INTRODUCTION

Entomopathogenic nematodes (EPNs) have been used as an alternative to chemical insecticides to control insect pests since they were discovered in the 1920s (Koppenhöfer, Shapiro-Ilan, & Hiltpold, 2020). Despite the industry’s offering of a variety of EPN products in the global market (Table 1), many researchers still focus on testing the insecticidal properties of indigenous EPN species and they are in high demand as biological products since last few decades (Acharya, Hwang, Mostafiz, Yu, & Lee, 2020; James, Malan, & Addison, 2018; Kerchev, Kryukova, Kryukov, & Glupov, 2017; Matuska-łyżwa, Żarnowiec, & Kaca, 2021; Platt, Stokwe, & Malan, 2018; Půža, Nermuť, Konopická, & Habuštová, 2021). Current EPN species are grouped into Heterorhabditidae and Steinernematidae families (Koppenhöfer, Shapiro-Ilan, & Hiltpold, 2020). Around a hundred Steinernema (Steinernematidae) and a dozen Neoaplectana (Steinernematidae) and Heterorhabditis (Heterorhabditidae) species have been identified (Koppenhöfer, Shapiro-Ilan, & Hiltpold, 2020; Lewis & Clarke, 2012; Shapiro-Ilan, Arthurs, & Lacey, 2017). These EPNs are obligated parasites, and their hosts are arthropods. The parasitism of these beneficial nematodes leads to the suppression of the immune system of the insect host (Cowles, 2011; Lacey et al., 2015; Lewis & Clarke, 2012; Shapiro-Ilan & Brown, 2013).
Nematode penetrates the host insect’s body through natural openings such as the mouth, anus, and spiracles or the intersegmental membranes of the insect cuticle (Bedding & Molyneux, 1982; Peters & Ehlers, 1994). It reaches the hemocoel, where symbiont bacteria (Xenorhabdus spp. in Steinernematidae and Photorhabdus spp. in Heterorhabditidae) are released from the EPN intestine (Askary, 2010; Baliadi, Sastrahidayat, Djauhari, & Rahardjo, 2011). The nutritious, rich hemolymph of the host insect helps in the rapid reproduction of these bacteria. Bacteria secrete many toxins and hydrolytic exoenzymes that cause sepsis or blood poisoning. It also ultimately leads to the host's death within 48-72 hours (El Aalaoui, Mokrini, Dababat, Lahlali, & Sbaghi, 2022).

Chemical pesticides can harm humans and the environment and cause secondary outbreaks of pests and their resistance. Moreover, common chemical pesticides are non-selective and eliminate all insects (Sharma, Sharma, & Hussaini, 2011). On the contrary, biological agents (EPNs, bacteria, and entomopathogenic fungi) are safe for humans and the environment. They have little or no effect on other non-targeted organisms (Jagodič, Trdan, & Laznik, 2019). EPN is safe for plants, pollinators, and vertebrates (Akhurst & Smith, 2002). Despite widespread use in fields, gardens, and pasture lands, no significant acute or chronic toxicity to humans or other vertebrates has been detected (Akhurst & Smith, 2002; Jagodič, Trdan, & Laznik, 2019). In this regard, the Environmental Protection Agency (EPA) in the USA and the same governmental organizations in India, Australia, and many European countries have exempted them from all registration requirements and relevant regulations (Ehlers, 2005; Ehlers & Hokkanen, 1996).

In laboratory experiments, mass-produced EPNs perform at a higher mortality rate of pests than local strains (Malan & Moore, 2016; Platt, Stokwe, & Malan, 2018) field results of EPN research show the high effectiveness of local strains. Commercial EPNs could be less effective due to local soil structure and moisture content. Infective juvenile EPNs are more active and effective as invading pests at 20–40% soil moisture and on sandy or sandy loam soils (Koppenhöfer, Shapiro-Ilan, & Hiltzold, 2020; Stuart, Barbercheck, & Grewal, 2015). The latter provides optimal levels of moisture and oxygen for EPNs (Matuska-Łyżwa, Żarnowiec, & Kaca, 2021; Stuart, Barbercheck, & Grewal, 2015). Any variation in soil conditions may decrease the efficacy of non-local EPNs. Therefore, researchers are looking to use aboriginal EPN strains due to their better adaptation to the local environment (James, Malan, & Addison, 2018).

