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Effective Strains of *Beauveria Bassiana* and *B. Pseudobassiana* used Against the Asian Locust (*Locusta Migratoria* L.*)* in Kazakhstan

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Abstract: The purpose of the study was to perform genetic identification of the strains of fungi of the *Beauveria* genus and evaluate the effect of abiotic factors on the growth and survival of colonies and the productivity of conidia of these fungi, as well as to determine the biological effectiveness of the semi-preparative form of the drug based on *Beauveria bassiana* in the field. Conventional and modern methods accepted in biology and mycology were used. Identification of strains of *Beauveria* fungi was carried out by determining the direct nucleotide sequence of the TEF region. Laboratory experiments were carried out to evaluate various factors on the diameter of colony growth, survival in deep and surface cultivation, the type of semi-preparative form of the drug based on *B. bassiana* and the time of its exposure, and the effect on the biological effects of drugs in the field. Statistical data processing was carried out in the R studio program using the P value. The productivity of conidia of entomopathogenic fungi during surface cultivation depended on the type of strain and the duration of cultivation of conidia. The diameter of the growth of *Beauveria* fungi colonies was influenced by the factors of the strain, the type of nutrient medium, the temperature of colony cultivation, and the number of days after the start of colony cultivation. The survival of conidia of the *B. bassiana* fungus on the wings of *Locusta migratoria* L. orrelated with the indicators of cultivation temperature and germination of conidia and cultivation time. The productivity of *Beauveria spp.* conidia correlate with the type of strain, the type of substrate, and the time of exposure. The conidia continued to germinate on the 25th day. Fungi grew better on a substrate based on millet. In the field, the mortality rate of *L. migratoria* L. and the biological effectiveness of biopesticides were influenced by the factors of the strain, the form of spraying with the bio preparation, and the time elapsed after treatment with the biopesticide.

Keywords: *Beauveria Spp.* Strain, Abiotic Factors, Fungi, Insect Mortality, Biological Effectiveness

Introduction

Subspecies of the migratory locust *Locusta migratoria* L. (Orthoptera: Acrididae) have been recorded in various parts of the world and all of them are the main agricultural pests, causing significant economic damage (Kim *et al*., 2020; EFSA Panel on Nutrition *et al*., 2021). In favorable environmental conditions, some locust species exhibit gregarious and migratory behavior, which leads to the formation of spectacular swarms (Peng *et al*., 2020; Klein *et al*., 2021; Siddiqui and Khan, 2021). In some countries, such as India, Pakistan, America, Australia, China, and Africa, pest control in the populations of locusts and grasshoppers are exercised during mass reproduction outbreaks (Salih *et al*., 2020). In Kazakhstan, the outbreak of 1999 caused damage to at least 200,000 ha of agricultural land in 2018 and the subsequent damage was estimated at $15 million (Malakhov *et al*., 2018). Chemical pesticides are traditionally an effective method of locust control. However, it has been shown that this practice leads to serious environmental pollution and also entails high costs for emergency response (Rehner *et al*., 2011; Kepler and Rehner, 2013; Kovač *et al*., 2020). To mitigate the serious environmental problems that have arisen as a result of the continued overuse of chemical pesticides, biopesticides based on entomopathogenic fungi such as *Beauveria bassiana* (Rehner *et al*., 2011; Kepler and Rehner, 2013; Kovač *et al*., 2020) and *Beauveria pseudobassiana* (González-Mas *et al*., 2019; Amobonye *et al*., 2020; Dannon *et al*., 2020) are increasingly being used to control insect outbreaks. The use of these biopesticides is an effective means of biological control of locust and grasshopper pests, helps to avoid chemical residues in agricultural products, destroys a large number of locusts in cloudy conditions, and changes their morphological phase transformation of insects, preventing the formation of dense wings and swarms of locusts (Wang *et al*., 2020; Kovač *et al*., 2021; Altimira *et al*., 2022). Entomopathogenic fungi are considered in this case mainly because these fungi are safer for humans, animals, and the environment (Valverde-Garcia *et al*., 2018; Medo *et al*., 2021; Pérez-González *et al*., 2021).

The purpose of the study was to identify effective strains of fungi of the *Beauveria* genus, assess the effect of abiotic factors on them (such as nutrient medium, temperature, and cultivation time), and evaluate the biological efficiency of these strains.

