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Occurrence of entomopathogenic fungi in soil of Santiago and Fogo islands (Republic of Cape Verde)

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Abstract: *Occurrence of entomopathogenic fungi in soil of Santiago and Fogo islands (Republic of Cape Verde).* The soil is a habitat for many entomopathogenic fungi (EPF) all over the world. The Galleria bait method has been chosen to isolate the EPF from soil samples. The common occurrence of EPF in agricultural land was confirmed by these studies, which concluded, that the soil of Cape Verde is a habitat for EPF, such as *Beauveria bassiana* and *Metarhizium anisopliae*. The presence of *B. bassiana* was recorded in the cultivable land of the island of Fogo, while *M. anisopliae* occurred in the soil of Fogo as well as Santiago. There were no records of EPF in woodlands. Statistically, more soil samples containing *B. bassiana* were derived from perennial crops. The amount of sand in the soil determined the presence of the fungus. On the other hand, *M. anisopliae* was present on less inclined grounds. *Fusarium* spp. was present in half of the examined soil samples. The isolation of native EPF and their subsequent application in developing countries may significantly contribute to reduce pest populations in agriculture effectively.

Key words: entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, *Fusarium* spp., soil

INTRODUCTION

The advantages of using biological methods to control crop pests were largely discussed and documented in different countries (Cock et al. 2009, Hussein et al. 2010). In the last few years there has been an increasing interest in native biological control agents. In Europe, in the first decade of the twenty-first century seven exotic species used in biological control were successfully replaced by native species. In this period, a total of 18 native species compared with six exotic ones were commercialized. The situation was different in the African continent, where from a total of 26 species commercialized as natural enemies, 25 were originate from material collected and initially bred in other continents (Cock et al. 2009).

The designing an effective biological control in developing countries with tropical and subtropical climates is a priority. In the regions, where government programmes (e.g. Programme of the Government of Cape Verde 2011–2016, 2010) predict a growth in agricultural output, an effective plant protection should be provided. What is more, the abuse of synthetic pesticides has been declared as being one of the environmental problems in these countries (Programme strategy of Cape Verde 2009–2012, 2010).

The Cape Verde Islands are of volcanic origin. The climate of this sub-sahelian archipel is dry and semidry (de Faria 1970). The intensive rainfall between August and October is responsible for erosion, especially of steeply declined regions (between 25 and 45%).

The islands of Santiago and Fogo both provide the climatic and ecological conditions for the development of agriculture.

The agriculture of irrigated grounds is showing a growing tendency, each year increasing the areas where drop by drop irrigation is used instead of the still dominating traditional flooding irrigation. The most popular on irrigated grounds are orchards (*Musa* spp., *Mangifera* spp., citruses) and vineyards (*Vitis* sp.)

Dryland farming, with its main crops being corn and legumes such as *Cajanus cajan*, still occupy 95% of all arable grounds of Cape Verde (National Adaptation Programme of Action on Climate Change 2008–2012, 2007).

Worldwide research indicates the effectiveness of microorganisms in the pest control, in case of permanent tropical crops, such as citruses, bananas, coconut palms, mango, guava, papaya and pineapple (Dolinski and Lacey 2007).

The significance of entomopathogenic fungi (EPF) was mainly documented as a key element of an integral defence of plants against pests. There are 1,200 described species of EPF. 12–15 of these species are used throughout the world in pest control (Kowalska and Pruszyński 2007), in the form of around 120 registered biopesticides (Tkaczuk 2008).

Boczek and Lipa (1978) pointed out, that entomopathogenic representatives of Ascomycetes occur mostly in tropical and subtropical regions.

It has been proven, that the place of origin predicts the behaviour of the fungi tested afterwards in the biological pest control (Bidochka et al. 2001). That is why the usage of local strains may contribute to a more effective pest control, especially in cases, where exotic organisms have not yield any satisfying results.

