The Influence of Entomophtorales Isolates on Aphids *Aphis fabae* and *Metopeurum fuscoviride*

JULIJA HALIMONA, LIGA JANKEVICA

Laboratory of Experimental Entomology, Institute of Biology, University of Latvia, 3 Miera Street, LV-2169, Salaspils, Latvia; e-mail: julija_halimona@inbox.lv

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Abstract: During this research the effect of entomopathogenic fungi was tested on aphids *Aphis fabae* and *Metopeurum fuscoviride*. Five entomopathogenic fungi isolates were used: *Conidiobolus obscurus* 79, *C. obscurus* 79-3, *C. obscurus* E 68, *C. thromboides* and *Basidiobolus ranarum*. The research proved all fungal isolates applied to be virulent.

Key words: Entomopathogenic fungi, mortality, aphids, pests, spraying.

Introduction

Aphids are among the most significant pest insects in forestry, agriculture and horticulture in the temperate climatic zones. Control of aphids has been done predominantly by using chemical insecticides, however this practice has caused problems for the environment (Botto 1999).

Entomopathogenic fungi are significant pathogens of the Homoptera (Humber 1989, Eilenberg et al. 2009), and many fungal species are responsible for epizootics that often successfully regulate aphid population (Lacey et al. 2001, Barta & Cagan 2006, Koval 2007).

The traditional approach in biological control with entomopathogenic fungi has been to apply the fungal material (usually conidia) to the cropping system, using an inundative or inoculative biological control strategy (Eilenberg et al. 2001).

The aim of this research was to estimate the virulence of *Conidiobolus obscurus* 79, *C. obscurus* 79-3, *C. obscurus* E 68, *C. thromboides* and *Basidiobolus ranarum* isolates against two aphid species: *Aphis fabae* and *Metopeurum fuscoviride*.

Methods

The study was carried out in the Institute of Biology (Salaspils) of the University of Latvia, in the laboratory of Experimental Entomology.

Two aphid species were used as experimental models: *Aphis fabae* Scopoli laboratory culture and *Metopeurum fuscoviride* Stroyan, collected in field.

Five entomopathogenic fungi isolates were used: 1) *C. obscurus* E 68 (= *Entomophthora thaxteriana*) isolated from *Aphis pomi* DEGEER (isolated by Jegina et. al. in 1984); 2) *Conidiobolus obscurus* 79 (Hall & Dunn) Ramaudiere & Keller, that was isolated from natural soil sample taken from apple-tree garden, it was proven that *C. obscurus* 79 isolate differs from *C. obscurus* E 68 by more intensive development of mycelium (up to 70 %) (Chudare 1989a); 3) *C. obscurus* 79-3, monosporous isolates of *Conidiobolus obscurus* 79 obtained through selection; 4) *C. thromboides* DRECHSLER (syn. *E. virulenta*) was isolated from *Neomyzus circumflexus* (Chudare 1998, Jankevica, Chudare 2003, Jankevica 2004); and *Basidiobolus ranarum*, isolated from *Myzus persicae* (Chudare 1989b). All before mentioned fungal cultures are a part of "Collection of bioagents, that limit the amount of plant pests", founded by the Institute of Biology, and all of them were multiplied on Malt extract agar and stored in refrigerator at +6 ±2°C temperature.

Aphids were placed in sterile plastic Petri dishes, 20 individuals in each. One feeding plant was added daily in Petri dishes during

experiment.

The mycelium of experimental fungi was scraped off from Malt extract medium and dissolved into sterile water. Suspensions of three different concentrations were prepared for each isolate: the initial concentration, 1:5 dilution and 1:50 dilution. For infection of aphids a pulveriser was held at the distance of 30 – 40 cm from Petri dish, spraying 0.2 g of entomopathogenic fungi suspension on its surface. Control individuals were sprayed with sterile water. Every experiment was repeated five times.

Table 1. Applied initial concentrations of fungal isolates.

Fungal isolate	Number of conidia/ml	Number of resting spores/ml	Number of germs/ml
Isolates sprayed on *A. fabae*:			
Conidiobolus obscurus 79	5.8×10^5	2.1×10^5	7.9×10^5
Conidiobolus obscurus 79-3	5.2×10^5	0.9×10^5	6.2×10^5
Conidiobolus obscurus E 68	5.3×10^5	0.3×10^5	5.6×10^5
Conidiobolus thromboides	3.6×10^5	0.6×10^5	4.2×10^5
Basidiobolus ranarum	2.2×10^5	0.5×10^5	2.7×10^5
Isolates sprayed on *M. fuscoviride*:			
Conidiobolus obscurus 79	2.5×10^5	0.3×10^5	2.8×10^5
Conidiobolus obscurus 79-3	1.3×10^5	0.5×10^5	1.8×10^5
Conidiobolus obscurus E 68	1.8×10^5	0.6×10^5	2.4×10^5
Conidiobolus thromboides	2.0×10^5	0.6×10^5	2.6×10^5
Basidiobolus ranarum	0.6×10^5	0.9×10^5	1.5×10^5

Experimental Petri dishes were provided with 19±2°C temperature and 16 hours lightning. Aphids were observed for 72 hours, the number of living and dead insects was noted in the protocol daily.

