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# Evaluation of the Efficacy of Entolek K Planteco® Biopesticide Based on *Akanthomyces Lecanii* Fungus Against Pest Orthopterans in Soybean Agroecosystems in Southeast Kazakhstan

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**Abstract:** Pest control services dealing with mass pests such as locusts and grasshoppers are forced to use insecticides of various origins to prevent crop damage. In most cases, mainly chemical insecticides are used. This method of regulating pest populations has many negative consequences. As a result of chemical treatments with broad-spectrum insecticides, not only pests are killed, but also non–target organisms, primarily entomophages and pollinators. One of the alternatives to the chemical method of control is the use of biopesticides based on entomopathogenic viruses, bacteria, and fungi. The impact of the biopesticide "Entolek K Planteco®" based on the fungus *Akanthomyces lecanii* on different species of locusts, grasshoppers, and katydids was evaluated in soybean plantings of southeast Kazakhstan. Locust and grasshopper mortality was 70% on the 3rd day after treatment and 98% on the 7th day, while katydid mortality on the same days was 50 and 70% respectively. Thus "Entolek K Planteco ®" proved efficient for controlling harmful locusts, grasshoppers and katydids. This is especially important in sensitive areas where the use of chemical insecticides is legally prohibited (water protection zones, specially protected natural areas, agricultural land for growing organic products.

**Keywords:** Biopesticide, *Akanthomyces Lecanii*, Locusts, Grasshoppers, Katydids

## Introduction

Harmful orthopterans, including locusts, grasshoppers, and katydids, cause significant damage to agriculture in Kazakhstan. Since 2000, which coincided with the peak of the biggest outbreak of Italian locusts in Kazakhstan ever, more than 100 articles on locust management have been published. This is about 30% of all locust publications in the country in the 105 years preceding this locust outbreak (Paly, 1970; Lachininsky *et al*., 2002; 2016; Kharchenko, 2010; Temreshev *et al*., 2013; Sergeev *et al*., 2016; Temreshev and Esenbekova, 2017; Kambulin, 2018; Temreshev and Makezhanov, 2020).

During mass outbreaks, chemical insecticides such as organophosphates and pyrethroids remain the first line of defense against such economic pests as locusts and grasshoppers (Christie, 1936; Solter *et al*., 2012; Dakhel *et al*., 2019). As a result of treatments with broad-spectrum chemical insecticides, not only pests are killed, but also non-target arthropods, primarily entomophages and pollinators (Canning, 1953; Fasulati, 1971; Sokolov, 2000; Hajek and Eilenberg, 2018; Gillott, 2005; Alzate and Gutiérrez, 2008; Smith *et al*., 2006; Antoniou *et al*., 2012; Seagraves and Lundgren, 2012; Temreshev *et al*., 2016a). Repeated applications of chemical insecticides, in addition to reducing the biodiversity of the beneficial entomofauna of crop fields, may cause the emergence of resistant pest populations (FAO, 2012; Sandhu *et al*., 2012; Arling *et al*., 2014; Zhang *et al*., 2018; Nurzhanov, 2019). In addition, some synthetic pesticide degradation products can accumulate in the soil, vegetation, tissues, and organs of humans and domestic animals, subsequently causing various teratogenic disorders (Garry *et al*., 2002; Sánchez-Bayo *et al*., 2013; Madu, 2015; Berheim *et al*., 2019; Garcês *et al*., 2020; Tudi *et al*., 2021).

