An Experimental Comparison of the Virulence of Fungal (Entomophtorales) Isolates on *Schizaphis graminum*

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Abstract: In this research the pathogeneity of five isolates – *Conidiobolus obscurus*, *C. obscurus* 15, *C. obscurus* J, *C. thromboides* and *Bazidiobolus ranarum* was tested on green bug aphids applying two methods: spraying and exposure methods. The research showed, that both methods can be applied for estimation of pathogeneity of fungi and cause the diseases of green bug aphids (*Schizaphis graminum*).

Key words: Entomopathogenic fungi, mortality, green bug aphid.

Introduction

Mycoses play an important role among diseases of insects and mites. There are over 500 fungi known to be associated with insect diseases from five classes of fungi, the Deuteromycetes, Zygomycetes, Oomycetes, Chytridiomycetes and the Trichomycetes. The largest number of pathogenic fungi is in class Zygomycetes (Dent 1993). Nowadays entomopathogenic fungi as the perspective bioagents are in the focus of scientists` attention in Latvia and other countries.

Physiological and morphological properties, the diversity of catabolical reactions, very short term of infection and the ability to develop and multiply on artificial medium establishes to entomopathogenic fungi practical value as the agents of biomethod in plant protection.

The aim of this research was to estimate the virulence of enthomopatogenic fungi *Conidiobolus obscurus*, *C. obscurus* 15, *C. obscurus* J, *C. thromboides and Bazidiobolus ranarum* isolates.

Methods

The study was carried on in the Institute of Biology (Salaspils) of the University of Latvia, in the Laboratory of the Experimental Entomology from October 2005 till March 2006.

Laboratory culture of aphids was used and kept on oats.

Entomopathogenic fungi cultures, used through present study, were a part of collection "Collection of bioagents, that limit the amount of plant pests", founded by the Institute of Biology.

Fungal cultures*:* 1) *Conidiobolus obscurus* (HALL & DUNN) REMAUDIERE & KELLER (= *Entomophthora thaxteriana*), isolated from *Aphis pomi* DEGEER, culture was multiplied on Malt extract agar; 2) *C. obscurus* J (isolated by Jēgina et al. 1984), obtained through choosing monosporous isolates. Culture was multiplied on Malt extract agar; 3) *C. obscurus* 15, obtained from natural culture, that was isolated from soil example taken in apple tree garden, through choosing monosporous isolates. *C. obscurus* 15 monosporous isolates differ from *C. obscurus* J by the growing intensity 70%. This factor is important at the time of cultivation (Chudare 1989a) 4) *C. thromboides* DRECHSLER (= *E. virulenta*) isolated from *Neomyzus circumflexus*, culture was multiplied on Malt extract agar (Čudare 1998, Jankevica, Cudare 2003, Jankevica 2004); 5) *Bazidiobolus ranarum*, isolated from *Myzus persicae*. Culture was multiplied on Malt extract agar (Chudare 1989b).

All the isolates were stored in refrigerator to the temperature + 8°C.

Two methods were used: spraying and exposure method. During the study death – rate dynamics was estimated. The infection of greenbugs was carried out using the following methods.

Spraying method

C. obscurus, C. obscurus J, *C. obscurus* 15, *C. thromboides* and *B. ranarum* suspensions of different concentrations of conidia and resting spores were used for aphids spraying. Cultures of entomopathogenic fungi had been grown for five days before harvest. From Petri dish, where fungi culture was grown, with disinfected scalpel upper mycelium layer was scraped off and suspended in sterile water (primary concentration suspension). Then, from obtained suspension was prepared 1:5 dilution, 1:10 dilution and 1:100 dilution.

Aphids were placed in sterile plastic Petri dishes (Ø 90 mm), 20 adults in each dish. Winged forms of aphids weren't used in experiments. At the beginning of experiment, and both at first and second counting days, food plant's sprout was placed in each Petri dish. Spraying was carried out with sprayer, hold at 30-40 cm from Petri dish. On the surface of every Petri dish was sprayed 0,2 g of suspension of entomopathogenic fungi. Control individuals were sprayed with water. Each variant had five replicates.

