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VIRULENCE OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BRONGNIARTII (SACCARDO) PETCH (ASCOMYCOTA: CORDYCIPITACEAE) TO LOCUSTS

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Annotation. *The article describes the results of the experiment conducted on identification of virulence of the entomopathogenic fungi Beauveria Bringniartii Sacc. to Asian migratory locust (Locusta migratoria migratoria L.) and Italian locust (Calliptamus italicus L).*

Keywords. *Bioinsecticide, Asian migratory locust, Italian locust, letal dose, microbial insecticide, locust nymphs.*

Аннотация. *В статье описаны результаты проведенного эксперимента по выявлению вирулентности энтомопатогенных грибов Beauveria Bringniartii Sacc. по отношению к азиатской перелетной саранче (Locusta migratoria migratoria L.) и итальянскому пруту (Calliptamus italicus L.).*

Ключовые слова. *Биоинсектицид, азиатская перелетная саранча, итальянский прус, смертельная доза, микробный инсектицид, нимфы саранчи.*

Annotatsiya. *Maqolada Beauveria Bringniartii Sacc. entomopatogen zamburug‘ining Osiyo (Locusta migratoria migratoria L.) va voha (Calliptamus italicus L.) chigirtkalariga nisbatan virulentligini aniqlash bo‘yicha o‘tkazilgan tajriba natijalari keltirilgan.*

Kalit so‘zlar. *Bioinsektitsid, Osiyo migratsiya chigirtkasi, italyan chigirtkasi, o‘ldiradigan doza, mikrobial insektitsid, chigirtka nimfalari.*

INTRODUCTION

A significant number of microbiological pesticides based on entomopathogenic fungi have been developed worldwide. As active ingredients, 12 species or subspecies (varieties) of mycoinsecticides and mycoacaricides have been used for induced and inoculating applications. Insecticides based on the fungal strains *Beauveria bassiana* (33.9%), *Metarrhizium anisopliae* (33.9%), *Isaria fumosorosea* (5.8%) and *B. brongniartii* (4.1%) are the most frequently used among 171 products.

RESEARCH METHODS

To conduct experiments and identify pathogens, it is necessary to maintain cultures of orthopterous insects in the laboratory. The laboratory population of the Asian migratory locust and the Italian locust is maintained using the following method. Locust egg pods are collected in the field. After collection, the eggs are kept at a temperature of 20–25 °C for 30–35 days to complete embryonic development, after which the diapausing eggs are placed in a refrigerator. Under such conditions, with optimal moisture content in the sand where the egg pods are laid, the eggs can be stored for 5–6 months without losing viability.

The required number of eggs for the experiment is removed from the refrigerator and placed in a thermostat, where the temperature is maintained at 20–25 °C for the first 2–3 days and 28–30 °C in the following days. The hatched nymphs are kept in a cage measuring 70×70×70 cm, with walls covered in mill gas for optimal air ventilation. For young nymphs of the Asian migratory locust and the Italian locust, 16 hours' photoperiod and an air temperature of 28-32°C are maintained. For older nymphs and imago of the Asian migratory locust, a 12-hour photoperiod and a temperature of 33–35 °C are used.

Green sprouts of wheat and barley, as well as reed and wheat bran, are used as for feeding for the Asian migratory locust. For the Italian locust, lettuce, dandelion, alfalfa, sweet clover, and wheat leaves are provided. The queen insects of the laboratory population are kept in a separate room.

The following media are used for culturing fungi: Czapek medium (sucrose – 30 g; $NaNO_3$ – 3 g; KH_2PO4 – 1 g; $MgSO_4 \cdot 7H_2O$ – 0.5 g; $FeSO_4$ – 0.01 g; water – 1 l; agar-agar – 2%), medium with peptone and yeast extract (KH_2PO_4 – 2 g; $(NH_4)_2SO_4$ – 1 g; $MgSO_4$ – 1 g; glucose – 20.0 g; yeast extract – 1.0 g; water – 1 l; agar-agar – 2%), and agarized beer wort. The wort obtained from the brewery is sterilized at 0.8 atm for 20 min, then filtered through cotton wool, diluted with water (2:1), mixed with 2% agar, and sterilized a second time in an autoclave under the same conditions.