Materials and Methods

The study was carried out in 2019-2021 at the Kazakh Research Institute for Plant Protection and Quarantine named after Zh. Zhiembayev (Kazakhstan) and in the Laboratory of Ecological Parasitology of the Institute of Systematics and Ecology of Animals of the Siberian Branch (SO) of the Russian Academy of Sciences (RAN) (Russia). As study materials, we used strains of an entomopathogenic fungus from the *Beauveria* genus from the collection of the biotechnology laboratory of the Kazakh Research Institute for Plant Protection and Quarantine collected in the foothill and steppe zones of Kazakhstan and Kyrgyzstan. We selected 10 strains from dead insects and the Russian BBK-1 strain was used as a reference (Table 1).

Deoxyribonucleic Acid (DNA) isolation and amplification of the Thyrotroph Embryonic Factor (TEF) region. The Cetyltrimethylammonium Bromide (CTAB) method was used for isolation (Kazartsev and Lednev, 2021), where the buffer solution contained 100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mm Ethylenediamine Tetraacetic Acid (EDTA), 2% CTAB and 100 g/mL of proteinase K. The Polymerase Chain Reaction (PCR) (Kazartsev and Lednev, 2021) was performed with primers TEF 1-f (5'–GCTCCCGGTCACCTGAYTTYAT-3' and TEF 1-r (5'-ATGACACCCACAGGACGGTCTG-3'). The identification of fungi was carried out by determining the direct nucleotide sequence of the TEF region, followed by determining the nucleotide identity with sequences deposited in the Gene Bank international database, as well as by constructing phylogenetic trees with the nucleotide sequences of reference strains (Fig. 1) (Chen *et al*., 2019).

Table 1: Strains and natural isolates of entomopathogenic fungi used in experiments to assess the biological activity of Asian locust larvae

No.	Strain name	Object of isolation	Place of isolation, year of isolation
	1	2	3
Foothill zone			
1	BCo_1-14	Coleoptera order	Almaty Region, Medeu, Zailiyskyi Alatau, 2014
2	BSc_1-15	Coleoptera order, Scolytidae family (*Ips hauseri*)	Almaty Region, Medeu, Zailiyskyi Alatau, 2015
3	BSc_2-15	Coleoptera order, Scolytidae family (*Ips hauseri*)	
4	BTr_1-16	Coleoptera order, Scolytidae family, *Trypodendron cirratum* genus	Republic of Kyrgyzstan, 2015
5	BPit-16	Coleoptera order, Scolytidae family, *Pityogenes spesivtsev* genus	
Steppe zone			
6	$BCa_{2(m)}$-09	Coleoptera order, Carabidae family	South Kazakhstan Region, Maktaaral District, 2009
7	$BCa_{3(m)}$-09	Coleoptera order, Carabidae family	
8	$BCo_{2(к)}$-09	Coleoptera order	Zhambyl Region, Kordaisky District, 2009
9	BScar-09	Scarabaeidae order	
10	BHy-09	Hymenoptera order	
11	BBK-1 (reference)	Orthoptera order, Acrididae family (*Calliptamus italicus)*	Novosibirsk Region, Karasuk District, 2000

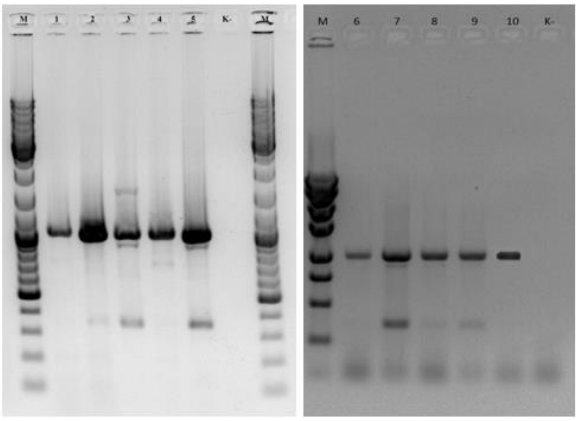

Fig. 1: Electrophoregram of the amplification products PCR at the TEF region: Notes: (1-10): Samples, numbering according to base pairs (bps); (M): Molecular weight marker (Fermentas) (100 to 10,000 bps, from 100 to 1,000 with 100 bps increment), (K-): Negative control sample