To the aphids was provided lighting, they were maintained in room temperature 18±2 after spraying. Insects were observed during 72 hours. The number of living and dead individuals was counted and written in the protocol daily.

Conidias and resting spores in suspensions of studied fungi were counted under light microscope using Goriajev`s chamber.

Exposure method

From each fungus culture were bored pieces (culture medium with fungus mycelium) using cork borer (Ø 1 cm). After each manipulation cork borer was sterilized. Four pieces of each fungus isolate were fixed to the coverlid of Petri dish (Figure 1). Every variant had five replicates.

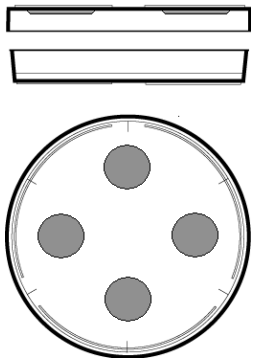

Figure 1. Disposition of fungal pieces on the coverlid of Petri dish.

In each Petri dish were placed 20 grown up aphid individuals. For every fungus culture were applied four exposure times - 15, 30, 45 and 60 minutes. Each two minutes the lids of Petri dishes were turned for 90 degrees clockwise. When exposure time was over, on the Petri dishes new sterile lids were placed and in each dish was placed oat's sprout. Group of control individuals didn't contact with entomopathogenic fungi and they were hold in the same conditions as treated individuals.

Before exposure experiment special Petri dishes were prepared, in each of them four cover slips were disposed. These Petri dishes were exposed the same way as dishes containing aphids. Conidias, dropped on cover slips, were fixed with 10% cotton blue lactophenol. The number of germs was calculated, counting conidias and resting spores in ten eyeshots.

Germs were counted in microscope by magnification of 40×. Area of each eyeshot is $\pi \times r^2$, where r – microscope lens radius (1 mm). Accordingly, total concentration of conidias was found after a formula:

$$P_{con.} = N_{con.} / \pi,$$

where $N_{con.}$ – the general amount of conidias in 10 eyeshots expressed in conidia/mm^2.

The same principle was applied also calculating the total amount of resting spores in the solution:

$$P_{rest.sp.} = N_{rest.sp.} / \pi,$$

where $N_{rest.sp.}$ – the general amount of resting spores in 10 eyeshots.

Data processing

The data were summarized in the MS Excel tables.

Corrected mortality

If some individuals of aphids from the control group have died during experiment time, then mortality percent was calculated and corrected according to the Abbot`s formula:

$P = 100 \times (P_0 - C)/(100 - C)$,

where P – corrected mortality, C – mortality percent in control, P_0 – observed mortality (Abbott 1925). Cumulative mortality is the total amount of dead individuals on the second and third day.

SD (standard deviation) was calculated using MS Excel functions.

Determination of LC_{50}

LC_{50} value is the number of virulent parts or concentration of pathogens, which kills 50% of test individuals (Lipa, Šližyņski 1973).

The percents of corrected mortality were transformed into probits in accordance with the proper table. Using interpolation method and logarithms of transformed mortality and concentration of germs, logarithm of graphic regression was traced (or probit).

Results and discussion

Influence efficiency of isolates of entomopathogenic fungi on aphids depending on infection methods applied:

Corrected cumulative mortality

The executed experiments shows, that *C. obscurus*, *C. obscurus* 15, *C. obscurus* J, *C. thromboides* and *B. ranarum* isolates were virulent and caused mortality of aphids.

Twenty-four hours after infection with entomopathogenic fungi insignificant mortality rate was observed: for spraying method 1-13.3% (Figure 2), for exposure method 2-16.3% (Figure 3). The explanation is, that on the first day the infection in greenbugs was on the initial phase. Mortality of aphids in control group during this time period did not exceeded 2%.