The elimination of these undesirable consequences is possible only through the search for new, highly effective, and at the same time environmentally safe methods of plant protection. It is the problems of environmental protection that determine the development of new non-chemical methods of plant protection, including biological control. Biological control is considered an alternative in the system of protective measures and at the same time, due to its specific features, it is the basis for the development of environmentally safe, economical, and long-term programs for the control of harmful organisms. Biological control of pests is based on the use of natural parasitic and predatory insects, fungal, bacterial, and viral microorganisms, and their waste products (Hajek and Eilenberg, 2018; Lord, 2005; Ganassi *et al*., 2010). The mechanism of action of biological plant protection products is manifested in the form of parasitism and destruction of harmful organisms by entomophages, bacteria, fungi, and viruses, as well as the use of their antagonistic properties concerning plant diseases (Hajek and Eilenberg, 2018; Glare *et al*., 2017). As a rule, biological protective agents have a narrow selective ability, thus they do not cause damage to humans and the environment in comparison with chemical pesticides (Streett and McGuire, 1990; Streett *et al*., 1997; Temreshev and Childebaev, 2015; Temreshev *et al*., 2016b; Dakhel *et al*., 2019).

There are several groups of microorganisms, which parasitize on locusts, grasshoppers, and katydids (Lomer *et al*., 2001; Hajek and Eilenberg, 2018; Dakhel *et al*., 2019; 2020; Lednev *et al*., 2020). Alpha-proteobacteria, entomophthora fungi, and microsporidia occasionally may make some impact as natural regulators of population dynamics (Chernyshev, 1961; Henry, 1981; de Faria and Wraight, 2007; Jaronski, 2014; Lednev *et al*., 2020). The most significant group of biopesticide agents for pest orthopteran control are entomopathogenic fungi (Jaronski, 2014; Sinha *et al*., 2016; Bakhvalov, 2001).

The Entomopathogenic fungus, *Akanthomyces lecanii* (Zimm.) *Lecanicillium lecanii* (Gams and Zare, 2001) is one of the potential microbial biocontrol agents which have a wide host range (Gams and Zare, 2001). This is a species of ascomycete fungus belonging to the genus *Akanthomyces* of the *Cordycipitaceae* family. Previously, its anamorph was allocated to the genus *Lecanicillium* or included in the *Verticillium*. The teleomorph was previously called *Córdyceps confragosa* (Vinit *et al*., 2018). These species have been divided into several new taxonomic entities, including *A. lecanii, A. longisporum, A. attenuatum, A. nodulosum*, and *A. muscarium* (Lipa *et al*., 1994; Goettel *et al*., 2008). For example, several recent papers, such as Kouvelis *et al*. (2004) who carried out mitochondrial DNA studies, refer to the name *L. muscarium*. *A. lecanii* itself appears primarily to be a pathogen of soft scale insects (Coccidae) (Goettel *et al*., 2008). This fungus was first described in 1861 and has a worldwide distribution. Insects are infected when they come into contact with sticky fungal spores, which then grow and invade the body, thus the internal organs are consumed, leading to the death of the host. In horticulture and agriculture, "*A. lecanii*" isolates were developed for controlling insect pests such as whitefly, thrips, and aphids, by R.A. Hall and H.D. Burges.

Biological pesticides based on *Lecanicillium* spp. are now marketed as "Mycotal" (now *L. muscarium*) and "Vertalec" (now *L. longisporum*) by Koppert company in the Netherlands (Fadayivata *et al*., 2014). Other biopesticides based on these fungi have been developed elsewhere for use in cash crops, oil seeds, soybeans, ornamentals, and vegetables (HPRTRK, 2018).

Nowadays, biopesticides based on the fungus *A. lecanii*, and in particular "Entolek K Planteco®" Emulsifiable Concentrate (EC, manufactured by "Biopreparat" Trading House, Russia) are gaining importance in protecting crops from a variety of pests (Koval, 2007; Yankouskaya and Voitka, 2016; Temreshev *et al*., 2019a; 2019b). However, their effectiveness against locusts, grasshoppers, and katydids has not been studied. In this study, we report the results of the field studies intended to fill this gap.