The influence of the strain factor, its origin, and exposure time on the productivity of the Beauveria genus fungi conidia with deep cultivation. We evaluated five strains of Beauveria from the foothill zone (BCo_{1-14}, BSc_{1-15}, BSc_{2-15}, BTr_{1-16}, BPit-16), five strains from the steppe zone (BCo_2(k)-09, BHy-09, BScar-09, BCa_2(m)-09, BCa_3(m)-09) and the reference strain (BBK-1) of the entomopathogenic fungus on the 3rd, 6th and 9th days after the start of strain cultivation. Deep cultivation of filamentous fungi was carried out on a modified Sabouraud medium. In sterile flasks with a volume of 750 mL with 100 mL of liquid medium, a piece of medium with a fungus culture was placed with a microbiological loop. The flasks were placed on a shaker and cultivated for 3-6-9 days. To assess the productivity of fungi in deep culture, 1-3 mL of an aqueous suspension of conidia was introduced into flasks with 100 mL of a liquid medium with a pipette. Sampling from Culture Liquid (CL) was carried out every 3 days. The blastospore titer was calculated according to the standard method using the Goryaev chamber (Kazartsev and Lednev, 2021).

The influence of the type of nutrient medium, cultivation temperature, cultivation time, strain, and its origin, on the growth diameter of colonies of the Beauveria fungus. We conducted several experiments aimed at determining the thermal preferendum of conidia of selected strains of the *Beauveria* fungus. Determination of the effect of temperature on the intensity of radial growth of fungi in surface culture Petri dishes with Sabouraud and Czapek nutrient media, which were then placed in thermostats (IPP 300, Memmert, Germany). We evaluated five strains of *Beauveria* from the foothill zone (BCo_{1-14}, BSc_{1-15}, BSc_{2-15}, BTr_{1-16}, BPit-16), five strains from the steppe zone (BCo_2(k)-09, BHy-09, BScar-09, BCa_2(m)-09, BCa_3(m)-09) and the reference strain (BBK-1) of the entomopathogenic fungus. The level of germination of the fungus conidia was determined at air temperatures of 20, 25, 30, and 35ºC. The increase was calculated for 29 days with an interval of 2 days. For this purpose, the diameter of the colony was measured in two directions. A four-fold repetition of the experiment was used for each strain (Kazartsev and Lednev, 2021).

The influence of air temperature, germination of conidia, and their exposure time on the survival of the conidia of the Beauveria fungus. The calculation of the rate of germination of conidia of the studied strains of fungi was carried out on the wings of locusts. Insect wings were immersed in an aqueous suspension of the fungus with a dilution of 5×10^6 for 10 sec. Then they were placed in a Petri dish with a moistened filter, in a thermostat at different temperature conditions (+20, 25, 30, and 35°C). The preparation was viewed after 12, 24, 36, 48, 60, and 72 h under a microscope in 10 fields of view, germinated and non-germinated conidia were counted using a Carl Zeiss Axioskop 40 light microscope at 25°C and 99% Relative Humidity (RH). The repetition was four-fold by 100 conidia (Seid *et al*., 2019).

The influence of strain factors, its origin, and type of nutrient substrate on the productivity of Beauveria conidia with surface cultivation. The study of productivity during surface cultivation was carried out on solid grain substrates (rice, peas, millet, and pearl barley). 10 mg of grain substrate was poured into glass flasks with a volume of 500 mL, topped up with 5 mL of distilled water. The flasks were covered from above with foil and kraft paper and sterilized in the mode of 0.8 atmospheres for 30 min. Then they were sterilized and shaken again. Then 1 mL of an aqueous suspension of the fungus with a titer of 1×10^5 was poured into the flasks and cultivation was carried out in a thermostat at a temperature of 25ºC. Sampling was carried out once in 5 days. The conidia were collected by flushing from the substrate. Then the titers were determined (Seid *et al*., 2019).

The influence of the semi-preparative form of the preparation based on the *Beauveria bassiana* factor and the time of exposure on biological efficiency in the field. In 2020-2021, the area of distribution of Asian locusts in the Zhambyl region was 22,210 and 25,730 ha. The biological effectiveness of a semi-preparative form based on the conidia of the fungus *B. bassiana* was evaluated on the larvae of younger ages of the Asian locust. In the field, the plots were processed in their natural stations using a Mikroner AU-8000 portable motor sprayer (the titer of the working suspension was at the rate of 5×10^7 conidia/ha). The treatment was carried out with an oil and water suspension of conidia of selected *B. bassiana* strains. The flow rate of the working fluid was 10 l/ha. The plot area was 300 to 400 m^2 with quadruple repetition. The treatment was carried out in the evening hours before sunset. At the main hospital of the biotechnology laboratory of the Kazakh Research Institute for Plant Protection and Quarantine, the treated locust larvae collected in the field were placed in plastic containers at room temperature and outdoors under gas fabric insulators (Seid *et al*., 2019). Insect mortality was taken into account as a percentage on days 5, 7, 9, 11, 13, 15, 17, 19, and 21 after the inoculation with the strain (Seid *et al*., 2019).

