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Research Article

THE OCCURRENCE OF ENTOMOPATHOGENIC FUNGI IN SOILS FROM FIELDS CULTIVATED IN A CONVENTIONAL AND ORGANIC SYSTEM

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ABSTRACT
The occurrence of entomopathogenic fungi, involved in regulating the population of insects preying on arable crops, depends on numerous factors, such as using crop protection chemicals, which are prohibited in the organic tillage system. Using two methods: selective medium and the *Galleria mellonella* bait method, the species composition and the occurrence of entomopathogenic fungi in soils deriving from organic and conventional fields sown with winter cereals were compared. Four entomopathogenic fungal species were identified in the samples: *Beauveria bassiana*, *Isaria fumosorosea*, *Metarhizium anisopliae* and *Lecanicillium* sp.. *M. anisopliae* was the most frequently detected one. *M. anisopliae* and *B. bassiana* formed more colony forming units in soils from organic fields, whereas *I. fumosorosea* in soils from the conventional ones.

Keywords: entomopathogenic fungi, soil, arable field, conventional system of cultivation, organic system of cultivation.

INTRODUCTION

Enthomopathogenic fungi participate in the regulation of insects populations, including agricultural pests. Soil, where they find the best growing conditions, is their major dwelling place. Moreover, insects infections take place there.

The enthomopathogenic fungi occurrence in the soil can depend, inter alia, on soil type [Tkaczuk and Miętkiewski 1996], on the cultivated plant species [Krysa et al. 2012, Tkaczuk 2008], or on the agricultural practices [Oliveira et al. 2013, Jabbour and Barbercheck 2009, Tkaczuk 2008, Quesada-Moraga et al. 2007, Hummel et al. 2002]. Because of their low resistance to environment changes, the degree of soil colonization by these fungi can be the indicator of its condition [Meiling and Eilenberg 2006]. Among the factors limiting the occurrence of these insect pathogens, there are plant protection chemicals, particularly fungicides and herbicides [Tkaczuk et al. 2013, Poprawski and Majchrowicz 1995]. Taking care of good soil conditions and the prohibition of chemical pesticides usage is an organic farming characteristic.

The transition from conventional to organic farming method predominantly favours the increase of the soil microorganisms diversity [Mäder et al 2002]. However, in the case of entomopathogenic fungi the impact of such changes has not been sufficiently elucidated yet. Organic cultivation has been shown to increase the abundance and diversity of entomopathogens in, soils in comparison to the conventional tillage [Klingen et al.2002; Mader et al. 2002], but other authors report no significant differences in assemblages of fungal entomopathogens in soils from conventionally cultivated fields after their transition to organic tillage [Jabbour and Barbercheck 2009].

Lubelskie Voivodeship, which was chosen as a research area, is characterized by a large number of organic farms, in comparison to other regions of the country [Kuś and Jończyk 2009]. Furthermore, the strong farm fragmentation is observed in this region and, therefore, due to the small agricultural area it is difficult for the farms to live off the agricultural production. Limited financial resources is the reason why mineral fertilization is here at one of the lowest levels in the country [Dziaduch 2010].

Studies on the occurrence of entomopathogenic fungi populations in soils from fields differing in the level of farming intensity can help to monitor the functioning of the agri-environmental packages. Knowledge of the species composition of entomopathogenic fungi in different regions of the country can be also useful in assessing the potential of individual fungal species to regulate the populations of crop pests [Meyling i Eilenberg 2006].

The aim of the study was to compare the species composition and the intensity of entomopathogenic fungi occurrence in soils from selected agricultural fields cultivated in the organic and conventional system in the Lublin voivodeship.

MATERIAL AND METHODS

Soil samples from 28 arable fields were collected in September 2012. Research plots were located in the north-estern region of Lubelskie Voivodeship, near the following locations:

Kuzawka, Łomnica, Liszna, Dołhobrody, Szuminka, Kołacze, Pożarów, Krynica, Skrychiczyn, Kępa, Połoski, Zahorów, Wereszczyn, Wola Wereszczyńska, Sławatycze, Różnaka, Suchawa, Iżyce, Brzozowiec, Janostrów, Kępa, Piszczac, Trojanów and Tarnów; 14 in organic field crops of winter cereals (rye, triticale, wheat and spelt) falling within agri-environmental program package (2.0) and 14 in conventional field crops (rye, wheat and triticale). Fields from the organic farms had been organically cultivated for at least four years. The objective was to obtain pairs of samples (conventional and organic) from farms at neighboring sites. These sites differed in agricultural practices and fertilizers and pesticide usage, but environmental factors, such as climate and soil conditions were as comparable as possible. At the conventional farms, cereals seeds were treated with fungicides, and insecticides and herbicides were sprayed as needed during vegetation period. Mineral fertilization of soil also was used on the conventional farms. On the organic farms, by contrast, no pesticides or mineral fertilizers were used.

