Efficacy of different entomopathogenic fungi collected in the field on bugs and codling moth

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Abstract

Entomopathogenic fungal strains against Codling moth and Stink bug were tested in the laboratory. Eleven isolates belonging to seven species were isolated from the larval stage of Leucoptera scitella *(Zeller).*

Beauveria bassiana *(Bals.-Criv.) Vuill. was the most abundant species representing 43 % of the total number of isolates collected in the field*.

The highest mortality rates were 90% on the second larval instar of the Codling moth was recorded by L. muscarium *1 (Petch) Zare & W. Gams, While* B. bassiana *killed more than 80 % of the larvae and it has the highest mortality rate (70 %) on the nymphs in the case of Stink bug.*

Keywords: Entomopathogenic fungi, *Cydia pomonella, Pentatoma rufipes*, *Leucoptera scitella*

Introduction

Codling moth and Stink bugs are among the important pest insects of organic fruits, which poses an imminent and serious threat to a variety of tree fruit (Geier, 1963, Kührt et al., 2006, Peusens & Beliën 2012). There is a growing interest to use naturally occurring entomopathogenic microorganisms especially fungi to control the pests in organic orchards. More than 700 species of fungi are considered entomopathogenic and many of them offer a great potential for pest management. This study was conducted to evaluate the bio-efficacy of different native species of fungi, collected from natural environments at the Lake of Constance, against the larval stage of *Cydia pomonella* and the nymphal stage of *Pentatoma rufipes*.

Material and Methods

Isolation of Fungi

Eleven fungal strains were isolated from dead larvae of *Leucoptera scitella* (Zeller) in the fields. The small larval integument was externally sterilized in 100% ethanol for about one minute and subsequently in a 2 % sodium hypochlorite solution for 50 sec. and allowed to air dry for another minute. Sterilized surface segments were put into PDA medium in Petri-dishes and incubated at room temperature in an incubator for ten days.

Morphology identification

The fungal colonies were observed and the fungal morphology was studied under a microscope by observing the colony features (colour, shape, size and hyphae).

Molecular conformation

The DNA Extraction of genomic DNA from the fungi was conducted by using DNeasy Plant Mini Kit. ITS1and ITS4 primers were used to amplify ribosomal internal transcribed spacer (ITS). PCR products were purified using the QIA quick PCR purification kit (Bao et al., 2012). The sequence was obtained in all samples compared with public sequences available in the

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GenBank database using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) confirming the taxonomy of all morphologically identified fungal specimens.

Insect sources

The Codling moth larvae were obtained as a starter population from the Institute of Biological Control, Julius-Kühn-Institut, at Darmstadt and reared in the laboratory on an artificial diet according to Bathon et al. (1991) at 25 ± 1 °C, 40-60% R.H and 18:6 L: D photoperiod. The Stink bug nymphs were collected from organic apple orchards at the Lake of Constance.

Experimental setup

All isolates were tested at concentrations listed in table 1 by using 0.05% Tween 80 and sterile distilled water to prepare the fungal spore suspension. Insects (second instar larvae and nymph) were sprayed (as a group of 5) with 0.5 ml of conidia suspensions. Controls were treated with sterile distilled water containing 0.05% Tween 80 Codling moth larvae were fed on artificial diet and the Stink bug nymphs were fed on a piece of an untreated organic apple fruit. Naturalis®-L was also used as positive control in the experiment. Treated insects were placed in 9-cm diameter Petri dishes and incubated in a climatic chamber at 24 ± 1°C, 70 ± 5% RH and 16:8 (L:D), each treatment was replicated six times.

Mortality was recorded after 12 days and the dead insects were surfaced-sterilised and incubated at 25 ± 1°C and 70 ± 5% RH to determine the cause of the mortality.

Results

Eleven native isolates belonging to seven entomopathogenic fungal species were obtained from the larval stage of *L. scitella* (Table 1).

Table 1: Fungal strains collected from different locations at the Lake of Constance (Germany).