Statistical processing of the received data. The statistical data processing was carried out in the R studio program using the P value (Dutbayev *et al*., 2006; Kuldybayev *et al*., 2021). We tested the reliability of five hypotheses.

Hypothesis 1 the type of strain, its origin, and exposure time do not have (H_o) or (H_A) influence on the productivity of conidia of fungi of the *Beauveria* genus during submerged cultivation.

Hypothesis 2 the type of nutrient medium, cultivation temperature, cultivation time, strain, and its origin does not have (H_o) or (H_A) influence on the growth diameter of *Beauveria* fungus colonies.

Hypothesis 3 air temperature, germination of conidia of the fungus Beauveria, and exposure time do not have (H_o) or have (H_A) influence on their survival.

Hypothesis 4 the type of strain, its origin, and the type of nutrient substrate do not have (H_o) or have (H_A) influence the productivity of *Beauveria* conidia during surface cultivation.

Hypothesis 5 the type of semi-preparative form of the drug based on *Beauveria bassiana* and its exposure time does not have (H_o) or (H_A) effect on its biological effects in the field.

Results

The results of genetic identification of strains in the TEF region showed that strains from the foothill zone of the Almaty region of Kazakhstan and Kyrgyzstan were representatives of the species *Beauveria pseudobassiana* (BCo_1-14; BPit-16; BSc_2-15; *B. bassiana* (BSc_{1-15}) and *B. varroae* (BTr_1-16). Hybrid strains of the South Kazakhstan and Zhambyl regions of Kazakhstan were represented by *B. bassiana* (BCa_2(m)-09; BHY-09, BCa_3(m)-09) and *B. pseudobassiana (*BScar-09 and BCo_2(k)-09). The results of the identification of 10 strains in the TEF region are shown in Fig. 2.

The influence of the strain factor, its geographical area, and time of exposure on the productivity of Beauveria conidia with surface cultivation.

Histograms of the distribution of the general sets of dependent variables showed that the data had an abnormal distribution (Fig. 2 and 3). In this regard, a nonparametric analog of the analysis of variance for the associated Kruskal-Wallis samples was used for the analysis (Fig. 3).

The evaluation of the conidia productivity of the *Beauveria* genus with deep cultivation showed that this variable significantly depended on both the strain factor and the duration of cultivation of the entomopathogenic fungi conidia (P<0.01). The strains similar in terms of productivity with deep cultivation of conidia to the BBK-1 reference strain (isolated from insects of the *Orthoptera* order, *Acrididae* family of *Calliptamus italicus* from the Karasuksky district of the Novosibirsk region of Russia) turned out to be BSC1-15 and BCa_3(m)-09 (Table 2).

The origin of first strain is based on the *Beauveria bassiana* fungus isolated from beetles of the *Coleoptera* order, *Scolytidae* family (*Ips hauseri*) in the Almaty region of Kazakhstan in 2014. The second strain of *B. bassiana* fungus was isolated from beetles of the *Coleoptera* order, *Carabidae* family, in the South Kazakhstan region in 2009 We can note that over time, the productivity of conidia increased, equaling 375,255,909 conidia on the 3rd day, 1,222,380,682 on the 6th day and 1,687,953,080 on the 9th day (Table 2).

In laboratory conditions, it was found that the diameter of the growth of *Beauveria* fungal colonies was statistically significantly influenced by the factors of the strain, the type of nutrient medium, the temperature of colony cultivation, and the number of days after the start of colony cultivation (P<0.01, Table 3). The strains were grown on Sabouraud and Czapek nutrient media and the average colony length was 22.6 and 23.0, respectively. Fungal colonies grew more intensively at a temperature of 20-25°C. At this temperature, the diameter of the colonies was 33.5-36.6 cm. At 30°C the growth of colonies slowed down reaching 20.1 cm and at 35°C it practically stopped (Table 3). The colonies of BSC_{1-15} and BCa_3(m) strains grew more intensively, compared with this indicator for the BBK-1 reference strain. The average diameter of the colonies was 25.7, 27.1, and 14.9 cm, respectively (Table 3).