## Materials and Methods

### *Identification of Orthopterocenosis in Soybean Crops*

As objects, we used various species of pest locusts (Acrididae: *Calliptamus italicus* (Linnaeus,1758)), grasshoppers (Eumastacidae: *Gomphomastax clavata*), crididae: *Calliptamus barbarus, Chorthippus apricarius* (Linnaeus, 1758), *Chorthippus biguttulus* (Linnaeus, 1758), *Epacromius tergestinus, Euchorthippus pulvinatus, Oedipoda caerulescens* (Linnaeus, 1758), *Stenobothrus fischeri)*, and katydids (Tettigoniidae: *Decticus verrucivorus* Linnaeus, 1758, *Tettigonia viridissima* Linnaeus, 1758, *Tessellana vittate*). Identification of locust, grasshopper, and katydid species was done using the available keys, and an assessment of their economic importance was carried out based on existing literature (Lockwood *et al*., 1999; Lachininsky *et al*., 2002; Sokolova *et al*., 2003; Storozhenko *et al*., 2004; Tao *et al*., 2006; Telli *et al*., 2014; Sergeev *et al*., 2016).

### *Laboratory Experiments*

The laboratory experiments were conducted in May 2019 in the Almaty region, SE Kazakhstan, on Italian Locust, non-swarming grasshopper, and katydid species.

Before conducting field trials of the biopesticide, we conducted its laboratory testing. For this purpose, we selected nymphs of III-IV instars of locusts (Acrididae: *Calliptamus italicus* (Linnaeus, 1758), grasshoppers (Acrididae: *Calliptamus barbarus, Chorthippus apricarius* (Linnaeus, 1758), *Chorthippus biguttulus*

(Linnaeus, 1758), *Epacromius tergestinus, Euchorthippus pulvinatus, Oedipoda caerulescens* (Linnaeus, 1758), *Stenobothrus fischeri*, Eumastacidae: *Gomphomastax clavata* and katydids (Tettigoniidae: *Decticus verrucivorus* Linnaeus, 1758, *Tettigonia viridissima* Linnaeus, 1758, *Tessellana vittata*.

The biopesticide was diluted to the concentration of 1%, at a ratio of 400 mL of water with the addition of 4 mL of "Entolek К Planteco®" EC. After that, the solution was applied to the bottom of a 1 l plastic glass for the treated group, and tap water was used for the untreated control group. Suspension is applied as a spray using a hand sprayer. The exposure for both the experimental and the control groups lasted for one, three, five, seven, and 10 min. For each group of test insects (locusts, grasshoppers, and katydids) three replications of 10 individuals each were made. To estimate the time after which full immobilization of the pests upon contact with the biopesticide occurs, we used a stopwatch after the insect was treated. The treated insects, which survived the first 10 min, were then placed in a clean plastic cup, fed, and examined daily for 10 days. Fresh food was continuously added to the treated and untreated control batches to prevent insect mortality due to starvation or cannibalism.

### *Experimental Plot and Design*

Treatment of experimental plots with biopesticide "Entolek К Planteco®" EC was carried out with the backpack sprayer CRS-25 (manufacturer Caiman, Japan).

The experimental design included four-hectare plots in 4 replications for both, treatment and untreated control (treated with water).

### *Field Experiments*

In the fields of soybean plantings in the form of "Baiserke Agro" LLP the impact of the biopesticide "Entolek К Planteco®" EC based on fungus *A. lecanii* was assessed on pest Orthopterans-locusts, grasshoppers and katydids.

During the field experiment, the weather was mostly cloudy, but no precipitation. However, 2-3 days were sunny and hot, with temperatures up to 32°C. The average temperature was 28°C. The water used to prepare the working solution of the biopesticide is ultra-fresh, of medium hardness, and slightly acidic in terms of pH (the pH of the water was 6.55, close to neutral). The VDM droplet size of the spray equipment used for the treatment was 150 microns. The height of the soybeans averaged 85 cm during field treatment, an estimated percentage canopy of 74%. The biopesticide was diluted to the concentration of 1%, similar to the laboratory experiment. The titer of the biopesticide was 1.8*109 Conidia Forming Units (CFU, information from product label)/ml (liquid complex of *A. lecanii* culture, and products of its metabolism (entomotoxins), manufactured by "Biopreparat" Trading House, Russia), the spraying dose rate was 2.5 L/ha. The assessment of the effectiveness and duration of the toxic action of the biopesticide was conducted per the "Methodological recommendations on the toxicological assessment of insecticides for locust control in the field", using modified Abbott's formula (Kurdyukov, 1987) where: EB = effectiveness of the biopesticide, A = the number of live insects after processing, *Ao* = number of live insects before treatment:

$$EB = \frac{A}{A_o} \times 100 \quad (1)$$

The predominance of locust, grasshopper, and katydid species was determined using the Balog Index, Di, which reflects the ratio of the Number of Individuals (NI) of any species to the total number of individuals of all species (N) in the habitat (Balogh, 1958):

$$Di = \frac{ni}{N} \times 100 \quad (2)$$

The analysis of variance was performed using the R studio software. The acceptable level of significance was determined using the P-value (Aphalo, 2017).

## Results

### *Laboratory Testing*

Results of laboratory tests of "Entolek К Planteco®" EC are presented in Tables 1 and 2, and in Fig. 1. The numbers in Table 1 and 2 indicate the number of insects that remained alive during the indicated period (3, 7, and 10 min after treatment with the biopesticide suspension). Locust and grasshopper nymphs were even less resistant than the three katydid species. Their death rate was 90-100% in 7 min after treatment (Fig. 2). Since the chitin of locust nymphs is even less dense than that of katydids, and their sizes are smaller, we can also follow the above pattern in this respect. Only Italian Locust *C. italicus* and blue-winged grasshopper *Oe. caerulescens* nymphs died a little slower than other species.

The first, second, and third columns show values treated with biopesticide, and the fourth column shows values untreated with biopesticide (Fig. 1).

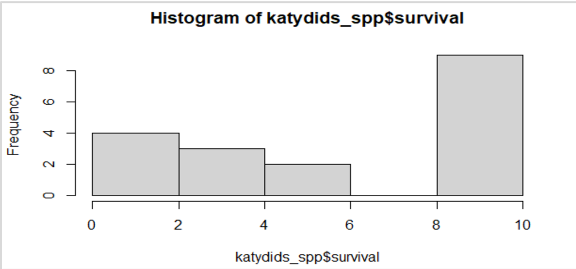


**Fig. 1:** The average distribution of a Survival variable in minutes after exposure to Entolek K Planteco® EC on nymphs of III-IV instars of different katydids species under laboratory conditions

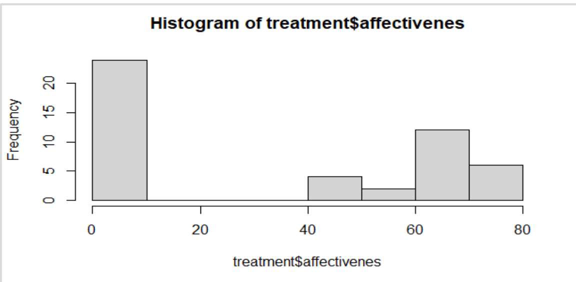


**Fig. 2:** The general distribution of biological efficacy of the biopesticide Entolek K Planteco® EC against pest katydids on soybean crops (Almaty region, village Arkabay, "Baiserke Agro" LLP, 2019)

### *Field Trials*

The field experiment was conducted in June 2019 in the Almaty region, SE Kazakhstan, on non-swarming grasshopper species and Italian Locust on a soybean farm.

Preliminarily, monitoring of soybean plantation areas for infestation by orthopteran pests was conducted. The species composition was determined and predominant locust, grasshopper, and katydid species were identified (Table 3).

After identifying species composition and abundance of target orthopteran pests, treatment to assess the biological efficacy of the biopesticide was carried out. Post-treatment monitoring of treated insects showed high biological efficacy of the tested biopesticide (Table 4, 5).

