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Natural Occurrence and Distribution of Soil borne Entomopathogenic Fungi in Shahrood Region, Northeast of Iran

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ABSTRACT

The study investigated the occurrence of soil borne entomopathogenic fungi (EPF) in potato, wheat, sugar beet, alfalfa fields and orchards. A total of 150 soil samples were collected and EPF were isolated using Galleria method. Soil pH was rages from 6.8 to 8.1 and soil texture was sandy, loam, sandy- loam, clay and sandy-loam-clay. Soil borne EPF occurred at 78% of soil samples from which 40% *Beauveria bassiana*, 21% *Metarhizium anisopliae* and 17% had both species. Occurrence and distribution of EPF was not significantly affected by pH and texture of soil samples. Although Shahrood region is located at dry climate but this study showed that its soil is rich of EPF. Fungal pathogens collected from this soil survey will serve as a source of potential biological control agents of soil borne pests.

Keywords: Entomopathogenic fungi, soil borne, *Beauveria bassiana, Metarhizium anisopliae*

INTRODUCTION

Microbial life within the soil ecosystem is a fascinating aspect of soil biology, and has recently caught the attention of microbiologists. High populations of beneficial soil borne organisms are characteristics of healthy soils (Magdoff, 2001). Microbial assemblages in agricultural soils are important for ecosystem services in sustainable agricultural systems, including pest control (Altieri, 1999). Soil-borne pests are serious enemies of some plants and chemical control of these pests can damage to soil ecosystem and reduce its fertility. Isolating entomopathogens from soil provides insight into the naturally occurring pathogen biodiversity and provides a pool of potential biological control agents (Bruck, 2004). The soil environment constitutes an important reservoir for a diversity of entomopathogenic fungi, which can contribute significantly to the regulation of insect populations (Keller and Zimmerman, 1989). Many fungal species belonging to Hypocreales (Ascomycota) inhabit the soil for a significant part of their life cycle. Of these, *Beauveria spp*., *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Paecilomyces spp*. are especially common (Keller and Zimmerman, 1989). There is evidence for higher population levels of entomopathogenic fungi in soils of organically farmed fields as opposed to conventionally farmed fields (Klingen *et al*., 2002). Our knowledge about local species composition and distribution is important if the

indigenous populations of entomopathogenic fungi in the soil are to be managed in ways to facilitate the control of pest insect populations within the agroecosystem.

MATERIALS and METHODS

Soil Sampling

Soil samples were collected with a garden spade to a depth of about 15 cm after removal of surface litter at potato, wheat, sugar beet, alfalfa fields and orchards of Shahrood region, north east of Iran during 2006-2007. A total of 150 (30 from each field) soil samples were collected. Soil texture and PH of samples were determined by soil laboratory.

Insect Culture

Galleria mellonella larvae used in this study were obtained from infested beehives in Shahrood and were used to initiate a continuous culture on natural wax.

Baiting Procedure

Each soil sample was thoroughly mixed and approximately 40 ml soil, moistened with distilled water (if the soil was too dry), was placed in glass Petri dishes (diameter 9 cm). Larvae of third or fourth instars (approximately four weeks after hatching) were used for baiting the soil samples. Prior to baiting, the larvae were immersed in 56 oC water for 15 sec. to minimize their ability to produce silk webbing in the soil (Woodring and Kaya, 1988). No food was provided for the larvae during the bait experiment. Each soil sample was baited with 10 larvae and incubated in the dark in closed cardboard boxes at ambient room temperature (20– 25 oC). During the first two weeks of baiting the Petri dishes were frequently shaken, inverted and left upside down. Once a week the soil was inspected for dead larvae. All dead larvae or pupae were washed three times in distilled water and transferred individually to Petri dishes provided with moist filter paper and incubated at room temperature. Incubated cadavers were inspected for presence of external fungal growth. The fungi were identified morphologically both by low magnifying stereomicroscope of cadavers and by preparing slides for light microscopy. Analyses were made of frequencies of occurrence the fungal isolates between the surveyed fields by standard chi-square tests.