The dependent survival variable of the conidia of the *Beauveria* fungus on the wings of locusts was statistically significantly influenced by factors of cultivation temperature, germination of conidia, and cultivation time (P<0.01, Table 4). The optimal temperature for the growth of conidia on the wings of locusts was 20-30°C (9.3-11.0 conidia) and at 35°C the number of conidia was statistically significantly reduced to 8.5 thousand. On average, 8.0 thousand conidia were germinated and 13.0 thousand were not germinated. After 12, 24, 36, and 48 h, the rate of germination of conidia on the wings of locusts was in the range of 9.8 to 10.4 thousand and after 60 and 72 h, it began to decrease to 9.5 to 9.8 thousand (Table 4).

Laboratory evaluation showed that the productivity of *Beauveria* conidia with surface cultivation was statistically significantly influenced by the factors of strain, type of solid substrate, and time of exposure (P<0.01, Table 4). The most productive strains were BSC_{1-15}, BCa_3(m)-09 and BBK-1 (reference), amounting to 525,546,875, 482,656,250 and 483,081,328,

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respectively. Conidia continued to germinate on the 25th day. Fungi grew better on a solid substrate based on millet (341,154,000), but they could also grow on a medium based on millet, pearl barley, rice, and peas (186 to 251 million conidia) (Table 5).

The mortality of Asian locust larvae in the field in 2021 was statistically significantly influenced by the factors of the strain, the form of spraying, and the time elapsed after treatment ($P<0.01$). The insect mortality in the *Beauveria bassiana* BSC_{1-15} and BCa_3(m)-09 strains and the BBK-1 strain (reference) (Russia) amounted to 61.1; 56.4 and 66.5, respectively. When used in the semi-preparative form of oil and water, this indicator was at the level of 63.0 and 57.8%. The insect mortality in laboratory and field conditions on days 15-21 after treatment was 77-89% (Table 6).