Fungi strains	Concentration spores/ml (*C. pomonella*)	Concentration spores/ml (*P. rufepis*)	Collection date	Original source Larvae *of L. scitella*
Lecanicillium lecani	$6.5*10^8$	$1.1*10^7$	April 19	in cocoon (winter)
Fusarium avenaceum 1	$4*10^6$	$2.9*10^7$	April 19	in cocoon (winter)
Fusarium avenaceum 2	$213*10^3$	$1.2*10^8$	April 19	in cocoon (winter)
Beauveria bassiana	$2.6*\ 10^8$	$1.2*\ 10^8$	April 19	in cocoon (winter)
Clonostachys rosea	$8*10^7$	$4.3*10^8$	April 19	in cocoon (winter)
Lecanicillium muscarium 1	$2.9*10^6$	$5.2*10^7$	April 19	in cocoon (winter)
Lecanicillium muscarium 2	$2.8*10^7$	$2.1*10^7$	April 19	in cocoon (winter)
Isaria farinosa 1	$10.4*10^7$	$2*10^6$	April 19	in cocoon (winter)
Isaria farinosa 2	$2.4*10^7$	$8.4*10^7$	April 19	in cocoon (winter)
Isaria farinosa 3	$5.2*10^7$	$3.8*10^7$	July 19	in mine (summer)
Cladosporium cladosporioides	$9.6*10^7$	$7.9*10^7$	July 19	in mine (summer)
Naturalis-L	2.5 ml/1l	2,5 ml/1l	-----	

There was a difference in the number of isolates collected among the seven fungal species (Table 2), whereas *B. bassiana* was the most abundant species representing 43 % of the total number of isolates collected in the field.

Our results showed that all isolates tested were able to infect the second larval instar of *C. pomonella* and also the second nymphal instar of *P. rufipes*. in the laboratory.

Table 2. Isolated fungi from *L. scitella* larvae

No.:	Isolated fungi	Number of Isolates	%
1	*Isaria farinosa*	8	4.91
2	*Cladosporium cladosporioides*	27	16.56
3	*Lacanicillium lecani*	4	2.45
4	*Fusarium avenaceum*	21	12.88
5	*Beauveria bassiana*	70	42.94
6	*Lecanicillium muscarium*	13	7.98
8	*Clonostachys rosea*	9	5.52
9	*Paecilomyces* sp.	11	6.75

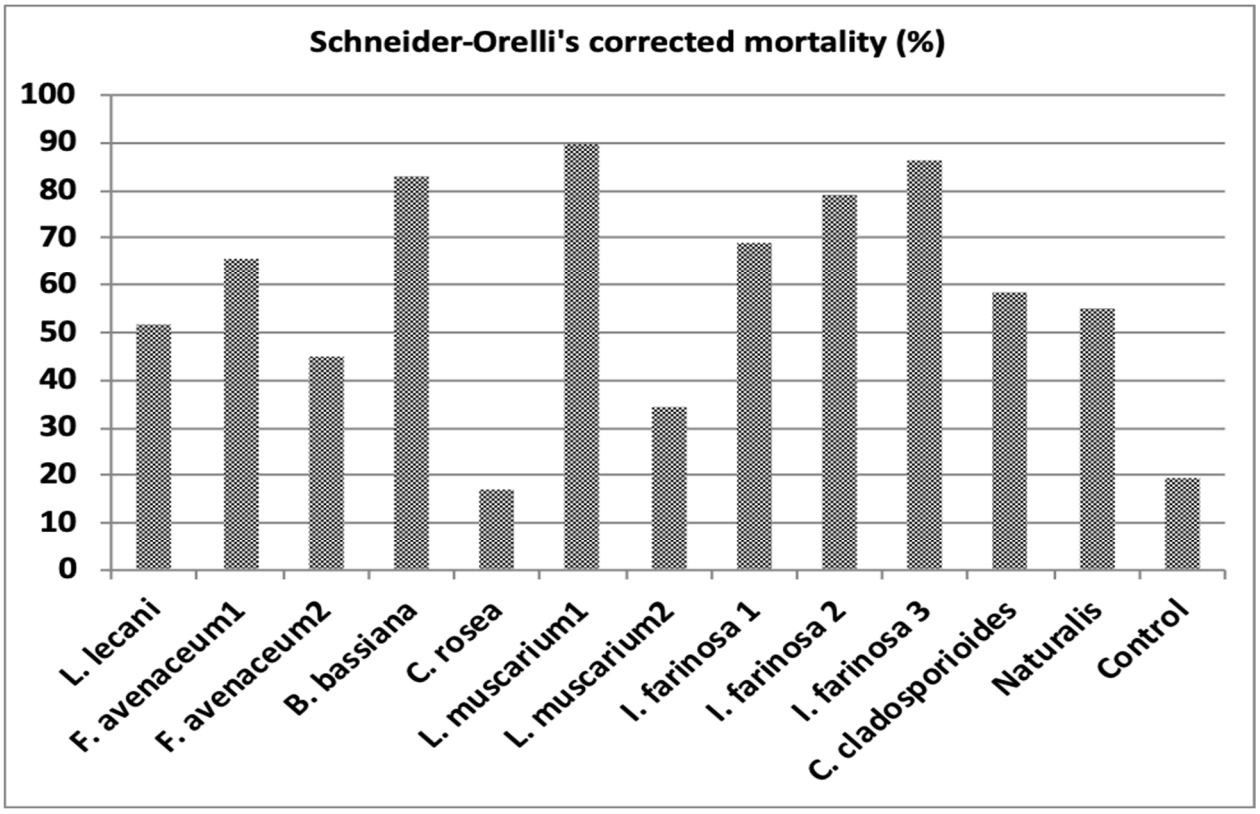

Fig. 1: Effect of different strains of fungi collected in the field on the second instar of the larval stage of *C. pomonella.*