In these studies, the presence of EPF in soil samples collected in agricultural and reforested areas of Santiago and Fogo was analyzed.

MATERIAL AND METHODS

Soil samples

The soil samples were collected in November and December 2011 from two islands of the Cape Verde archipelago. Seven localities were visited on the islands of Santiago (63.6%) and four on Fogo (36.4%).

44 soil samples were collected (four samples in each location). In each location, 2 kg of soil was collected at four points around the plant and mixed to obtain homogeneous sample. The samples were collected from 15 cm below the surface, previously having removed the plant waste.

The soil was sampled in three different types of habitat: reforested areas (27.3% of localities), irrigated agricultural land (54.5%) and rainfed agriculture (18.2%).

Reforested areas are highlands with a steep decline. The highlands of Santiago are mainly covered in *Jacaranda mimosifolia*, *Lantana camara* and eucalyptuses. In Fogo, however, *Cupressus* sp., *Acacia* sp., *Pinus* sp. and *Eucalyptus* sp. stand out.

Irrigated agricultural land is covered with perennial crops such as *Musa* spp., *Mangifera* sp., *Citrus* sp., *Vitis* sp.

Rainfed areas represent agricultural land with perennial crop of pigeon pea *Cajanus cajan*.

The soil samples were transported in clear plastic bags to the laboratory of the National Institute for Research and Agricultural Development (INIDA) located in São Jorge dos Órgăos (Santiago island) where they were sieved through a 3 mm mesh.

The percentage of sand in the soil samples was determined by the Analytical Laboratory of Soil, Water and Plants (LASAP) located in São Jorge dos Órgăos.

Isolation and identification of EPF

The soil samples were studied in the laboratory for the presence of EPF using the Galleria bait method (Zimmermann 1986). The last larval instar of *Galleria mellonella* (L., 1758) was used.

The larvae of *G. mellonella* (Lepidoptera: Pyralidae) were originated in laboratory in INIDA. They were produced based on an artificial diet according to the recommendations of Dr. Charles Waturu Nderito (2010).

A total of 455 larvae of *G. mellonella* was buried in 52 sterile closed plastic containers. Each box carried 400 g of soil and four larvae or 1,200 g of soil and 12 larvae. The containers were incubated at 25°C ±1°C for 30 days.

The mortality of larvae was recorded three times a week. The dead larvae were sterilized on the surface with 5% sodium hypochlorite and 70% alcohol followed by two washes in sterile, distilled water and kept separately for four days in moist chambers at 25°C. The fungi developed on the cadavers were subsequently cultivated for seven days in PDA medium in the dark and identified microscopically using taxonomic keys described by Barnett and Hunter (1987) and Watanabe (2009).

In order to statistically analyze the results, the SPSS programme was used.

RESULTS AND DISCUSSION

The mortality of larvae of *G. mellonella* introduced into soil samples reached 40.5%.

EPF were found in soil originate from Cape Verde. 57.8% of the collected soil samples contained EPF.

Besides EPF, in 37.7% of samples the presence of other potential entomopathogenic organisms such as rhabditid nematodes was noted. In 4.5% of soil samples no potential natural enemies were found.

EPF were represented by isolates of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae). Each species was isolated from 34.6% of soil samples (Fig. 1). The occurrence of *Beauveria* and *Metarhizium* in the soil from the islands of Santiago and Fogo confirms the cosmopolitan character of the aforementioned species of fungi (Meyling and Eilenberg 2007, Sánchez--Peńa et al. 2011).

In addition, in 50.0% of samples *Fusarium* spp. was found. 19.2% of soil samples contained fungi with unsporulated mycelium (Fig. 1).

From some of the samples, two or three different species of fungi were isolated. 7.7% of samples contained both aforementioned species of EPF.