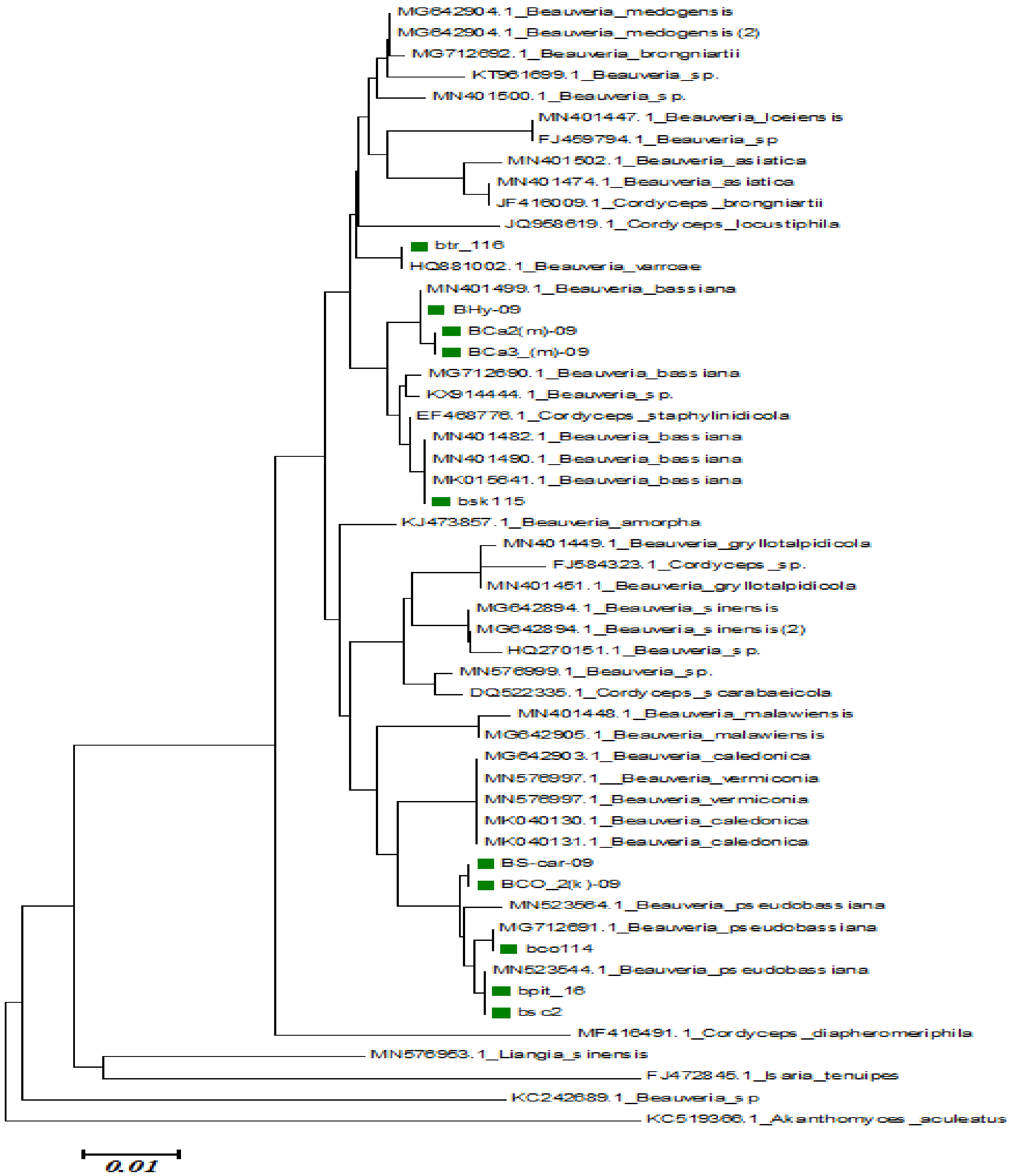

Fig. 2: Phylogenetic tree based on the analysis of the TEF fragment of the region of the *Beauveria* genus

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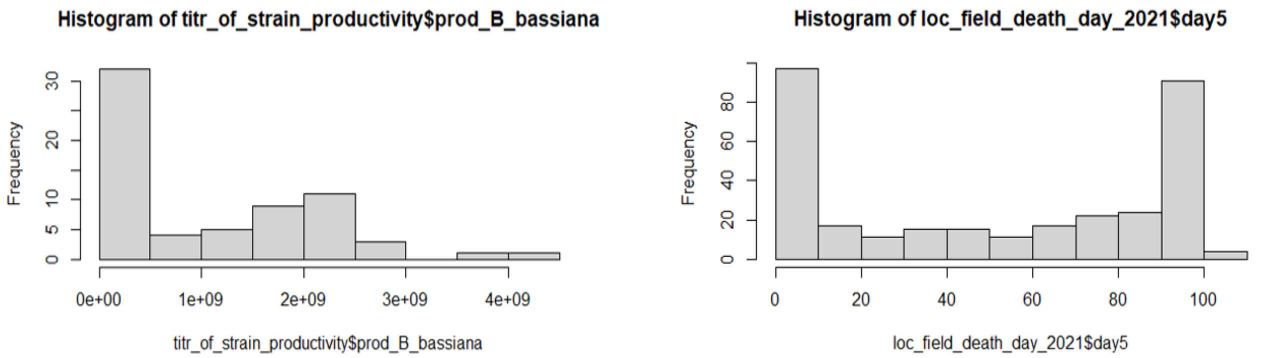

Fig. 3: The distribution of general sets of dependent variables: Note: A is the variable productivity of *Beauveria* conidia with surface cultivation; B is the diameter of growth of *Beauveria* fungus colonies

Table 2: The effect of the strain factor and the time of exposure on the productivity of *Beauveria* conidia with surface cultivation

Factor	Factor level	Number of conidia, pcs	P value
Strain	BSC_{1-15} based on *Beauveria bassiana* (Kazakhstan)	2,078,020,833	<0.01***
	BCa_3(m)-09 based on *B. bassiana* (Kazakhstan)	1,738,046,750	
	BBK-1 (reference) based on *B. bassiana* (Russia)	2,237,270,833	
Days after cultivation	3	375,255,909	<0.01***
	6	1,222,380,682	
	9	1,687,953,080	

Note: In this and subsequent tables, *weak correlation; **medium correlation, ***strong correlation at 99% confidence interval

Table 3: Influence of such factors as the nutrient medium, cultivation temperature, cultivation time, strain, and the strain geographical zone, on the growth diameter of the *Beauveria* fungus colonies

	Factor levels	Colony diameter, cm	P value
Nutrient medium	Sabouraud	22.60	<0.01***
	Czapek	23.00	
Cultivation temperature, ºC	20.0	33.50	<0.01***
	25.0	36.60	
	30.0	20.10	
	35.0	0.60	
Strain BSC_{1-15} based on *Beauveria bassiana* from the foothill zone	25.7	<0.01***	
Strain BCa_3(m)-09 based on *B. bassiana* from the steppe zone	27.1		
BBK-1 (reference) based on *B. bassiana*	14.9		
Days after cultivation	3.0	2.40	<0.01***
	5.0	4.60	
	7.0	8.90	
	9.0	11.50	
	11.0	14.30	
	13.0	19.00	
	15.0	21.90	
	17.0	25.20	
	19.0	29.00	
	21.0	31.00	
	23.0	33.60	
	25.0	36.40	
	27.0	39.00	
	29.0	42.80	