The first column shows the values of untreated biopesticides, in columns 2, 3, 4, and 5, the values of the biological effectiveness of the biopesticide (Fig. 2).

## Discussion

Results of laboratory tests "Entolek K Planteco®" EC are presented in Tables 1 and 2, Fig. 3. Katydids *D. verrucivorus* and *T. viridissima* showed slightly higher resistance than *T. vittata*. It can be assumed that this difference is largely due to the thickness of the chitin cuticle and the size of the studied insects, which to some extent protects them from the effects of the biopesticide. The first two katydid species have denser chitin cuticles and larger sizes than the third species.

Locust and grasshopper nymphs were even less resistant than the three katydid species. Their death rate was 90-100% in 7 min after treatment (Fig. 4). Since the chitin of locust nymphs is even less dense than that of katydids, and their sizes are smaller, we can also follow the above pattern in this respect. Only Italian Locust *C. italicus* and blue-winged grasshopper *Oe. caerulescens* nymphs died a little slower than other species.

As in laboratory experiments, locust pests were affected more quickly in field trials than katydid.

As can be seen from Table 3, katydids *T. viridissima* and *T. vittata*, Italian Locust *C. italicus*, non-swarming grasshopper *C. barbarus*, several species of grasshoppers of the *Chorthippus* genus, and *Stenobothrus fischeri* were predominant among pest orthopterans in soybean field (Fig. 5).

The maximum pest mortality rate on day 7 after treatment with 70.3% for katydids and 98.1% for locusts and grasshoppers respectively in the field. Ten days after application, the efficacy of the biopesticide remained almost at the same level (Table 4, 5). It should be noted that already on the 3rd day after treatment the mortality rate was 50.1% for katydids and 70.2% for locusts. For a biopesticide, such efficacy can be considered quite high.

It should be noted that the efficacy assessment was somewhat difficult both by the rapid decomposition of dead Orthoptera and the fact that the corpses were quickly destroyed by necrophagous insects (ants, beetles, etc.) and birds (Fig. 6, 7).

In the laboratory, an overgrowth of collected dead locust and grasshopper specimens with fungal mycelium in a humid chamber was noted (Fig. 8).

In general, the rapid action of the biolpesticide on harmful Orthoptera is explained by the presence of a significant amount of entomotoxins in the culture fluid of the preparation. Due to this, the first signs of biolpesticide action appear very quickly.

**Fig. 3:** Nymphs of katydids, dead from biopesticide Entolek K Planteco®

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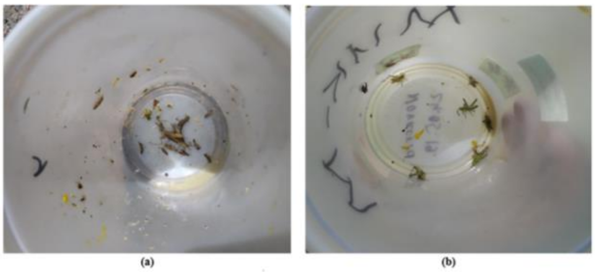