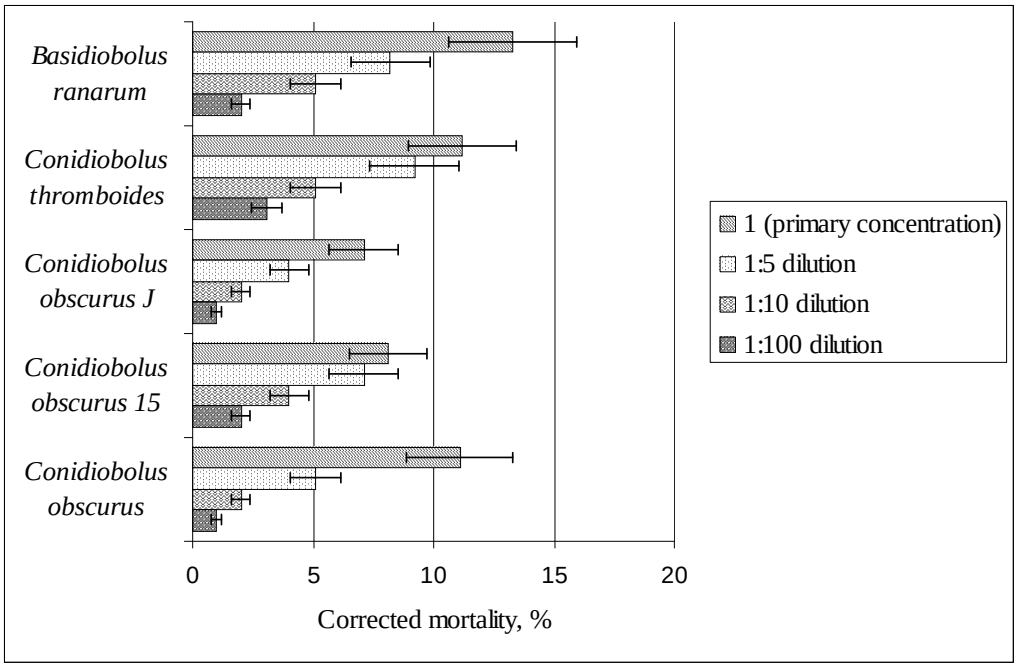

Figure 2. The dependence of corrected mortality of aphids after 24 hours from the beginning of exposure to different concentration of suspension of entomopathogenic fungi. Spraying method.

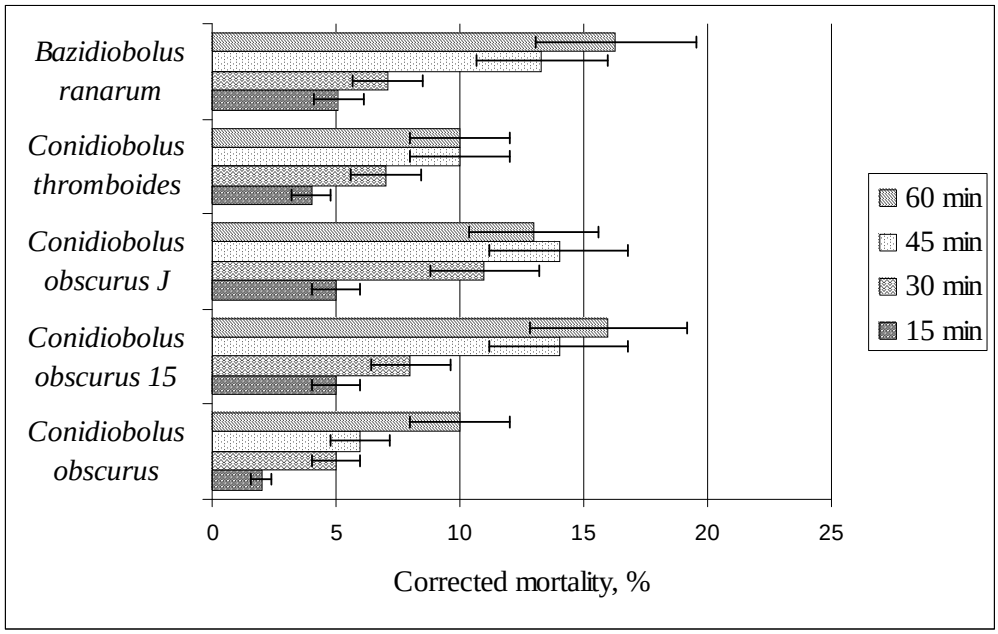

Figure 3. The dependence of corrected mortality of aphids after 24 hours from the beginning of exposure of entomopathogenic fungi. Exposure method.

