотесттердегі өлім мөлшері шамамен 50–70 %; 3 изолят (AF 15, AF 22 және as 36) құрттардың өлімін 40 %–дан астырмады. Скрининг нәтижелері бойынша AF 29, AF 57 және KP 76 изоляттары *A. obscurus* қарсы ықтимал биобақылау агенті ретінде одан әрі зерттеуге жақсы үміткер екенін көрсетті.

Кілт сөздер: энтомопатогендік нематодтар, *Steinernematids, Agriotes obscurus, Galleria mellonella*, биологиялық бақылау.

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Инсектицидный потенциал энтомопатогенных нематод Северного Казахстана

Целью исследования является создание биопрепарата на основе хищных нематод для борьбы с вредителями картофеля. Выделено 15 изолятов энтомопатогенных нематод: 13 выделено из хвойных и смешанных лесов; 2 из почв сельскохозяйственных полей: 1 с картофельного поля, 1 с поля, засеянного многолетними травами. Нематоды были идентифицированы как *Steinernematids* на основании цвета трупов личинок *Galleria mellonella* и морфологии самцов. Были выделены изоляты энтомопатогенных нематод из различных местообитаний (леса, сельскохозяйственные поля, луга) Акмолинской и Павлодарской областей. Был проведен скрининг изолятов на личинках семенного щелкуна (*Agriotes obscurus*). Установлено, что проволочник чувствителен ко всем изолятам. Из 15 протестированных изолятов 12 оказались наиболее вирулентными по отношению к проволочнику: около 50–70 % смертности в лабораторных биотестах; 3 изолята (AF 15, AF 22 и AS 36) не могли вызвать более 40 % средней смертности личинок. Результаты скрининга показали, что изоляты AF 29, AF57 и KP76 являются хорошими кандидатами для дальнейших исследований в качестве возможного средства биоконтроля против *A. obscurus*.

Ключевые слова: энтомопатогенные нематоды, *Steinernematids*, *Agriotes obscurus*, *Galleria mellonella*, биологический контроль.