RESULTS and DISCUSSION

A total of 143 fungal isolates were obtained from the 150 soil samples baited. EPF were found in 78% (117 out of 150) of soil sampled (Table-1). The fungal species isolated were M. anisopliae and B. bassiana. Out of 143 EPF isolated, 86 isolates were B. bassiana and 58 isolates were M. anisopliae (Table 2). Although the number of B. bassiana in the soil samples was higher than M. anisopliae (except of potato fields) but there was no significant differences between their frequencies in the different fields (figure1and table 2). These results are in agreement with findings of other studies. Klingen et al (2002) in their study

found three EPF from soil samples including Tolypocladium cylindrosporum, M. anisopliae and B. bassiana. They mentioned that 35% of soil samples had EPF. Sosa-Gomez et al. (2001) found M. anisopliae, B. bassiana and Paecilomyces lilacinus in soybean fields. Three EPF including B. bassiana, M. anisopliae and P. tenuipes were found in Pacific Northwest nursery soils by Bruck (2004). Some of important agricultural pests spend at least one stage of their life cycle in the soil and some of them feed on underground parts of plants. Chemical control of soil-born pests is difficult and has side effects on soil ecosystem. An alternative control method is to use soil-born entomopathogenic fungi. Although entomopathogenic fungi need high relative humidity to cause infection and their use in dry climates is limited but soil environment can provide enough moisture for them. The results of this study revealed that entomopathogenic fungi are occur naturally in agricultural soils of Shahrood region which has dry climate therefore can provide good resources for biological control of soil-born pests.

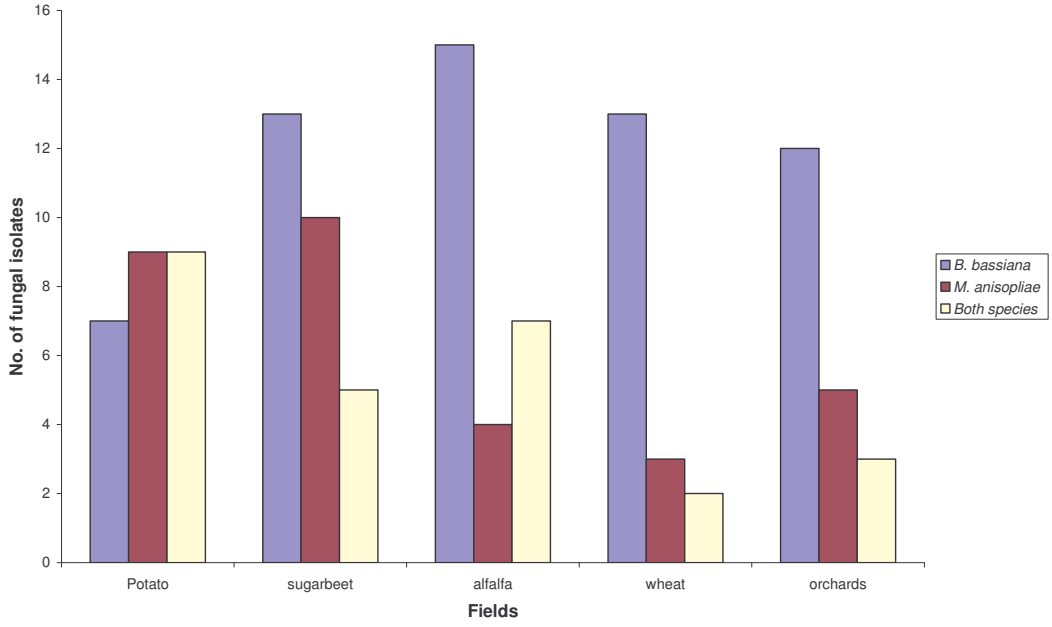

Figure 1: Occurrence of *B. bassiana* and *M. anisopliae* in soil sampled from different fields

Table 1: Soil properties, samples having fungi and number of fungal isolates collected from different fields

Fields	Soil texture	Soil pH	No. of soil samples having fungi	No. of fungi isolated
Potato	sandy, loam, sandy-loam-clay	6.8-8.1	25	34
Sugar beet	sandy-loam-clay	7.38-7.85	27	33
alfalfa	sandy-loam-clay	7.64-7.9	26	33
Wheat	sandy, loam, sandy-loam	6.8-7.8	18	20
Orchards	sandy- loam	7.55-7.93	21	23
Chi-square			2.44 $^{n.s}$	4.93 $^{n.s}$

n.s: Not significant

Table 2: Frequencies of occurrence of entomopathogenic fungi in soil samples from different fields

Fields	Samples having only *B. bassiana*	Samples having only *M. anisopliae*	Samples having both species	No. of *M.anisopliae* isolates	No. of *B.bassiana* isolates
Potato	7	9	9	18	16
Sugar beet	13	10	5	15	18
alfalfa	15	4	7	11	22
Wheat	13	3	2	5	15
Orchards	12	5	3	8	15
Chi-square	2.99 $^{n.s}$	6.25 $^{n.s}$	6.30 $^{n.s}$	9.15 $^{n.s}$	1.52 $^{n.s}$

n.s: Not significant

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