After 72 hours corrected mortality of aphids, caused by entomopathogenic fungi, in spraying experiment achieved 20.2-72.0% (Figure 4), in exposure experiment – 3.3-75.3% (Figure 5). The most effective fungi culture in spraying experiment was *C. obscurus* (with maximal caused aphids` corrected mortality 63.4%), *C. obscurus* 15 (with maximal caused aphids` corrected mortality 72%) and *C. obscurus* J (with maximal caused aphids` corrected mortality 66.7%). In exposure experiment the most effective fungus was *B. ranarum*, which caused maximal aphids` corrected mortality 75.3%.

Previous studies (Jankevica, Čudare 2003) also showed, that *C. thromboides* and *B. ranarum* have high pathogeneity on aphids. For instance, cumulative mortality of *Aphis gossypii* after 48 hours caused by *C. thromboides* was 95.8% and *B. ranarum* – 91.4%.

Virulence of entomopathogenic fungi plays an important role in pest control. Fungi from Entomophtoraceae family can cause mortality of aphids 30-100%. Virulence decreases, if fungus culture is reared on artificial medium for a long time. For instance, entomopathogenic fungus *Entomophtora aphidis* at once after distribution caused 95% mortality of treated aphids. In same queue year after cultivating on the artificial medium killed only 36% of aphids. Passaging of entomopathogenic fungi through insects strengthens their virulence (Jegina et al. 1977).

Isolates of entomopathogenic fungi, used in experiments, did not cause 100% cumulative mortality of aphids even using the highest concentrations of germs. This could be explained by, that these isolates were cultivated on artificial medium for a long time and more than a year was not passaged through insects.