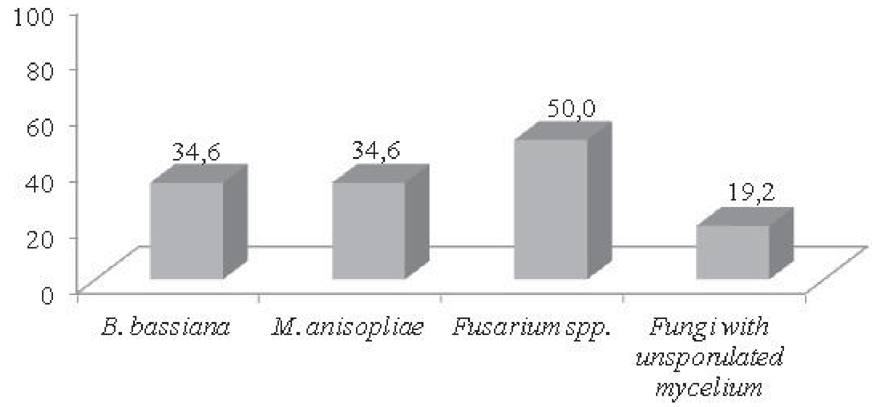

FIGURE 1. Percent of soil samples from Cape Verde containing fungi (November and December 2011)

The fungus *B. bassiana* was found on the island of Fogo (Table 1), in soil samples taken from perennial crops, represented by vineyards (75.0% of samples) and pigeon pea *C. cajan* cultivated in rainfed areas (85.7%).

According to Tkaczuk (2008), cultivating plants from family *Fabaceae* favours the occurrence of EPF. But Tkaczuk's results obtained in Poland indicated a dominance of *M. anisopliae* in soils, where *Fabaceae* were cultivated.

There was no recorded presence of *B. bassiana* in the soil of Santiago. Earlier studies, however, showed that 5% of adult weevils *Cosmopolites sordidus* (Coleoptera: Curculionidae) found on banana plantations in Santiago were infected by *B. bassiana* (Nascimento, not published). This fact may indicate the presence of endophyte strains of *B. bassiana* associated with banana crops (Dolinski and Lacey 2007).

TABLE 1. Distribution of fungi in soil of different habitats on the Santiago and Fogo islands in Cape Verde, 2011

Fungal species	Percent of samples containing fungi				
	Reforestation		Irrigated agricultural land		Rainfed areas (Fogo)
	Santiago	Fogo	Santiago	Fogo	
Beauveria bassiana	0.0 a	0.0 a	0.0 a	75.0 b	85.7 b
Metarhizium anisopliae	0.0 a	0.0 a	40.0 ab	100.0 b	14.3 a
Fusarium spp.	50.0 a	66.7 a	40.0 a	50.0 a	57.1 a
Fungi with unsporulated mycelium	50.0 a	33.3 a	30.0 a	0.0 a	0.0 a

Values in lines followed by the same letters are not significantly different.

A significant Pearson's correlation was shown between the presence of *B. bassiana* in soil samples and the presence of sand ($r = 0.647$; $p < 0.01$). On average, there was more sand in samples originate from Fogo than there was in samples from Santiago. Marjańska-

-Cichoń et al. (2005) pointed out, that sandy soil is richer in entomopathogenic fungi than argillaceous soil. But in sandy soil from Poland *M. anisopliae* was dominating.

The presence of the species *Metarhizium* was confirmed in all soil samples collected on the island of Fogo on the vine plantation and in 14.3% of soil planted with pigeon pea. On the island of Santiago the presence of *M. anisopliae* (40.0%) was related to banana plantations. All of these terrains had an inclination of no more than 25%.

A significant Pearson's correlation ($r = -0.714$; $p < 0.01$) was found between the presence of *M. anisopliae* and the inclination of the terrain.

The EPF were isolated from soil samples collected from lots occupied by orchards, vine and *C. cajan*. These perennial crops create beneficial circumstances for the survival of EPF, more so given the stability of the environmental conditions and, consequently, the presence of potential hosts, as mentioned by Chandler et al. (1997) and Tkaczuk (2008).