The number of germs (conidia and resting spores) in experimental fungal suspensions was counted with Goriajev's chamber (Table 1). Conidia and resting spores were counted diagonally in 50 squares. Concentration of germs in 1 ml of suspension was calculated (Pimenova et all, 1971). Corrected cumulated mortality of aphids was estimated according to the Abbott's formula (Abbott 1925):

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

where P – corrected cumulative mortality, C – per cent of dead individuals in control group, P_0 – observed cumulative mortality per cent.

Also median lethal concentration (LC_{50}) was counted (Finney 1971). As fungal conidia are the main infective agent, the number of conidia was taken in account calculating lethal concentrations.

Results

One day after infection with fungi aphid mortality rate was lower than 12.4 % for *A. fabae* lower than 9.2 % for *M. fuscoviride*. The first day's results also showed the highest birth rate: 2.6 – 4.0 individuals for *A. fabae* (Figure 1) and 1.8 – 3.4 individuals for *M. fuscoviride* (Figure 2). On the third day of experiment, corrected cumulative mortality caused by fungi reached 51.2 – 91.7 % for *A. fabae* (Figure 3) and 35.8 – 70.4 % for *M. fuscoviride* (Figure 4). *A. fabae* was proven to be more susceptible to fungal infection. *C. thromboides* isolate was the most effective against aphids in both cases.

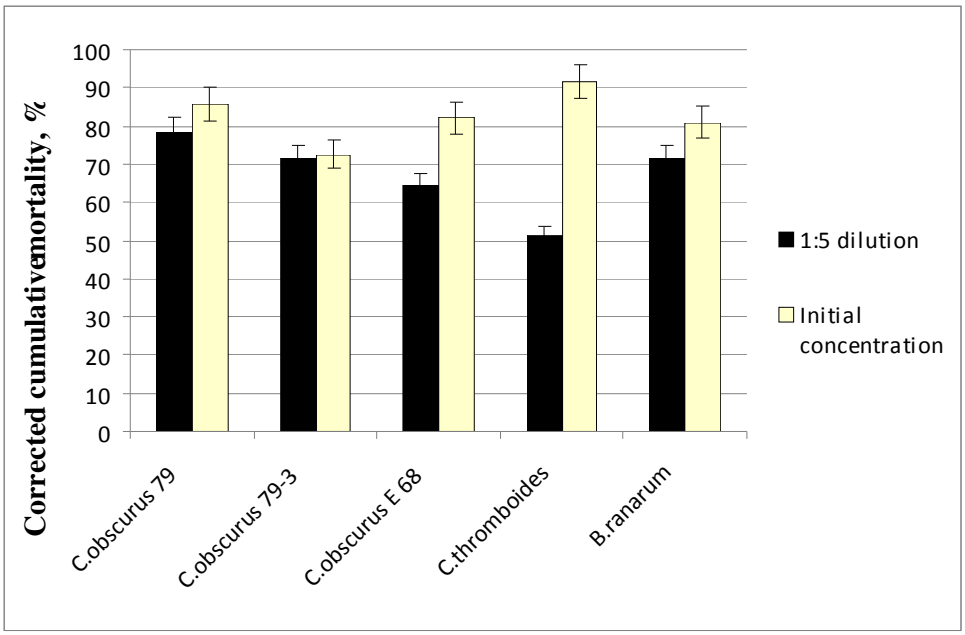

Figure 1. The dependence of corrected cumulative mortality (%) of *Aphis fabae* aphids from concentration of applied suspension of entomopathogenic fungi 72 hours after spraying.