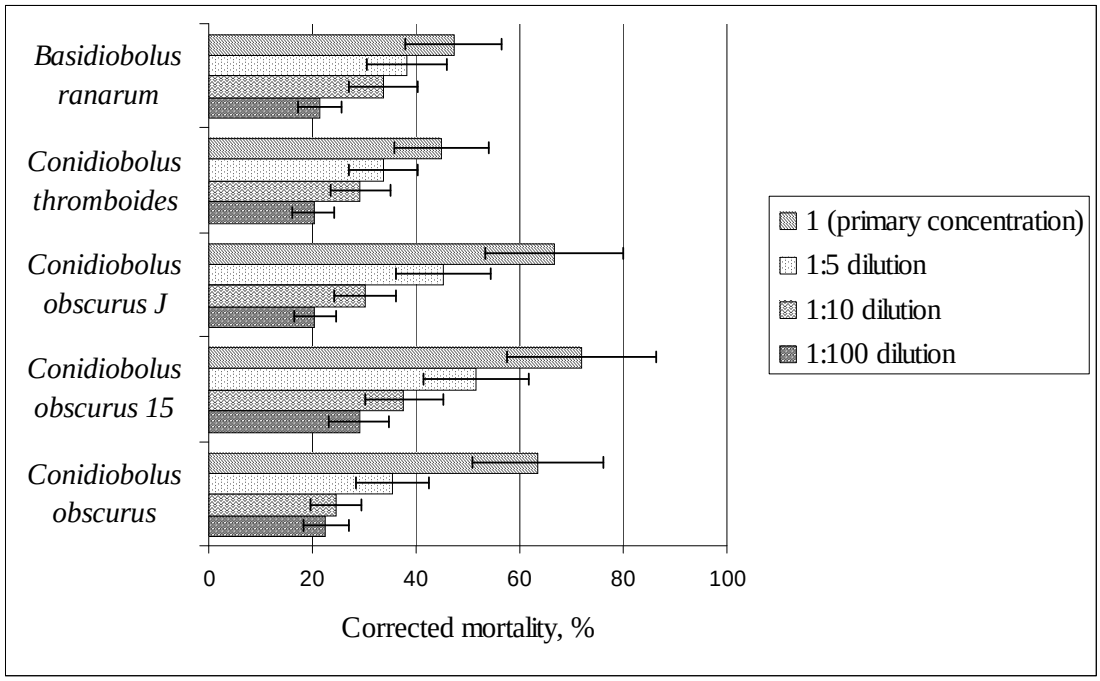

Figure 4. The dependence of corrected mortality of aphids after 72 hours from application of concentration of solution of entomopathogenic fungi. Spraying method.

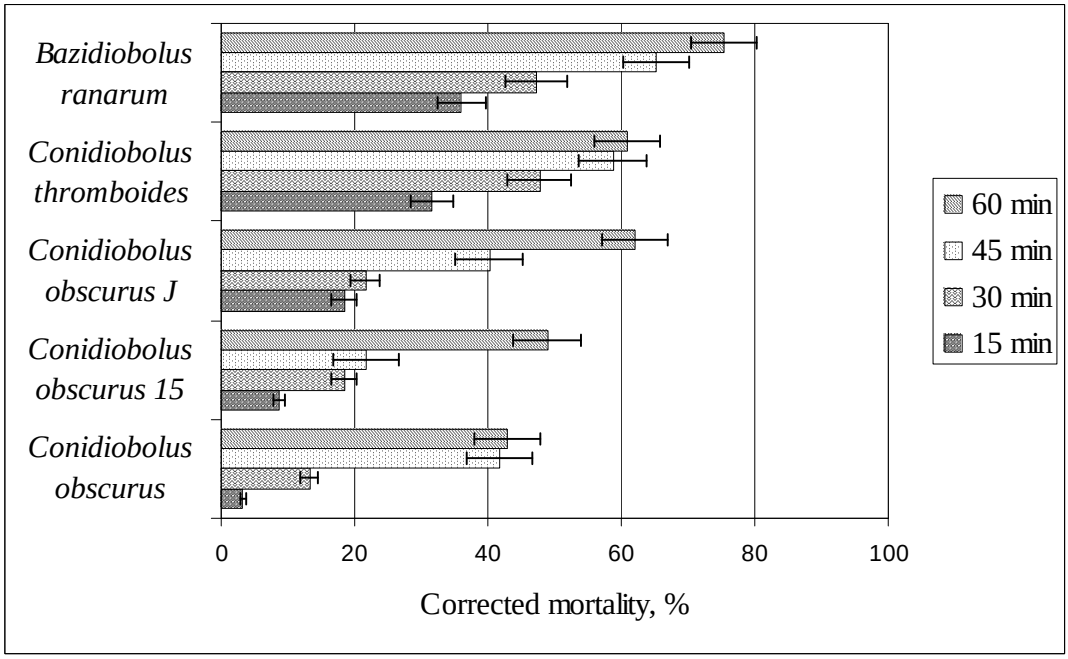

Figure 5. The dependence of corrected mortality of aphids after 72 hours from the beginning of exposure to entomopathogenic fungi. Exposure method.

Both in spraying and exposure experiments concentrations of germs in isolates of fungi (concentration of conidias + concentration of resting spores) increased proportionally. In spraying experiment fungal titres of equal dilution degree didn't differ substantially (Figure 6). The same can be attributed to the exposure experiment, where number of germs per eyeshot for all five isolates of fungi was similar for identical exposure times (Figure 7).