Table 4: Influence of various factors of temperature, germination of conidia, and exposure time on the dependent variable of survival of conidia of the *Beauveria bassiana* fungus

Factor	Factor levels	Germination of *Beauveria* conidia on the wings of locusts, thousand	P value
Cultivation temperature, °C	20	9.3	<0.01***
	25	10.2	
	30	11.0	
	35	8.5	
Germination of conidia	Germinated	8.0	<0.01***
	Non-germinated	13.1	
Hours	12	10.1	<0.01***
	24	10.4	
	36	9.8	
	48	10.0	
	60	9.8	
	72	9.5	

Table 5: Effect of strain factor, solid substrate, and time of exposure on the productivity of *Beauveria* conidia with surface cultivation

Factor	Factor levels	Number of conidia	P value
Strain $BSC_{1\text{-}15}$ based on *B. bassiana*		525,546,875	<0.01***
Strain BCa_3(m)-09 based on *B. bassiana*		482,656,250	
BBK-1 (reference) based on *B. bassiana*		483,081,328	
Days	5	61,717,038	<0.01***
	10	131,049,787	
	15	202,419,389	
	20	359,363,849	
	25	524,658,878	
Substrate	millet	341,154,000	<0.01***
	pearl barley	251,618,238	
	rice	244,054,119	
	peas	186,540,795	

Table 6: Influence of *B. bassiana* strain factors, the strain geographical zone, the form of spraying, and time after treatment on the mortality of Asian locust larvae in laboratory and field conditions, 2021

Factor	Factor levels	Larval mortality, %	P value
The strain of the *B. bassiana* fungus	$BSC_{1\text{-}15}$	61.1	<0.01***
	BCa(m)-09	56.4	
	BBK-1 (reference)	66.5	
	control	0.0	
Form of spraying	Oil	63.0	<0.01***
	water	57.8	
Insect mortality (%), day after inoculation with the strain	5	1.1	<0.01***
	7	6.9	
	9	23.0	
	11	47.8	
	13	66.3	
	15	77.5	
	17	85.8	
	19	88.9	
	21	89.4	

Table 7: The effect of the factor of the *Beauveria bassiana* strain, the semi-preparative form of the preparation, and the time of exposure on the *biological efficiency*

Factor	Factor level	Biological efficiency of Beauveria, %	P value
Strain	$BSC_{1\text{-}15}$	61.1	<0.01***
	BCa_3(m)-09	56.4	
	BBK-1 (reference)	66.5	
	Control, without treatment	0.0	
Semi-preparative form	oil	61.1	<0.01***
	Water	57.5	
	control	0.0	
Titer	5x10-7	59.5	<0.01***
	Control, without treatment	4.4	

The indicator of the biological effectiveness of the treatment of Asian locust larvae in the field in 2021, was also statistically significantly influenced by the factors of the strain, the form of spraying, and the time elapsed after treatment (P<0.01). This indicator for the *Beauveria bassiana* strains BSC_{1-15} and BCa_3(m)-09 and BBK-1 (reference) (Russia) amounted to 61.1, 56.4, and 66.5, respectively. When used in the semi-preparative form of oil and water, this indicator was at the level of 61.1 and 57.5%. (Table 7).

Discussion

Chen *et al*. (2019) identified 231 isolates on the dead insects bearing anamorphic or teleomorphic structures of fungal fruit in the conditions of seven types of vegetation of the Gaoligong Mountains at a height difference from 600 to 3,800 m. The following species were identified: *Beauveria asiatica, B. bassiana, B. brongniartii, B. caledonica, B. medogensis, B. pseudobassiana* and *B. sinensis, B. baoshanensis sp. nov., B. malawiensis, B. rudraprayagi* and *B. yunnanensis sp. nov.* Most isolates were identified as *B. bassiana* and *B. pseudobassiana* (86.1%). Kazartsev and Lednev (2021) studied the distribution and genetic diversity of 91 isolates of Beauveria collected during long-term accounting in boreal forests of the north of the European part of Russia. Based on morphological analysis and sequence analysis of TEF and Bloc loci, *B. pseudobassiana, B. bassiana, and B. caledonica* species with populations of 81, 11 and 8%, respectively, were identified (Kazartsev and Lednev, 2021). Strains from the foothill zone of Kazakhstan and Kyrgyzstan were represented by such species as *Beauveria pseudobassiana, B. bassiana, and B. varroae*. The hybrid strains of Southern Kazakhstan were represented by *B. bassiana* and *B. pseudobassiana*. The evaluation of the conidia productivity of the *Beauveria* genus with surface cultivation has shown that this variable significantly depends on both the strain factor and the duration of cultivation of the entomopathogenic fungi conidia (P<0.01). The strains BSC_{1-15} and BCa_3(m)-09 are close to the BBK-1 reference strain in terms of conidia productivity. The diameter of the growth of *Beauveria* fungus colonies is statistically significantly influenced by the factors of the strain, the type of nutrient medium, the temperature of colony cultivation, and the number of days after the start of colony cultivation (P<0.01).