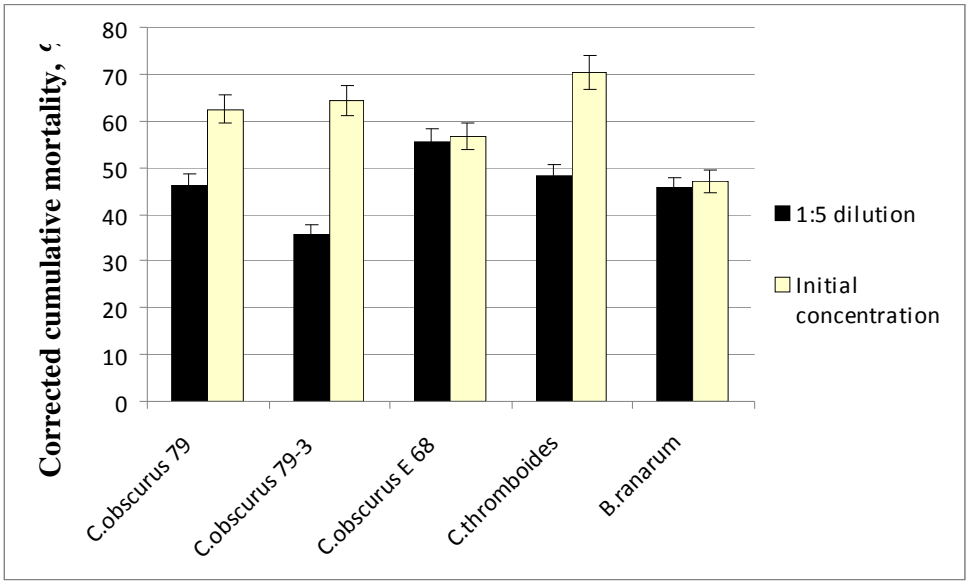

Figure 2. The dependence of corrected cumulative mortality (%) of *Metopeurum fuscoviride* aphids from concentration of applied suspension of entomopathogenic fungi 72 hours after spraying.

72 hours after spraying aphid individuals had practically no offspring born. Whereas in control group average birth rate figures increased by 4.2 individuals for *A. fabae* and by 3.4 individuals – for *M. fuscoviride* aphids. Our data confirm the results of other studies, that entomopathogenic fungi cause the decrease of aphid fecundity (Liu et al. 1999, Zaki 1998).

It was observed that concentration of entomopathogenic fungi isolates had an impact on aphids' birth rate – the higher concentration, the fewer individuals were born.

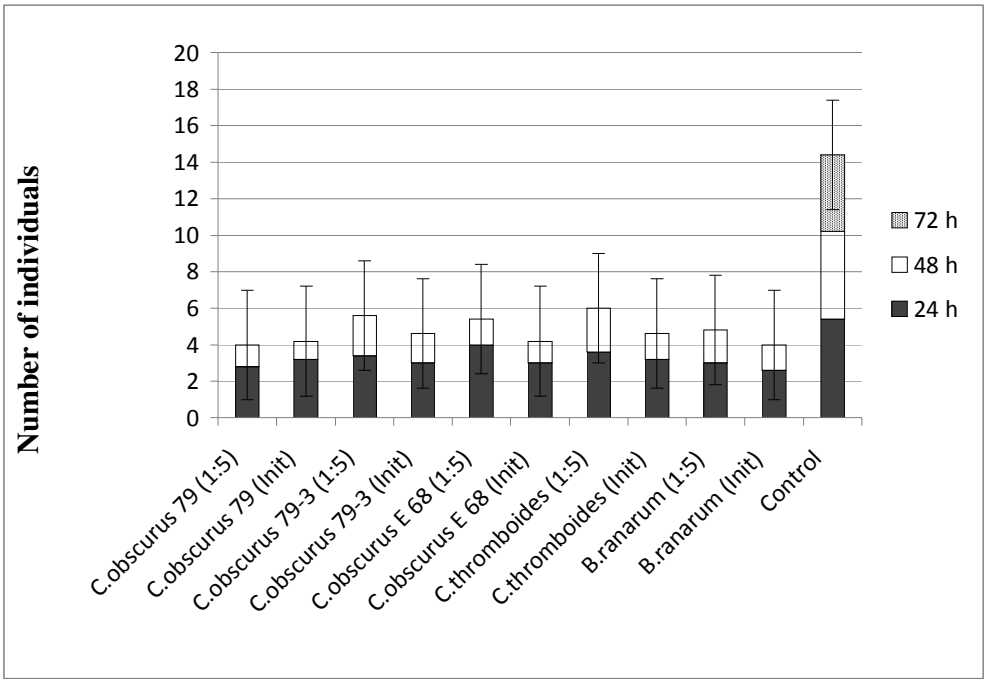

Figure 3. The dependence of average birth rate (number of individuals) of *Aphis fabae* aphids from concentration of applied suspension of entomopathogenic fungi.