Fungal isolates caused different mortality rates in both Codling moth and Stink bug in the laboratory tests. In the case of codling moth, the mortality rates ranged between 19-90 % depending on the tested strain.

The highest mortality rates of 90, 86 and 83 % on larvae of *C. pomonella* was recorded by *L. muscarium 1*, *I. farinosa 3* and *B. bassiana* strains, respectively (Fig. 1). While *B. bassiana* has the highest mortality rate (70 %) on the nymphs in the case of stink bug and the mortality rates ranged between 11-70% (Fig. 2).

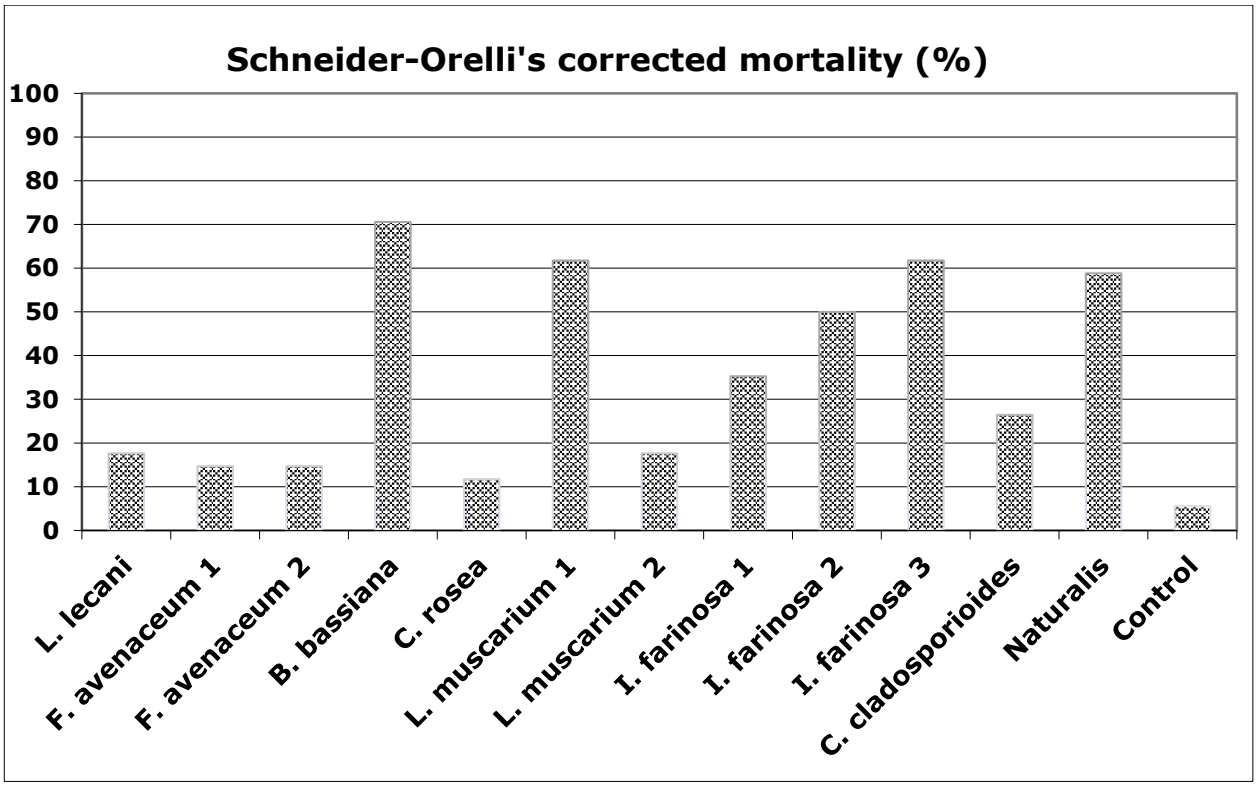

Fig. 2: Effect of different strains of fungi collected in the field on the second instar of the larval stage of *P. rufipes.*

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