Bioinsecticide production in developing countries of Central Asia, such as Kazakhstan, is a fast-growing sector with several benefits for local farmers but should account for local environmental conditions. For example, Kazakh steppes, one of the main agricultural areas in Central Asia, are usually dry during the vegetation period (average precipitation in May-August is 180–200 mm in northern Kazakhstan) (Eisfelder, Klein, Niklaus, & Kuenzer, 2014; Kusainova, Mezentseva, & Tusupbekov, 2020; Propastin, Kappas, Erasmi, & Muratova, 2007; Salnikov, Turulina, Polyakova, Petrova, & Škakova, 2015). Therefore, the potential effectiveness of EPN can be lower in Kazakhstan soil conditions. Moreover, foreign bioproducts are usually expensive for Kazakh farmers due to production and logistic costs. Nevertheless, in the last decade, demand for bioproducts has increased, especially for potato and vegetable producers (Aipova, Abdykadyrova, & Kurmanbayev, 2019). Potato (Solanum tuberosum L.) is Kazakhstan’s main non-grain agricultural product (Po, Sinha, & Naeem, 2018). Primary potato production was 3,912,000 tonnes in Kazakhstan in 2019, which was 1% of global production (FAO, 2021). In some years, the percentage of insect pest-damaged potatoes in the field may reach around 50% (Bechinski, Sandvol, Carpenter, & Homan, 1994; Kuhar, Doughty, Speese III, & Reiter, 2008). This circumstance required the identification of local nematodes, which, in the case of high efficacy, can be mass-produced by local industries.

This research aims to evaluate the effectiveness of two aboriginal EPN isolates compared to mass-produced EPNs in laboratory conditions on larvae of Tenebrionidae.
MATERIALS AND METHODS

The experiment was conducted in the LLP “Scientific-Analytical Center “Biomedpreparat” laboratory in Stepnogorsk, Akmola region, Kazakhstan, September-December 2021. A laboratory experiment was conducted with larvae of darkling beetles (Coleoptera: Tenebrionidae) as a model of potatoes and vegetable pests. The larvae of Tenebrionidae are a common pest of stored products and have similarities in body structure with common crop and potato pest wireworms (Elateridae) (Neville, 1975). Moreover, Tenebrionidae has a faster lifespan (a few weeks), which is beneficial for this experiment. The larvae of darkling beetles were obtained from a local garden shop.

Two industrially produced EPN isolates of Entonem® S. carpocapsae (Bioproduct S.C.), Capsanem® S. feltiae (Bioproduct S.F.) and two local isolates, AF29 (S. feltiae), KP76 (S. carpocapsae) from the collection of LLP “Scientific-Analytical Center “Biomedpreparat” (Stepnogorsk, Kazakhstan) were used in this experiment. To produce IJ, the local isolates were cultured on egg yolk medium 1 (Tahir & Shaheen, 2020). Bioproducts S.F. and S.C. were prepared into ready-to-use packages.

The experiment was conducted in small pots with soil (Fig. 1). The dark chestnut soil with a pH of 8.0 was collected in the Pavlodar region, near Pavlodar city (latitude: 53.017506; longitude: 76.000745). The soil was pre-sterilized at 120°C. Next, 35 grams of soil was put in a pot, and the soil moisture was brought to 20%. One larva and one potato slice (1 cm³) were put in each pot. Nematodes were introduced into the soil at doses of 100, 200, 300, and 400 IJs per 1 cm² of the pot surface with 1 ml of distilled water. Each dose of nematodes was carried out in 10 replicates. The experiment was run three times. The control was 10 pots with larvae moistened with 1 ml of distilled water without EPN.

The pots (with soil, larvae, potatoes, and EPN) were weighed and randomly placed on the thermostat. The temperature of the thermostat was 25°C. Next, all the pots were covered with parafilm, through which holes were made. Every three days, the pots were weighed, and the evaporated moisture was replaced with distilled water.

The accounting of dead larvae was carried out on the 7th day of the experiment. The dead larvae were collected in separate Petri dishes and incubated in a thermostat for 48 hours before being autopsied to determine the presence of nematodes.

The statistical analysis was done with two-way ANOVA by R software version 4.1.2 (R Core Team, 2021). The predictor variables were nematode isolates and treatment doses; the response variable was insect mortality percentage. In addition, a Tukey’s HSD multiple comparison test (p ≤ 0.05) was conducted to compare differences between treatments.

Table 1. List of available products containing entomopathogenic nematodes

| Nematode species          | Product name     | Company and country of origin |
|---------------------------|------------------|-------------------------------|
| **Steinernema carpocapsae** | Mioplant         | Novartis, Austria             |
|                          | Boden-Nutzlinge  | Phone-Poulenc, Germany        |
|                          | Biosafe          | SDS Biotech, Japan            |
|                          | Proactant Ss     | Biocontrol, USA               |
|                          | Biosafe-N        | Thermo Trilogy, Columbia      |
|                          | BioVector        | Thermo Trilogy, Columbia      |
|                          | Helix            | Novartis, Canada              |
|                          | Bouncer          | Curative Microbes Pvt, India  |
|                          | Carpocapsae-System | Canada                      |
|                          | Capsanem         | Koppert BV, Netherlands       |

| **Steinernema feltiae**   | Exhibit          | Novartis, Switzerland         |
|                          | Stealth          | Novartis, UK                  |
|                          | NemaSys          | Micro Bio, UK                 |
|                          | Entonem          | Koppert BV, Netherlands       |
|                          | X-GNAT           | E.C. Geiger, USA              |
|                          | NemaShield       | Bioworks, USA                 |
|                          | Owinema SC       | Owiplant, Poland              |