To determine the virulence properties of entomopathogenic fungi, target insects were treated by spraying with a suspension of spores. A Goryaev chamber was used to determine the spore concentration in the suspension. Different solutions (ranging from 1×10^3 to 1×10^8) were prepared, and the insects in the experimental variants were treated accordingly. In the control group, insects were treated with plain water. The studied insects were kept in special insectaries and fed with plants.

RESULTS

Initial assessments showed that Italian locust nymphs were the most susceptible, while Asian migratory locust nymphs were the most resistant to the disease caused by *B.brongniartii* [Nurzhanov, Latchininsky, 1987]. Further studies have shown that the susceptibility of Asian migratory locusts to this mycosis is highly dependent on temperature and humidity conditions. Thus, at high ambient temperatures (30-33 ° C), the death of infected individuals does not occur due to the following reasons: rapid growth and molting of nymphs; inhibition of the development of fungal spores; slow shedding of spores germinating with the nymphal skin. At optimal temperatures, the virulence of the fungus against fourth-instar Asian migratory locust nymphs depends on air humidity. Thus, Asian migratory locust nymphs stored in glass containers with high air humidity are more susceptible to mycosis (Table 1). By the tenth day, the mortality rate in these cases is 95%, while the percentage of dead nymphs kept in gauze cages with low air humidity is only 41.1%.

Table 1

Virulence of *B.brongniartii* strain VD-85 against fourth-instar nymphs of the Asian migratory locust at different air humidity.

Conditions	Number of nymphs	Relative humidity (%)	Mortality of nymphs (%):			
			2 day	6 day	10 day	15 day
Gauze cages	40($10\times^4$)	60-75	0	0	41,1±3,2	70,6±4,1
Glass cages	80 ($10\times^8$)	80-85	2,5±1,6	27,5±7,5	95,0±3,8	95±3,8

Study of the relationship between the mortality of 2nd-stage Asian migratory locust nymphs and the infectious dose of fungal spores showed that the insecticidal activity of the fungus increases with an increase in titer from 2×10^5 to 2×10^7 spores / ml, but not higher, mortality reached 100%. With an increase in suspension titers from 2×10^3 to 2×10^7 spores/ml, a similar increase in mortality was not observed (Fig. 2).

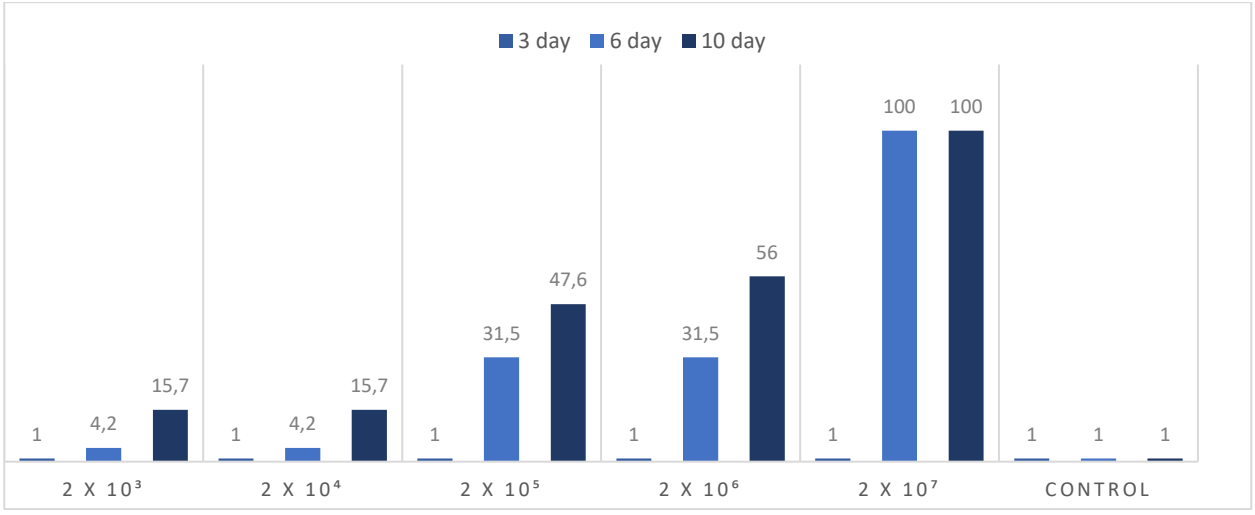

Fig. 2. Mortality of Asian migratory locust nymphs treated at the second instar (strain VD85; 28–29 ° C, humidity 80–85%, 25 individuals per variant)