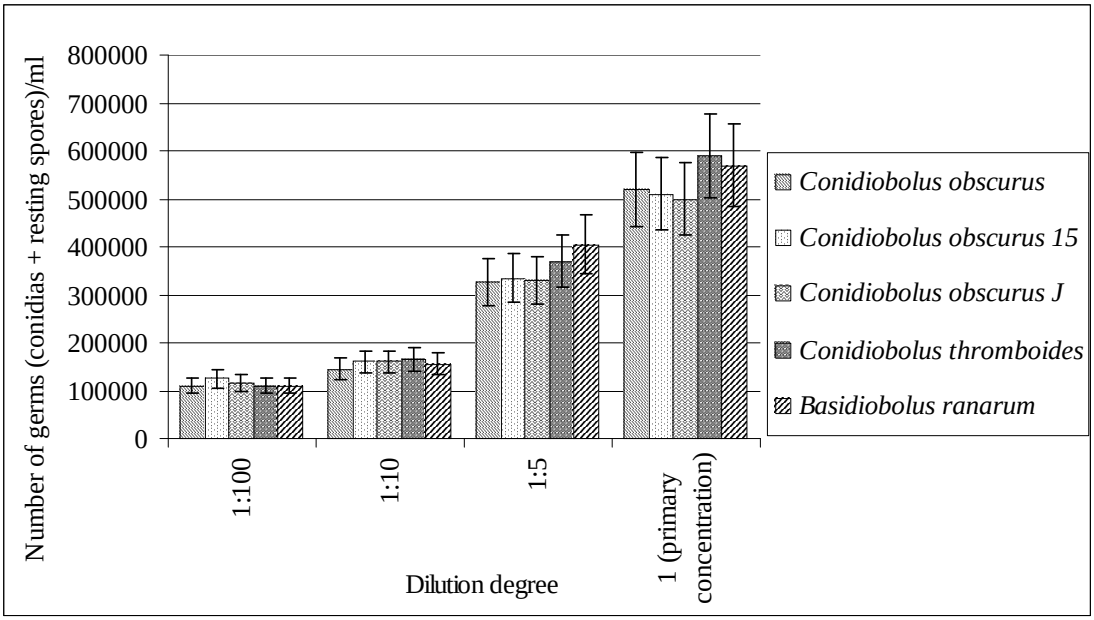

Figure 6. The dependence of concentration (number of germs per 1 ml) of suspension of isolates of entomopathogenic fungi from dilution degree. Spraying method.

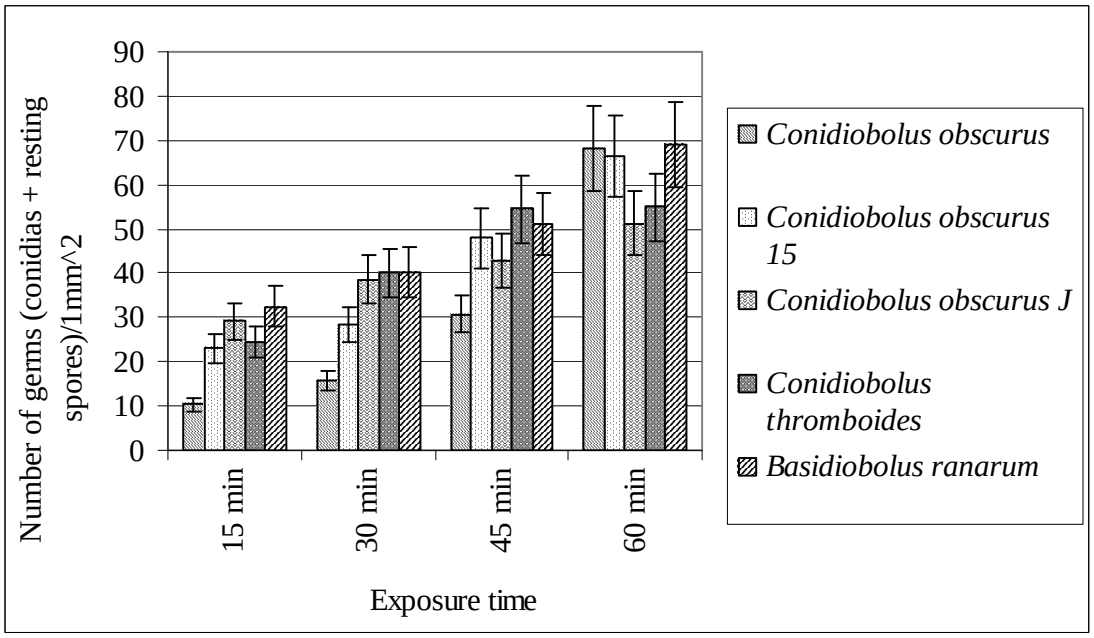

Figure 7. The dependence of concentration (number of germs) of isolates of entomopathogenic fungi from exposure time. Exposure method.