The samples were taken using a shovel, to a depth of up to 15 cm, from 10–15 random points on the tested field. The mixed samples were prepared from the collected material and stored in plastic bags at the temperature of 3–4 ^{o}C. Immediately before starting the experiment in the laboratory, the soil was sieved to separate the larger particles of impurities, and dried up to a moisture content of approximately 25–30% (which is optimal for fungal growth and limits the growth of entomopathogenic nematodes).

Fungi were isolated from the soil using two methods: the insect bait method and the selective medium. The Greater Wax Moth (*Galleria mellonella* L.) in the penultimate instar larvae, coming from a laboratory culture in the Plant Protection Department from Siedlce University of Natural Sciences and Humanities, was used as a bait insect. Five plastic boxes of 200ml capacity were filled with the soil taken from each field. Ten *G. mellonella* larvae were put into each box, then the boxes were placed in an incubator in the temperature of 20–22 ^{o} C. The first mortality control was conducted after 7 days, and then at the three-day intervals until the death of all larvae. Dead larvae with symptoms of fungal infection were transferred directly into moist Petri dishes. Dead larvae, without symptoms of fungal infection, were surface-sterilized in 1% sodium hypochlorite solution, and then rinsed three times in distilled water. After that, the larvae were put in Petri dishes with moistened filter paper. Microscopic preparations from fungal mycelium and spores which grew on the insects surface provided the basis for the determination of fungal species.

To examine the concentration of colony-forming units (CFU) of entomopathogenic fungi in tested soil samples, a selective medium developed by Strasser et al. was applied (1996). This is a commonly used method for the isolation of entomopathogenic fungi from soil environment [Tkaczuk 2008, Meyling and Eilenberg 2006, Keller et al.2003]. Two grams of soil were weighed out of each sample, then 18 ml of distilled water with addition of 0.05 Triton X-100, which reduces the surface tension, were added. The resulting solution was vigorously shaken for 30-40 seconds. Then, 0.1 ml of the soil solution was poured out on a selective medium and spread using a glass spatula. The selective medium consisted of: 1 litre of water, 20 g of glucose, 18 g of agar and 10g of peptone. After sterilization and cooling, the following selective components were added to the medium: 0.6 g of streptomycin sulfate, 0.005g of chlortetracycline, 0.05 g of cycloheximide and 0.1 g of dodine. These components were limiting the growth of saprophytic bacteria and fungi and foster the growth of entomopathogenic fungi. Experiment has been performed in three Petri dishes per sample. Dishes were transferred into incubators at temperature of 22 °C, and after 10–12 days colonies of individual fungal species were counted. The results were expressed as a number of colony-forming units (CFU) of entomophatogenic fungi in 1 g of soil. The results concerning density of CFU in soil were analyzed statistically by performing a 2-factorial analysis of variance and detailed comparison of average values was made using the Tukey's test at significance level α = 0.05.

RESULTS AND DISCUSSION

A total of four species of entomopathogenic fungi were isolated from the tested soils: *Beauveria bassiana, Isaria fumosorosea, Metarhizium anisopliae* and species of the genus *Lecanicillium*. All of them commonly occur and infect insects in soils from cultivated fields [Tkaczuk 2008, Tkaczuk and Mietkiewski1996, Bajan et al. 1995, Steenberg 1995, Miętkiewski et al. 1991, Vänninen et al. 1989, Keller and Zimmerman 1989].

The use of Galleria bait method enabled the isolation all four species from soil samples collected from organic fields, and only three from conventional cultivations: *I. fumosorosea*, *M. anisopliae*, and *Lecanicillium* sp. (Table 1 and Table 2). Frequency of individual species occurrence, isolated using insect bait method, is presented in Figure 1. In both cases, *M. anisopliae* was the most frequently isolated fungus. It was detected in 92% of the soil either from organic or conventional fields. *I. fumosorosea* occurred in 50% of soil from conventional fields, and in 57,1% of soil samples from organic cultivations. Using this method no *B. bassiana* occurrence was detected in soil from conventional cultivations, whereas it was isolated from 37% of soils from organic fields. Fungal species from *Lecanicillium* genus was detected in 7,1% of the tested soils, both from conventional and organic fields.