Source: Askary, Nermuthacek, Ahmad, & Ganai (2017), Caamano, Cloyd, Solter, & Fallon (2008), and Sharma, M., Sharma, A., & Hussaini (2011)
Fig. 1. Pot with soil, potato slice and larvae of *Tenebrionidae* (A) and replications (B) and part of prepared, parafilm sealed pots with added EPN doses before putting them to a thermostat for incubation (C).

Fig. 2. Mean larval mortality rate of name of insect by nematode species and bioinsecticides Entonem® (Bioproduct S.C.) and Capsanem® (Bioproduct S.F.) under controlled conditions (doses, ±SE).
RESULTS AND DISCUSSION

The larvae in control plots were alive during all experiments, except one out of ten died the third time. There was a difference in larval mortality between aboriginal and bioproduct EPNs and between applied doses of IJ. The strain AF29 showed high (67-83%) larval mortality by all treatments, but the treatment effect of AF29 was lower compared with treatments of commercial EPNs (Fig. 2). The effectiveness of KP76 was the lowest among all doses and did not reach 50%. Bioproduct S.F. (300 and 400 IJ/cm²) and Bioproduct S.C. (400 IJ/cm²) resulted in the highest larval mortality rate (Fig. 2). However, Bioproduct S.C. at the dose of 100 IJ/cm² was 47%, which was lower than in the strain AF29 and Bioproduct S.F. at the same dose of 67% and 93%, respectively. Microscopy of the dead bodies of larvae from all treated pots showed alive newborn nematodes (Fig. 3).

Fig. 3. Dissection of Tenebrionidae’s larvae infected with nematodes: A) KP76, B) AF 29, C) Bioproduct S.C., D) Bioproduct S.F.
Application of different EPNs strains and their doses against larvae shows a significant effect (Table 2, p-value <.0001). An average EPN strain from bioproducts had a high mortality rate of larvae. The larvae mortality rate by Bioproduct S.F. is the highest compared to other isolates, proved by statistical analysis (Table 3). However, the efficacy of local isolate AF29 and Bioproduct S.C. is not significantly different, Lsmean 76.7 and 79.2, respectively (Table 3). The mortality rate increases dramatically with AF29 doses (Table 4). Higher amounts of KP76 and Bioproduct S.F. do not significantly differ larva mortality rate. The effectiveness of Bioproduct S.C. doses greater than 200 IJ/cm$^2$ is also not statistically different.

In the present study, one of the two aboriginal EPN isolates had significant pest mortality, which can be competitive with industrial bioproducts. This experiment's findings agree with previous studies where insect mortality exceeded 50 percent (Alves, De O. Neves, Alves, Moino Jr, & Holiz, 2012; De Carvalho Barbosa Negrisol, Negrisol Júnior, Bernardi, & Garcia, 2013; Fallet et al., 2022; Javed, Khanum, & Khan, 2020; Navarez et al., 2021). A laboratory experiment in Petri dishes with new EPN isolates Steinernema affine, and cholashanense from Pakistan showed 67-75% mortality of adult beetles (Coleoptera: Tenebrionidae) at a dose of 150 IJ per insect (Javed, Khanum, & Khan, 2020). In this experiment, low doses of 100 IJ/cm$^2$ of AF29 and Bioproduct S.C. and 200 IJ/cm$^2$ of Bioproduct S.F. caused 76% and 86% mortality, respectively. The research on irrigated sweet potato fields in North Florida that were infected with wireworms (Coleoptera: Elateridae) resulted in a high effect of new EPN strains (Seal et al., 2020). S. carpocapsae, Steinernema sp. Mexican strain and two Heterorhabditis sp. caused 67-100% mortality at doses of 50 and 100 IJ/cm$^2$ (Seal et al., 2020). Moisture content in irrigated soil, as well as the high moisture content in our soil pots, probably increased EPNs infectivity, as it was recorded in many laboratory and field studies (Blatt & Barry, 2020; El Aalaoui, Mokrini, Dababat, Lahlali, & Sbaghi, 2022; Jaffuel et al., 2019; Öğretmen, Yüksel, & Canhilal, 2020; Sandhi, Shapiro-Ilan, & Reddy, 2020). In contrast with AF29, the KP76 strain did not show high efficacy even at high doses. A few experiments also showed poor pest control of some EPN strains (Ensafi et al., 2018; Sandhi, Shapiro-Ilan, Sharma, & Reddy, 2020). In a shade house experiment, for example, the indigenous S. feltiae sp. from Montana was only able to kill 25% at a rate of 400 IJ/cm$^2$ (Navarez et al., 2021; Sandhi, Shapiro-Ilan, & Reddy, 2020). Perhaps the tough chitin cuticle and immune defenses of Tenebrionidae larvae were a strong barrier to KP76’s penetration into the host, and thus an increase in dose rate does not affect insect mortality rates (El Aimani et al., 2022; Garriga, Morton, & Garcia-del-Pino, 2018; Labaude & Griffin, 2018; Öğretmen, Yüksel, & Canhilal, 2020).