The large difference between the options in the final calculations indicates that Orthoptera nymphs are resistant to low doses of infection (from 2×10^3 to 2×10^4), an increase in these doses (2×10^5 and 2×10^6) leads to a fairly rapid development of the

disease, and higher doses (up to almost 2 × 10^7) double the effectiveness. The insecticidal activity of the fungus does not appear on the 3rd day.

Age-related resistance of Asian migratory locust nymphs to the disease caused by *B.brongniartii* was also revealed. Thus, with increased air humidity on the 6th day of the count, the mortality of IV instar nymphs was 27.5% (Table 1), and the mortality of II instar nymphs was 100% (Fig. 2). Thus, for the successful use of fungal insecticides against Asian migratory locust nymphs, it is necessary to take into account their age, as well as the temperature and relative humidity of the air. Data on the mortality of the second instar nymphs of the Italian locust, treated with suspensions with different titers of fungal spores, indicate that the insecticidal activity of the fungus in this case is noted already on the third day, and on the seventh day of the count, mass death of nymphs was observed. As the infectious load increases, the rate of insect death and the final percentage of death increase. The mortality of insects increases sharply, 2-3 times, with an increase in titer to 1×10^6 and 1×10^7 spores/ml (Fig. 2.1).

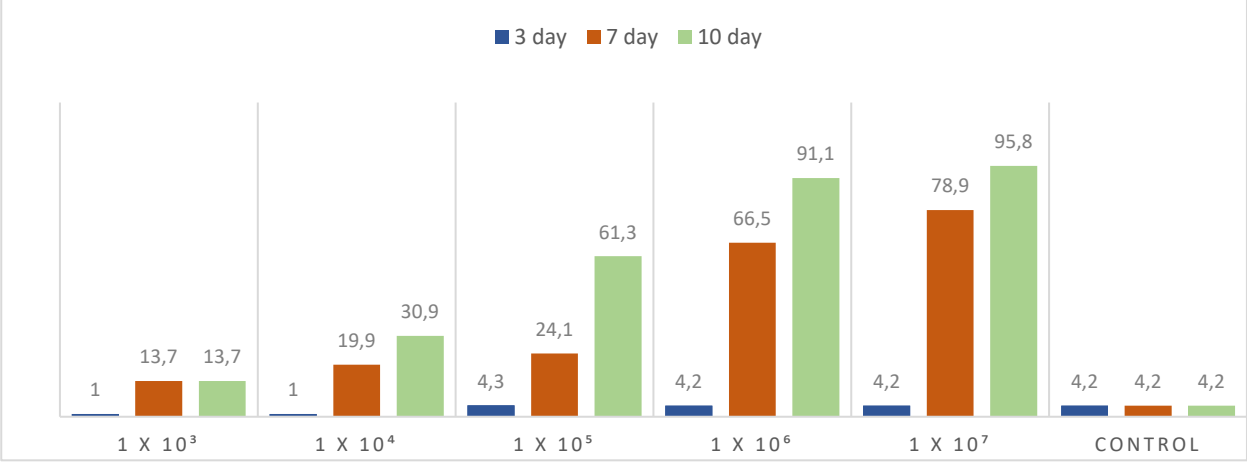

Fig. 2.1. Mortality of Italian locust when treating second instar with different titers of spores of strain VD85 (laboratory experiments, 25 individuals per variant).

Treatment of the fourth instar with a suspension of 3×10^3 –3×10^7 spores/ml showed that the mortality of older instar was higher than the mortality of the second instar (Fig. 2.2). In the first days, infected fourth instar nymphs were more sensitive to the pathogen than the second instar, LD_{50} (letal dose) was 6.3×10^6 spores/ml and 10.8×10^6 spores/ml, respectively. By the end of the experiment, the mortality of the

second instar increased. Thus, on the 10th day, the LD_{50} of the fungus for II instar was 1.7×10^5 spores/ml, and for IV instar – 2.3×10^5 spores/ml. After treatment of the second-instar with suspensions with titers of 1×10^3 and 1×10^4 spores/ml, 54.16% and 49.16% respectively, remained alive after 20 days, some of them developing to imago. A decrease in the average weight of sick individuals was noted compared to healthy ones. At the same time, treated insects at the stage of fourth-instar with a spore suspension with a minimum concentration continued in the imago phase.