M. anisopliae caused most fungal infestations of *G. mellonella* as well (Table 1 and Table 2). Percentage of insect larvae infected by individual species of fungi in soils from conventional fields was not significantly different from that observed in soils from the organic fields (Figure 2). *M. anisopliae* is often considered as a dominant in soil from cultivated fields, however, it is rarely isolated from natural habitats [Bidochka et al. 1998, Chandler et al. 1997]. According to Vänninen [1996] it is the most tolerant species to agricultural treatments, such as plowing or using pesticides. It tolerates a periodic absence of potential hosts in the environ-

Table 1. *G. mellonella* larvae mortality (%) in soils from fields cultivated in the organic system

Mortality factor	Study plot													
	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	12a	13a	14a
Entomopathogenic fungi														
Beauveria bassiana	–	–	4	2	–	2	2	–	–	–	2	–	–	–
Isaria fumosorosea	86	80	26	–	–	4	–	–	4	12	2	–	–	–
Metarhizium anisopliae	–	10	8	80	98	30	92	76	42	66	46	82	98	92
Lecanicillium sp.	–	–	–	–	–	–	–	–	–	–	–	–	2	–
Total	**86**	**90**	**38**	**82**	**98**	**36**	**94**	**76**	**46**	**78**	**50**	**82**	**100**	**92**
Fungi of unproved entomopathogenic abilities														
Aspergillus sp.	–	–	4	–	–	2	–	–	–	4	–	–	–	2
Gliocladium sp.	–	–	–	–	2	6	–	–	–	–	–	–	–	–
Fusarium sp.	–	2	–	4	–	6	2	–	2	–	–	–	–	–
Mucor sp.	–	–	–	–	–	4	–	2	–	–	2	–	–	4
Unfrutiful mycelium	12	6	30	4	–	30	2	2	14	6	6	4	–	–
Total	**12**	**8**	**34**	**8**	**2**	**48**	**6**	**4**	**16**	**10**	**8**	**4**	**–**	**6**
Other causes														
Nematodes	–	–	14	2	–	2	–	18	16	2	20	2	–	2
Unspecified causes	2	2	14	8	–	14	–	2	22	10	22	12	–	–
Total	**2**	**2**	**28**	**10**	**–**	**16**	**–**	**20**	**38**	**12**	**42**	**14**	**–**	**2**

Table 2. *G. mellonella* larvae mortality (%) in soils from fields cultivated in the conventional system

Mortality factor	Study plot													
	1b	2b	3b	4b	5b	6b	7b	8b	9b	10b	11b	12b	13b	14b
Entomopathogenic fungi														
Beauveria bassiana	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Isaria fumosorosea	2	80	–	6	10	–	–	–	–	–	14	96	16	2
Metarhizium anisopliae	96	14	78	82	64	94	92	98	82	80	44	–	64	4
Lecanicillium sp.	–	–	2	–	–	–	–	–	–	–	–	–	–	–
Total	**98**	**94**	**80**	**88**	**74**	**94**	**92**	**98**	**82**	**80**	**58**	**96**	**80**	**6**
Fungi of unproved entomopathogenic abilities														
Aspergillus sp.	–	–	–	–	–	–	–	–	–	–	–	2	–	8
Gliocladium sp.	–	–	–	–	–	–	–	–	–	–	–	–	6	2
Fusarium sp.	2	–	4	–	–	2	2	–	6	4	–	–	4	2
Mucor sp.	–	–	6	2	2	–	2	–	2	12	2	–	–	2
Unfrutiful mycelium	–	6	4	10	4	2	2	–	8	4	22	–	10	20
Total	**2**	**6**	**14**	**12**	**6**	**4**	**6**	**–**	**16**	**20**	**24**	**2**	**20**	**34**
Other causes														
Nematodes	–	–	–	–	8	–	–	–	–	–	18	–	–	36
Unspecified causes	–	–	6	–	12	2	2	2	2	–	–	2	–	24
Total	**–**	**–**	**6**	**–**	**20**	**2**	**2**	**2**	**2**	**–**	**18**	**2**	**–**	**60**