Although in this research laboratory experiment, the use of aboriginal EPN shows promising effects, there was only one field study of aboriginal EPN use in the southeastern part of Kazakhstan that was found in open access (Temreshev, Makezhanov, Yeszhanov, & Tursynkulov, 2020). Still, the results were preliminary without clearly explaining applied doses, methods, and infectivity rate. Therefore, further field experiments are required to investigate the local soil and climate conditions on aboriginal EPN efficacy. The next step of this study is to experiment in a potato field with several years of EPN application to account for diverse weather conditions that might affect aboriginal EPN’s infectivity.

Table 2. Two-way ANOVA summary of main effects and their interactions for larva mortality of Tenebrionidae

| Variables | df | F-value | P-value |
|-----------|----|---------|---------|
| Intercept | 1  | 5046.0  | <.0001  |
| Nematode  | 3  | 119.9   | <.0001  |
| Dose      | 3  | 19.4    | <.0001  |

Remarks: df = degree of freedom, F-value = value on the F distribution
Table 3. Results of multiple comparison test between aboriginal and bioproduct EPN strains. Different letters in group column indicate significant difference between nematode species (Tukey’s HSD test p ≤ 0.05)

| Nematode               | Lsmean | SE  | Lower and upper CL | Group |
|------------------------|--------|-----|--------------------|-------|
| KP76                   | 40.8   | 3.67| 33.4-48.2          | a     |
| AF 29                  | 76.7   | 3.67| 69.3-84.1          | b     |
| Bioproduct S.C.        | 79.2   | 3.67| 71.8-86.6          | b     |
| Bioproduct S.F.        | 93.3   | 3.67| 85.9-100.7         | c     |

Remarks: Lsmean = least-squares means, SE = standard error of the mean, Lower and upper CL = the lower and upper bounds of the confident intervals for the mean

Table 4. Results of multiple comparison test between applied doses of IJ within each EPN strains. Different letters in group column indicate significant difference between doses (Tukey’s HSD test p ≤ 0.05)

| Dose             | Lsmean | SE  | Lower and upper CL | Group |
|------------------|--------|-----|--------------------|-------|
| KP76 100IJ/cm²   | 33.3   | 4.08| 15.8-50.9          | a     |
| 200IJ/cm²        | 36.7   | 4.08| 19.1-54.2          | a     |
| 300IJ/cm²        | 46.7   | 4.08| 29.1-64.2          | a     |
| 400IJ/cm²        | 46.7   | 4.08| 29.1-64.2          | a     |
| AF 29 100IJ/cm²  | 66.7   | 4.08| 49.1-84.2          | a     |
| 200IJ/cm²        | 76.7   | 4.08| 59.1-94.2          | ab    |
| 300IJ/cm²        | 80.0   | 4.08| 62.4-97.6          | ab    |
| 400IJ/cm²        | 83.3   | 4.08| 65.8-100.9         | b     |
| Bioproduct S.C. 100IJ/cm² | 46.7   | 4.08| 29.1-64.2          | a     |
| 200IJ/cm²        | 86.7   | 4.08| 69.1-104.2         | b     |
| 300IJ/cm²        | 86.7   | 4.08| 69.1-104.2         | b     |
| 400IJ/cm²        | 96.7   | 4.08| 79.1-114.2         | b     |
| Bioproduct S.F. 100IJ/cm² | 86.7   | 4.08| 69.1-104.2         | a     |
| 200IJ/cm²        | 93.3   | 4.08| 75.8-110.9         | a     |
| 300IJ/cm²        | 96.7   | 4.08| 79.1-114.2         | a     |
| 400IJ/cm²        | 96.7   | 4.08| 79.1-114.2         | a     |

Remarks: Lsmean = least-squares means, SE = standard error of the mean, Lower and upper CL = the lower and upper bounds of the confident intervals for the mean
CONCLUSION

In conclusion, the first time aboriginal isolate *S. feltiae* AF29 from north Kazakhstan showed high mortality of *Tenebrionidae* larvae and had the potential to be used as a biological control agent for potato and vegetable pests. However, subsequent field research is needed to test AF29 efficacy on different pests in northern Kazakhstan’s irrigated and non-irrigated croplands.

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