There were no EPF in the soil samples collected in reforested areas in Santiago and Fogo (Table 1). The reforested areas of Cape Verde are located in steeply inclined terrains and suffer from strong water erosion during the rainy season. For the record, these studies were conducted at the end of the rainy season.

The results of the studies are consistent with the results of Sánchez-Peńa et al. (2011), who showed, that wooded areas are not always a source of natural enemies. On the other hand, Quesada-Moraga et al. (2007) isolated both *M. anisopliae* and *B. bassiana* from soil originate from wooded areas in Spain. In Poland, in the soil from forested habitats the *B. bassiana* was definitely dominating (Tkaczuk 2008).

The presence of a *Fusarium* species was frequently noted (40.0–66.7%) in soil samples collected on both islands (Table 1).

The common practice of isolating *Fusarium* from soil samples using the Galleria bait method should be brought to attention. As Wenda-Piesik (2011) points out, *Fusarium* is an example of a phytopathogenic fungus also able to induce lethal reaction in insects. The dualistic properties of the various species of Fusarium are the subject of studies in the biological control of mosquitoes and nematodes *Radopholus similis*. Dolinski and Lacey (2007) assessed the potential of *Fusarium* spp. in the biological control of *C. sordidus*.

It is to be noted, that the survival and pathogenicity of EPF depends on various abiotic factors. The conidia of some of the isolates of *B. bassiana* lose their pathogenicity after three hours of insolation (Kowalska and Pruszyński 2007). Therefore, the usage of native entomopathogens in the biological control of different pests may be profitable. Native isolates are adapted to the local climatic conditions, as well as to the local entomofauna (Tangchitsomkid and Sontirat 1998, Dolinski and Moino Jr. 2006), which is why they are more effective against pests than introduced isolates.

The EPF are used, on a large scale, all over the world in the biological control of various pests of crops, such as aphids, locusts, trips, whiteflies (Cavalcanti 2006). The development of plant protection in the Cape Verde should follow the example and investigate the influence of isolated strains of *B. bassiana* and *M. anisopliae* on pests occurring on the islands.

CONCLUSIONS

The occurrence of *B. bassiana* and *M. anisopliae* in agricultural land of Cape Verde was confirmed. The presence of *B. bassiana* was recorded in the soil with higher sand content, while *M. anisopliae* occurred on less inclined fields.

The frequent presence of *Fusarium* spp. drew attention and should be studied more.

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Streszczenie: *Występowanie grzybów owadobójczych w glebach wysp Santiago i Fogo (Republika Zielonego Przylądka).* Gleba jest siedliskiem wielu grzybów owadobójczych (EPF) na całym świecie. Do izolowania EPF z prób glebowych zastosowano metodę owadów pułapkowych. Powszechność występowania EPF na terenach rolnych została potwierdzona niniejszymi badaniami, w wyniku których stwierdzono, że gleby Republiki Zielonego Przylądka są siedliskiem EPF, takich jak *Beauveria bassiana* i *Metarhizium anisopliae*. Obecność *B. bassiana* zarejestrowano w glebie wyspy Fogo a występowanie *M. anisopliae* zarówno na wyspie Fogo, jak i Santiago. Nie stwierdzono EPF na terenach zalesionych. Statystycznie więcej prób glebowych zawierających *B. bassiana* pochodziło z terenów pokrytych uprawami roślin wieloletnich, przy czym istotnym czynnikiem wpływającym na obecność grzyba była zawartość piasku w glebie. *M. anisopliae* był natomiast obecny na terenach o mniejszym nachyleniu terenu. *Fusarium* spp. był obecny w połowie przebadanych prób glebowych. Izolowanie EPF rodzimych z późniejszym ich zastosowaniem w krajach rozwijających się może w istotny sposób przyczynić się do skutecznego ograniczenia liczebności populacji szkodników w uprawach.

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