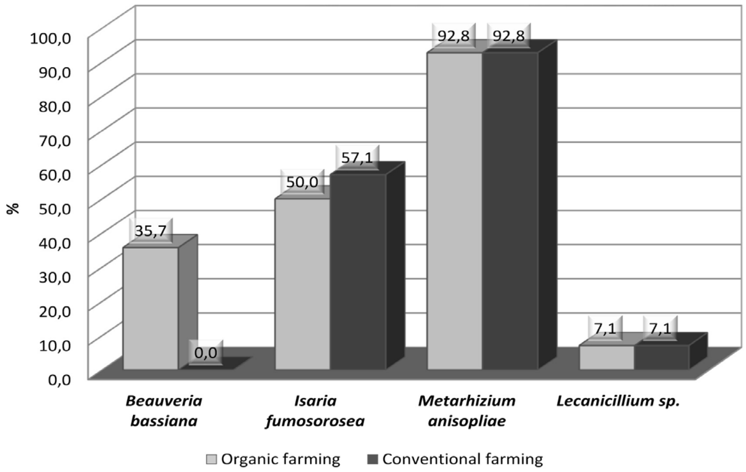

Figure 1. The frequency of isolation of entomopathogenic fungi in soils from fields cultivated in the organic and conventional system (Galleria bait method)

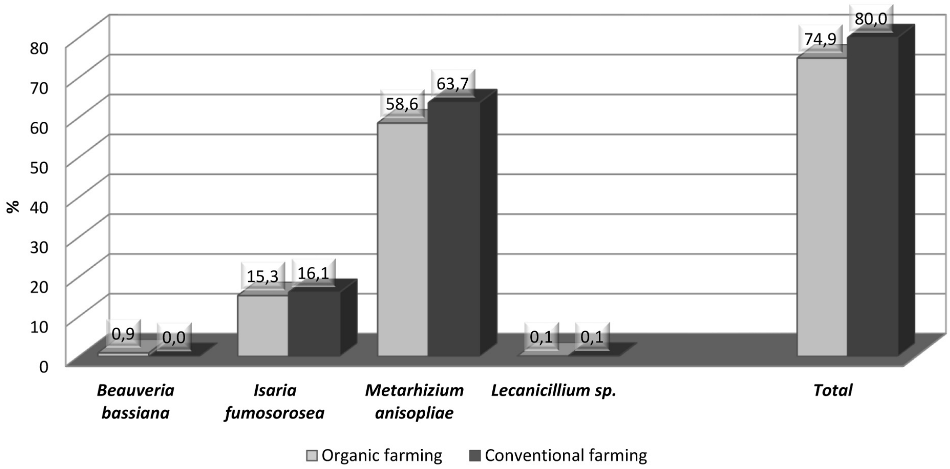

Figure 2. Mortality *G. mellonella* larvae in the soil of the fields cultivated in the organic and conventional system (the average of all samples)

ment too. Comparing occurrence of *M. anisopliae* in soils from fields of varied cultivation systems, Meyling et al. [2011] found no significant differences in occurrence of this fungus in soil from organic and conventional cultivations.

Using the insect bait method for isolation of entomopathogenic fungi from soil, Tkaczuk [2008] and Tkaczuk et al. [2012] indicate two species: *M. anisopliae* and *I. fumosorosea* as dominants in agricultural soils of Poland. Contrary to these results in soils from numerous countries such as Spain, Denmark, Italy or Albania dominant species is *B. bassiana* [Qesada-Moraga et al. 2007, Tarasco and Poliseno 2005, Tarasco et al. 1997].

The use of selective medium method provided slightly different results in comparison to the insect bait method. Using selective medium method both in the case of soils from organic and conventional fields three species were isolated: *B. bassiana*, *I. fumosorosea*, and *M. anisopliae* (Table 3 and Table 4). Concerning the frequency of individual species isolation this method was more effective than the insect bait method (Figure 3). In contrast to Galleria bait method, this method allowed to detect the colony-forming units of *B. bassiana* fungus in soils from conventional cultivations.