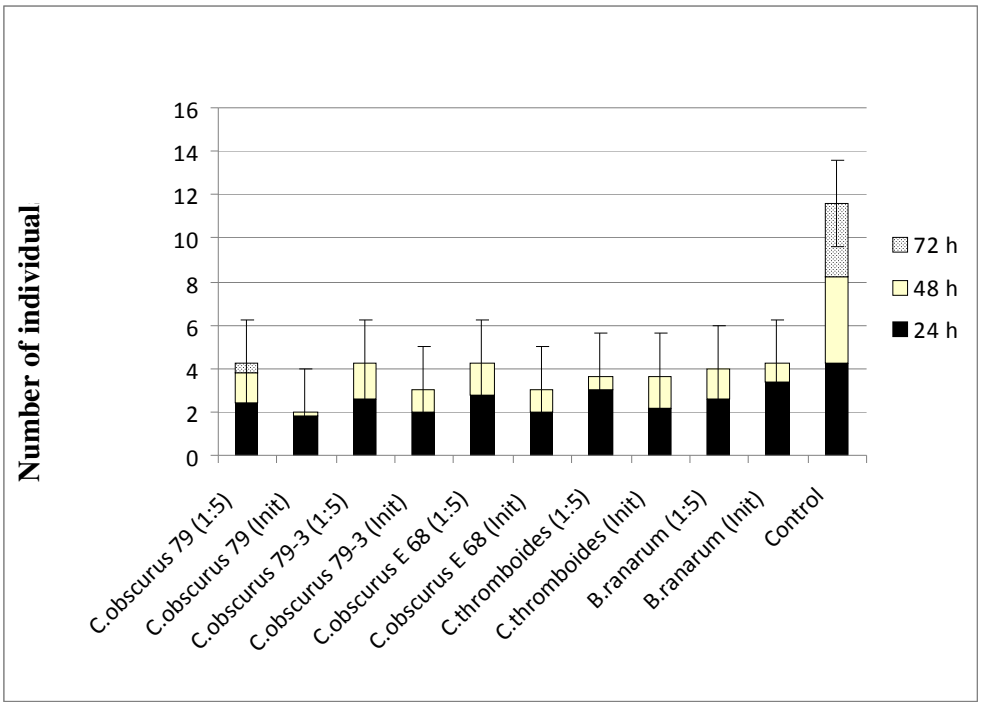

Figure 4. The dependence of average birth rate (number of individuals) of *Metopeurum fuscoviride* aphids from concentration of applied suspension of entomopathogenic fungi.

The highest virulence against *A. fabae* (LC_{50} = 0.6×10^5 conidia/ml) showed isolate *C. obscurus* 79-3 (Table 2). The lowest result was obtained from *C. thromboides* isolate (LC_{50} =

2.9×10^5 conidia/ml).

When applied against *M. fuscoviride*, the highest 50 % lethal pathogenity (Table 3) was registered for *C. obscurus* E 68 isolate (LC_{50} = 0.2×10^4 conidia/ml). The lowest mortality was caused by *C. obscurus* 79-3 (LC_{50} = 1.7×10^5 conidia/ml).

Table 2. LC_{50} of entomopathogenic fungi, sprayed on *A. fabae*.

Isolates of fungi	LC_{50}, conidia/ml
Conidiobolus obscurus 79	0.3×10^4
Conidiobolus obscurus 79-3	0.6×10^5
Conidiobolus obscurus E 68	1.4×10^5
Conidiobolus thromboides	2.9×10^5
Basidiobolus ranarum	1.5×10^5

Table 3. LC_{50} of entomopathogenic fungi, sprayed on *M. fuscoviride*.

Isolates of fungi	LC_{50}, conidia/ml
Conidiobolus obscurus 79	1.6×10^5
Conidiobolus obscurus 79-3	1.7×10^5
Conidiobolus obscurus E 68	$0.\ 2\times10^5$
Conidiobolus thromboides	1.6×10^5
Basidiobolus ranarum	1.6×10^5

Discussion

Experiment results show that all applied entomopathogenic fungi are virulent and cause the disease of aphids.

Mortality of aphids on the forst day did not differ significantly from the control group. The explanation can be, that on the first day the development of infection in insect tissues had just begun. The mycelium of entomopathogenic fungi had not managed to fill host insect's body cavities and thus affect substantionally aphid mortality and reproductive functions.

The fact that isolates of entomopathogenic fungi did not cause 100 % mortality of aphids on the third experiment's day, even applying the highest concentrations of fungal suspensions, can be explained by long cultivation of fungi on artificial mediums and storage in the refrigerator.

The second reason is probably the temperature regime, on which depends the speed of infection's development. According to other researches, temperature is a significant factor while carrying on the experiment (Liu et al. 1999, Xu, Feng 2002, Jaronski 2010). Probably, by raising incubation temperature it is possible to accelerate the development of fungal mycelium and thus – to raise mortality rate of aphids.

Though Feng et al. (1991) Suggest that the development of fungal infection seems to be better correlated with host density than with climatic factors.

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