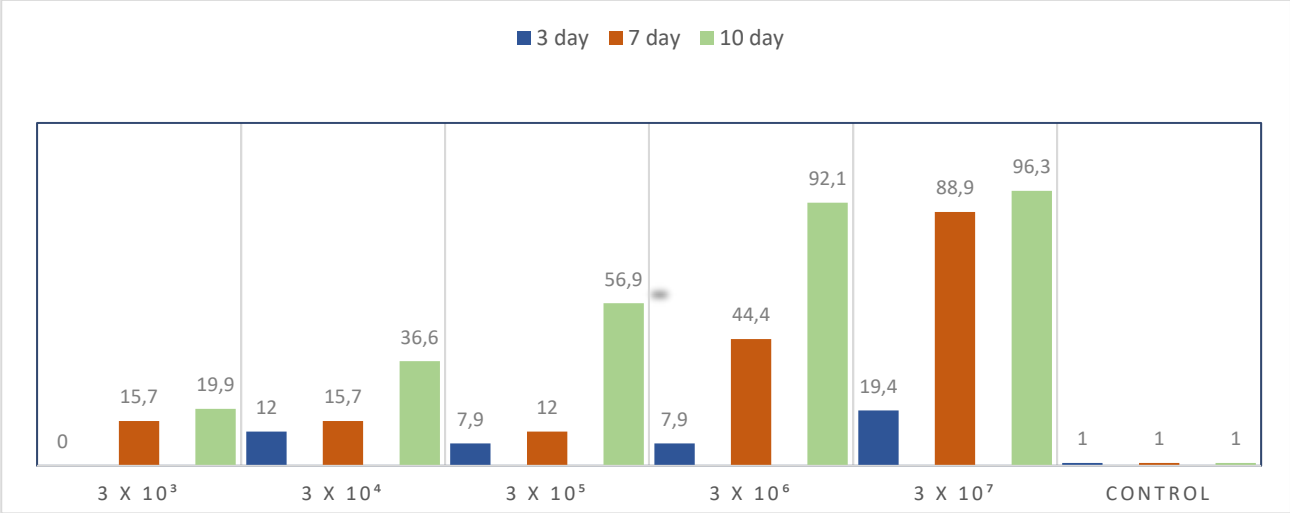

Fig. 2.2. Mortality of Italian locust when treating IV instar with different titers of spores of strain VD-85 (laboratory experiments, 25 individuals per variant)

Thus, laboratory experiments have confirmed the highly virulent properties of *B.brongniartii* against the II and IV instars of the Italian locust. No age-related resistance of nymphs to the pathogen was observed. Depending on the titer of the fungal spore suspension, infected individuals die within 10–15 days, starting from the second day after treatment. It has been established that the eggs of the Italian locust and Asian locust are not affected by the *B.brongniartii* fungus, but the hatching of nymphs from them was slower (Table 1.1). Thus, after treating the eggs with a fungal suspension with a titer of 1×10^7 spores/ml, the hatching of nymphs began only on the 6th day. In the control variant, 48.6% of nymphs hatched on the 6^{th}–7^{th} day. While 27.12% of nymphs hatched from the treated eggs. Under the influence of the fungus, a small mortality rate (1.2%) of eggs was observed. It was also established that the eggs

of the Asian migratory locust were resistant to *B.brongniartii* when egg capsules were laid in sand treated with a suspension of fungal spores (Table 2.2).

Table 1.1

The effect of the VD-85 strain of the *B.brongniartii* fungus on the viability of eggs and hatching of Italian locust nymphs (25-28 ° C)

Varieties	Number of samples	Hatching (%) after treatment:			
		6 days	7 days	8 days	9 days
Eggs treated with a suspension of fungal spores + Triton-X-100	81	7,5±4,8	27,2±5,2	71,0±9,5	97,8±1,4
Eggs treated with clean water + Triton-X-100	54	36,9±13,7	48,6±11,0	82,7±11,9	100

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