Medo i Cagan [2011], who examined occurrence of entomopathogenic fungi in soils of Slovakia, have also found differences in the obtained results using different methods. The authors reported that *M. anisopliae* was isolated approximately four times more frequently using selective medium than insect bait method. Bruck [2004], however, reported the greater sensibility of insect bait method for *B. bassiana*. Keller et al. [2003] observed no significant differences using these two methods to examine the occurrence of entomopathogenic fungi in soils from farmlands in Switzerland. According to Miętkiewski et al. [1991] in order to determine the abundance of entomopathogenic fungi in the environment in a more full way, it is advised to use simultaneously several species of insects as a trap, because susceptibility to infections is different in various insects.

In soils collected from organic fields entomopathogenic fungi formed, on average, 5,8×10^3 CFU in 1 gram of soil (from 2,7 to 11,9×10^3 on individual fields), and slightly less in conventionally cultivated soils: from 1,3 to 8,6×10^3, on average, 5,3×10^3 g^{-1} (Table 3 and Table 4, Figure 4). *M. anisopliae* and *B. bassiana* occurred in significant higher density in soils from organic fields, forming 2,7×10^3 g^{-1} and 1,0×10^3 g^{-1} CFU respectively. In soils from conventional cultivations *M. anisopliae* formed 2,1×10^3 g^{-1} CFU, whereas *B. bassiana* formed 0.6×10^3 CFU in 1 gram of soil. *I. fumosorosea* was the only species forming more CFU in soils from conventional cultivation (on average 2,6×10^3 g^{-1}) than in soils from organic fields (on average 2.1×10^3) (Figure 4).

I. fumosorosea fungus is a common species in soils from different environments in Poland [Miętkiewski et al. 1992, 1998]. However, it is rarely isolated from soils in other European countries [Tkaczuk 2008, Keller et al. 2003, Chandler et al. 1997, Vänninen 1996, Steenberg 1995].

Table 3. The density of colony forming units of entomopathogenic fungi (CFU×10^3 g^{-1}) in soils from fields cultivated in the organic system

Fungal species	Study plot													
	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	12a	13a	14a
Beauveria bassiana	0,7	–	1,3	–	1,5	1,8	1,5	–	0,3	–	2,0	0,5	2,0	2,0
Isaria fumosorosea	5,0	4,5	2,3	0,2	–	0,3	0,2	3,3	3,0	4,2	3,2	0,7	–	3,2
Metarhizium anisopliae	6,2	1,2	3,8	2,5	2,3	1,2	5,0	2,2	1,5	1,8	1,0	5,5	2,2	1,0
Total	**11,9**	**5,7**	**7,4**	**2,7**	**3,8**	**3,3**	**6,7**	**5,5**	**4,8**	**6,0**	**6,2**	**6.7**	**4,2**	**6,2**

Table 4. The density of colony-forming units of entomopathogenic fungi (CFU×10^3 g^{-1}) in soils from fields cultivated in the the conventional system

Fungal species	Study plot													
	1b	2b	3b	4b	5b	6b	7b	8b	9b	10b	11b	12b	13b	14b
Beauveria bassiana	0.5	–	0.8	1.2	0.7	1.5	2.3	–	–	–	0.2	–	0.5	0.3
Isaria fumosorosea	4.1	4.1	0.2	3.3	1.2	–	–	1.0	4.7	4.3	1.8	4.6	3.3	4.0
Metarhizium anisopliae	4.0	1.0	0.3	–	2.2	3.8	4.0	5.5	1.0	1.2	4.6	–	1.2	1.0
Total	**8.6**	**5.1**	**1.3**	**4.5**	**4.1**	**5.3**	**6.3**	**6.5**	**5.7**	**5.5**	**6.6**	**4.6**	**5.0**	**5.3**

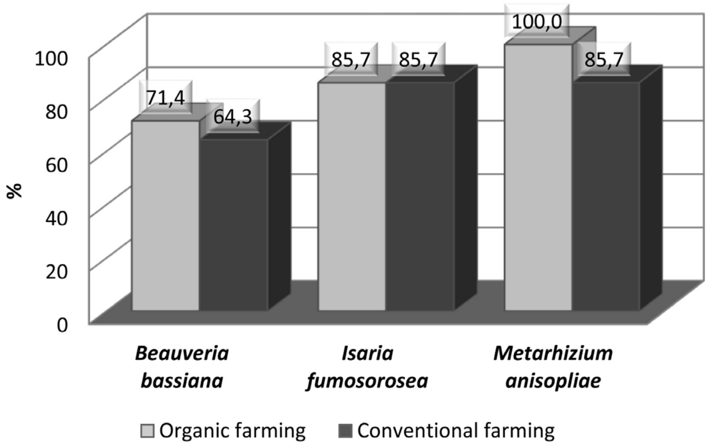

Figure 3. The frequency of isolation of entomopathogenic fungi in soils from fields cultivated in the organic and the conventional system (isolation on selective medium)