Analysis of LC_{50}

The highest pathogeneity (LC_{50} = 2.6×10^5 germs/ml) showed culture *C. obscurus* 15. Then follows *C. obscurus J* with 50% lethal pathogeneity 3.3×10^5 germs/ml and *C. obscurus* with LC_{50} = 4.0×10^5 germs/ml (Table 1).

The lowest pathogeneity had *C. thromboides* (LC_{50} = 8.9×10^5 germs/ml) and *B. ranarum* (LC_{50} = 7.6×10^5 germs/ml).

Table 1. LC_{50} of entomopathogenic fungi. Spraying method.

Isolates of fungi	**LC_{50}, germs/ml**
Conidiobolus obscurus	400 000
Conidiobolus obscurus 15	260 000
Conidiobolus obscurus J	330 000
Conidiobolus thromboides	890 000
Basidiobolus ranarum	760 000

Isolates *B. ranarum* (LC_{50} = 41.8 germs/mm^2) and *C. thromboides* (LC_{50} = 42 germs/mm^2) had proved themselves as the most pathogenic (Table 2). The lowest pathogeneity (LC_{50} = 79.4 germs/mm^2) showed isolate *C. obscurus 15*.

Table 2. LC_{50} of entomopathogenic fungi. Exposure method.

Isolates of fungi	**LC_{50}, germs/mm^2**
Conidiobolus obscurus	58.9
Conidiobolus obscurus 15	79.4
Conidiobolus obscurus J	49.0
Conidiobolus thromboides	42.0
Basidiobolus ranarum	41.8

Different results, obtained applying these both different methods, can be explained by demands of *C. obscurus*, *C. obscurus* 15 and *C. obscurus* J entomopathogenic fungi to the humidity, because humidity amount in spraying experiment was increased.

Conclusions

It was experimentally found out, that *C. obscurus*, *C. obscurus* 15, *C. obscurus* J, *C. thromboides* and *B. ranarum* cultures were virulent and caused death of aphid imagos.

Isolates of entomopathogenic fungi *B. ranarum* (LC_{50} = 41.8 germs/mm^2) and *C. thromboides* (LC_{50} = 42.0 germs/mm^2), using exposure method, after 72 hours caused 60-75% corrected mortality of aphids.

Isolates of *C. obscurus* (LC_{50} = 4.0×10^5 germs/ml), *C. obscurus* 15 (LC_{50} = 2.6×10^5 germs/ml) and *C. obscurus* J (LC_{50} = 3.3×10^5 germs/ml), using spraying method, after 72 hours caused 63-72% corrected mortality of aphids.

Both spraying and exposure methods can be applied for estimation of pathogenity of fungi and did not differ significantly.

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Kopsavilkums

Pētījums tika veikts LU Bioloģijas institūta (Salaspils) Eksperimentālās entomoloģijas laboratorijā no 2005. gada oktobra līdz 2006. gada martam. Pētījuma mērķis bija novērtēt entomopatogēno sēņu *Conidiobolus obscurus, C. obscurus* 15, *C. obscurus* J, *C. thromboides un Bazidiobolus ranarum* patogenitāti. Mērķa sasniegšanai tika veikta entomopatogēno sēņu virulences pārbaude, izmantojot laputu *Schizaphis graminum* laboratorijas kultūru. Tika izmantotas divas metodes: miglošana un eksponēšana. Pētījuma gaitā tika izpētīta laputu mirstības

dinamika. Laputu inficēšana veikta pamatojoties uz dažādu pētnieku aprakstītām metodēm. Iegūtie rezultāti liecina, ka *C. obscurus, C. obscurus* 15, *C. obscurus* J, *C. thromboides un B. ranarum* izolāti ir virulenti un izraisa laputu saslimšanu.

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