Orquídea Pérez-González *et al*. (2021) studied the effect of cultivation temperature, pH, light, culture, and substrate medium with additives on vegetative growth and formation of conidia and the virulence of strains of Beauveria sp against insects of the Lepidoptera and Coleoptera genera. Dextrose, peptone, and yeast agar with wheat bran or amaranth flour, at 23-25°C, with a pH of 7.5-8.5 and dim light were optimal for radial growth of the fungus colony. The light and pH for most of the conidia formation depended on the strain of the fungus. The biological efficacy of fungal strains against Phyllophaga sp. (Coleoptera: Scarabaeidae) and Spodoptera exigua Hübner and Trichoplusia ni Hübner (Lepidoptera: Noctuidae) was 91.1-96.0%.

The temperature value on growth was also noted in studies by Valverde-Garcia *et al*. (2018), where the growth of hyphae of the *Beauveria bassiana* strains (Bals. -Criv.) Vuill was better adapted to the temperature regime of 10 to 25°C. The temperature factor was also noted in the work of Seid *et al*. (2019) where the hyphae of *B. bassiana* showed the best optimum growth and productivity at temperatures from 8 and 12°C and up to 32°C in vitro (Seid *et al*., 2019).

Medo *et al*. (2021) tested the virulence of 46 soil isolates of Beauveria spp. against *Ostrinia nubilalis*. All strains had been collected from the soil by the Galleria mellonella bait method. A suspension of conidia with a concentration of 107 conidia per ml was used to determine virulence against O. nubilalis larvae of the 4th age at a temperature of 25°C. After 14 days, the mortality of larvae was in the range of 34-96%. The mortality did not depend on the habitat or any other environmental parameters of the places from which the isolates had been obtained. According to our data, the mortality of Asian locust larvae in the field in 2021 was statistically significantly influenced by factors of strain, the form of spraying, and time elapsed after treatment. The mortality of Asian locust larvae in the field in 2021 was statistically significantly influenced by the factors of the strain, the form of spraying, and the time elapsed after treatment (P<0.01). The insect mortality in the *Beauveria bassiana* BSC_{1-15} and BCa_3(m)-09 strains amounted to 55-65%.

The dependent survival variable of the conidia of the *Beauveria bassiana* fungus on the wings of locusts is statistically significantly influenced by factors of cultivation temperature, germination of conidia, and cultivation time (P<0.01). The productivity of *Beauveria* conidia is influenced by the factors of strain, type of substrate, and time of exposure (P<0.01). The most productive strains were BSC_{1-15}, BCa_3(m)-09 and BBK-1 (reference), amounting to 525,546,875, 482,656,250 and 483,081,328, respectively. Conidia continued to germinate on the 25th day. The fungi grow better on a substrate based on millet.

Conclusion

It has been established that the strains from the foothill zone of Kazakhstan and Kyrgyzstan are represented by the species *Beauveria pseudobassiana, B. bassiana, and B. varroae*. The hybrid strains of Southern Kazakhstan are represented by *B. bassiana* and *B. pseudobassiana.*

The productivity of conidia of entomopathogenic fungi during surface cultivation depended on the strain type and the duration of conidia cultivation. The growth diameter of *Beauveria* fungus colonies was influenced by factors of the strain, the type of nutrient medium, the temperature of colony cultivation, and the number of days after the start of colony cultivation. The survival rate of conidia of the fungus *Beauveria bassiana* on the wings of the locust *Locusta migratoria* correlated with the factors of cultivation temperature, conidial germination, and cultivation time. The productivity of *Beauveria spp.* correlated with the strain type, substrate type, and exposure time. Fungi grew better on the millet-based substrate. In the field, the mortality of *Locusta migratoria* L. and the biological effectiveness of biopesticides were influenced by strain factors, the form of spraying with a biological product, and the time elapsed after treatment with a biopesticide. In our future studies, we plan to study the morphological and cultural characteristics and pathogenicity of isolates of fungi of the *Beauveria* genus.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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