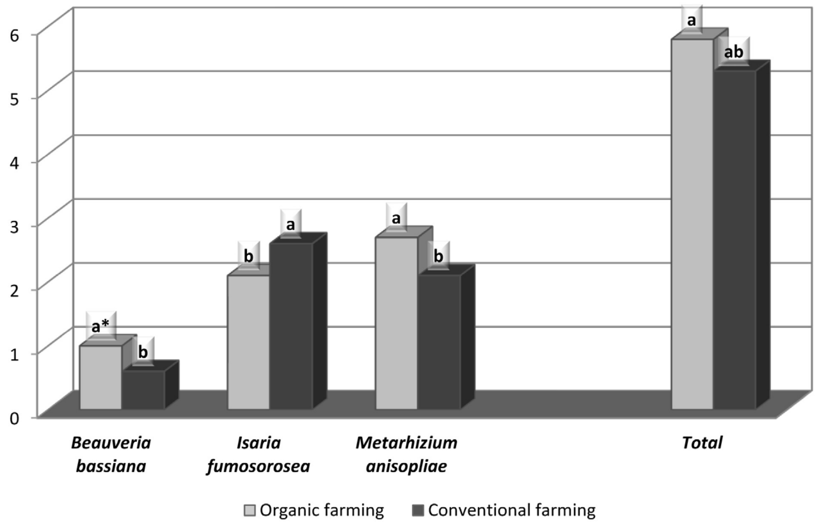

Figure 4. The density of colony forming units of entomopathogenic fungi (CFU×10^3 g^{-1}) in soils from fields cultivated in the organic and conventional system

The occurrence of entomopathogenic fungi in soils from cultivated fields may depend on soil type [Tkaczuk 2008]. In sandy soils, which are common in Poland, *I. fumosorosea* and *M. anisopliae* are the dominant species, in clay soils *B. bassiana*, *I. fumosorosea*, and *M. anisopliae* dominate. The dominance of the *B. bassiana* species is more typical to organic soils. The dominance of *I. fumosorosea* and *M. anisopliae* and lower prevalence of *B. bassiana* in sandy soils was also reported by Tkaczuk and Miętkiewski [1996] and Kleespies et al. [1989].

Hummel et al. [2002] using insect bait method in a long-term field study found that the application of certain pesticides significantly reduces the occurrence of entomopathogenic fungi in the soil. According to some authors, the discontinuance of the use of chemical plant protection products in organic cultivations can have a positive effect on the occurrence of these fungi [Klingen et al. 2002, Vänninen and Hokkanen 1988, Miętkiewski et al. 1997]. Tkaczuk [2008] found that fungus *I. fumosorosea* was the most resistant to pesticides of the studied species under *in vitro* conditions. It can be assumed that *I. fumosorosea* is the best species to be concomitantly used with pesticides in integrated crop protection systems.

A more frequent occurrence of *B. bassiana* species in soil from organic fields may be the result of using organic fertilizers, such as manure or green manure, which enrich the soil with organic matter. Using higher doses of manure may favorably affect the efficiency of *B. bassiana* as a biological control agent of soil pests [Rosin et al. 1996].

The presence of individual entomopathogenic fungi in different cultivations is an indicator of their capability to survive in such environments. Research on the species composition of soil microorganisms in fields cultivated in different tillage may be useful for selecting the suitable species for biological pest control. Generally, indigenous dominants are the most suitable for this purpose [Meyling and et al. 2011].

CONCLUSIONS

1. *M. anisopliae* was the most frequent entomopathogenic fungus in soils from both organic and conventional fields. It infected the largest number of *G. mellonella* larvae as well.
2. Entomopathogenic fungi formed on average more colony forming units in 1 g of soil in soil from organic cultivations than in soil from conventional fields.
3. *M. anisopliae* and *B. bassiana* occurred in higher density in soils from organic fields, whereas *I. fumosorosea* formed more CFU in soils conventionally cultivated.

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