**Fig. 4:** Nymphs of grasshoppers dead from biopesticide Entolek K Planteco®

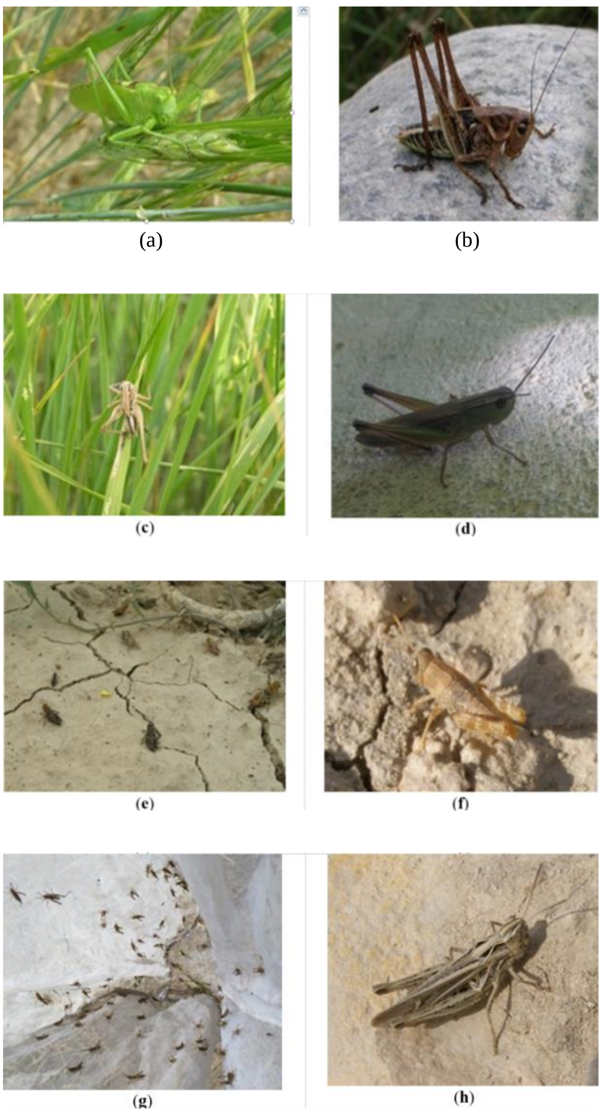


**Fig. 5:** Locusts, grasshoppers and katydids in soybeans: (a) Tettigonia viridissima, adult; (b) Decticus verrucivorus, nymph; (c) Tessellana vittata; (d) Chorthippus karelini, adult; (e) Calliptamus italicus, nymphs; (f) Calliptamus barbarus, nymph; (g) Ch

**Fig. 6:** Specimen of grasshoppers, dead from biopesticide Entolek K Planteco®

**Fig. 7:** Specimens of Italian locust, dead from biopesticide Entolek K Planteco®

**Fig. 8:** Specimen of grasshopper with fungal mycelium in a humid chamber

**Table 1:** Survival in minutes after exposure to Entolek K Planteco® EC on nymphs of III-IV instars of different katydids species under laboratory conditions

| | Biopesticide treatment | | | Untreated control | | |
|---|---|---|---|---|---|---|
| Katydids species | 3 min | 7 min | 10 min | 3 min | 7 min | 10 min |
| *Decticus verrucivorus* | 5 | 3 | 0 | 10 | 10.00 | 10 |
| *Tettigonia viridissima* | 5 | 3 | 0 | 10 | 10.00 | 10 |
| *Tessellana vittata* | 3 | 0 | 0 | 10 | 10.00 | 10 |
| *P-value* | Treatment factor | | | | <0.05 | |
| | Katydids species factor | | | | <0.05 | |

**Table 2:** Survival in minutes after exposure to Entolek K Planteco® EC on nymphs of III-IV instars of different locust and grasshopper species under laboratory conditions

| Locust and grasshopper's species | Experiment | | Control | |
|---|---|---|---|---|
| | 3 min | 7 min | 3 min | 7 min |
| *Gomphomastax clavata* | 3 | 0.00 | 10 | 10 |
| *Calliptamus italicus* | 4 | 1.00 | 10 | 10 |
| *Chorthippus apricarius* | 3 | 0.00 | 10 | 10 |
| *Chorthippus biguttulus* | 3 | 0.00 | 10 | 10 |
| *Euchorthippus pulvinatus* | 3 | 0.00 | 10 | 10 |
| *Oedipoda caerulescens* | 4 | 1.00 | 10 | 10 |
| *Stenobothrus fischeri* | 3 | 0.00 | 10 | 10 |
| *P-value* | Locust and grasshoppers species t factor | <0.05 | | |
| | treatment factor | <0.05 | | |

**Table 3:** Locust, grasshopper*, and katydid abundance and predominance in soybean crops (Almaty region, Arkabay district, "Baiserke Agro" LLP, area 4 ha)

| | Imago, spec. | | Nymphs, spec. | | |
|---|---|---|---|---|---|
| Taxa | ♂ | ♀ | Family Tettigoniidae | Total, spec | Balog domination index |
| *Decticus verrucivorus* | 5 | 6 | 12 | 23 | 21,29 |
| *Tettigonia viridissima* | 8 | 7 | 24 | 39 | 36,11 |
| *Tessellana vittata* | 9 | 10 | 27 | 46 | 42,60 |
| Total | 22 | 23 | 63 | 108 | 100 |
| Family Acrididae | | | | | |
| *Calliptamus italicus* | 11 | 9 | 67 | 87 | 15,60 |
| *Calliptamus barbarus* | 13 | 12 | 69 | 94 | 16,84 |
| *Chorthippus apricarius* | 19 | 22 | 21 | 70 | 12,54 |
| *Chorthippus biguttulus* | 18 | 15 | 27 | 60 | 10,75 |
| *Chorthippus karelini* | 16 | 17 | 35 | 58 | 10,40 |
| *Epacromius tergestinus* | 10 | 13 | 18 | 41 | 7,34 |
| *Euchorthippus pulvinatus* | 18 | 21 | 26 | 55 | 9,85 |
| *Oedipoda caerulescens* | 12 | 14 | 16 | 42 | 7,53 |
| *Stenobothrus fischeri* | 12 | 10 | 29 | 51 | 9,15 |
| Total | 129 | 133 | 308 | 558 | 100 |

**Table 4:** Biological efficacy of the biopesticide Entolek K Planteco® EC against pest katydids on soybean crops (Almaty region, village Arkabay, "Baiserke Agro" LLP, 2019)

| | Mortality, %, day after treatment | | | |
|---|---|---|---|---|
| Treatment | 3 | 5 | 7 | 10 |
| Entolek K Planteco® | 50,1±1,7 | 64,9.00±3,1 | 70,3±4,7 | 70,3±4,7 |
| Untreated control | 0,0 | 0,0.00 | 0,0 | 0,0 |
| *P-value* | treatment factor mortality, %, | <0.05 | | |
| | day after treatment factor | <0.05 | | |

**Table 5:** Biological efficacy of the biopesticide Entolek К Planteco® EC against pest locusts and grasshoppers on soybean crops (Almaty region, village Arkabay, LLP "Baiserke Agro", 2019)

| | Mortality, %, day after treatment | | | |
|---|---|---|---|---|
| Treatment | 3 | 5 | 7 | 10 |
| Entolek К Planteco® | 70,2±1,8 | 76,3±3,3 | 98,1.00 ±4,9 | 98,1 ±4,9 |
| Untreated control | 0,0 | 0,0 | 0,0.00 | 0,0 |
| *P-value* | treatment factor mortality, %, | | <0.05 | |
| | day after treatment factor | | <0.05 | |

## Conclusion

The biopesticide "Entolek К Planteco®" EC is efficient against pest locust, grasshopper, and katydid species, which are quite numerous in soybean crops. The results of laboratory and field tests revealed a sufficiently high efficacy and quick action, which is necessary when carrying out protective measures against this group of pests.

Specifically, this biopesticide can be applied to soybean crops grown in areas where the use of chemical insecticides is legally prohibited (water protection zones, specially protected natural areas, agricultural land for organic production, etc.).

Katydids *D. verrucivorus* and *T. viridissima* showed slightly higher resistance than *T. vittata*. It can be assumed that this difference is largely due to the thickness of the chitin cuticle and the size of the studied insects, which to some extent protects them from the effects of the biopesticide. The first two katydid species have denser chitin cuticles and larger sizes than the third species.

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## Author's Contributions

All authors equally contributed to this study.

## 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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