

Fungal Diversity and the Occurrence of Antagonistic Fungi in Organic and Conventional Farming Systems in Oman	العنوان:
Kazerooni, Elham Ghasemi	المؤلف الرئيسي:
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2017	التاريخ الميلادي:
مسقط	موقع:
1 - 121	الصفحات:
948468	رقم MD:
رسائل جامعية	نوع المحتوى:
English	اللغة:
رسالة دكتوراه	الدرجة العلمية:
جامعة السلطان قابوس	الجامعة:
كلية العلوم الزراعية والبحرية	الكلية:
عمان	الدولة:
Dissertations	قواعد المعلومات:
التنوع الفطري، المحاصيل الزراعية، الزراعة العضوية، الزراعة التقليدية، سلطنة عمان، المكافحة الحيوية، النظم الزراعية	مواضيع:
<a href="http://search.mandumah.com/Record/948468">http://search.mandumah.com/Record/948468</a>	رابط:

لإستشهاد بهذا البحث قم بنسخ البيانات التالية حسب إسلوب الإستشهاد المطلوب:

إسلوب APA

Fungal Diversity and the Occurrence Kazerooni, E. G., Al Sadi, A. M., و Rethinasamy, V. (2017).  
of Antagonistic Fungi in Organic and Conventional Farming Systems in Oman  
(رسالة دكتوراه غير منشورة). جامعة السلطان قابوس، مسقط. مسترجع من  
<http://search.mandumah.com/Record/948468>

إسلوب MLA

Kazerooni, Elham Ghasemi, Abdullah Mohammed Al Sadi, و Velazhahan Rethinasamy. "Fungal  
Diversity and the Occurrence of Antagonistic Fungi in Organic and Conventional Farming Systems  
in Oman" رسالة دكتوراه جامعة .دكتوراه رسالة  
<http://search.mandumah.com/Record/948468>

## 5. Discussion

Soil biodiversity is an important factor in ecosystem regulation (Guo et al., 2015; Rangel et al., 2015; Tardy et al., 2015; Thomson et al., 2015; Van Geel et al., 2015). Evaluation of microbial community and diversity in soil can help us improve soil health and quality and give us feedback about the influence of ecosystem and global changes. However, we are dealing with an open system and various factors such as agricultural practices may have effect on natural occurrence of beneficial microorganisms or reduce their occurrence rates (Heilmann-Clausen et al., 2015; Tardy et al., 2015; Thomson et al., 2015).

Believing that land use and soil type have an impact on microbial communities and activities, this investigation was carried out to evaluate soil microbial community in different farming systems in Oman. Similarly, the presence of antagonistic fungal species in these soils was quantified and characterized based on direct plating and molecular methods. Additionally, the potential of these antagonistic fungi to control damping-off of cucumber was examined.

In the first part of the study, we compared the efficiency of direct plating and pyrosequencing in estimating fungal diversity in soil. Several studies using molecular techniques and cultivation-based methods have described the fungal communities present in different farming systems (Al-Sadi et al., 2015b; Van Geel et al., 2015; Kazeeroni and Al-Sadi, 2016).

Fungal diversity was higher with the pyrosequencing than the direct plating method. These dissimilarities may be because many of the fungi are not cultivable. Some studies showed that approximately 1% of the total microbes could be detected by culture-based methods (Sugiyama et al., 2010). In addition, the high temperature in the Omani desert, which sometimes exceeds 50°C in summer, could have affected fungal diversity in soil by killing or suppressing several fungal species that are heat sensitive (Abed et al., 2013; Classen et al., 2015; Costa et al., 2015). This in turn could have affected the number of fungal species recovered from soil by culture-dependent methods. Also the absence of *Chytridiomycota* and *Basidiomycota* in culture-based

method could have been because they either need specific media or their presence was limited as evidenced by pyrosequencing analysis (only 3.51% and less than 0.77% of the total soil population, respectively) (Gleason et al., 2007; Yee et al., 2016). Therefore, the detection of the precise diversity of fungi in a habitat using culture-based techniques is still challenging. Pyrosequencing recovered more taxa that were not revealed by the culture-based method, implying that this approach will speed up the detection of very rare fungal species (Huang et al., 2015; Liu et al., 2017).

Microbial communities get influence from land use practices. Cultivation techniques, fertilization and pesticides are major factors that lead to significant changes in soil microbial communities (Tardy et al., 2015; Thomson et al., 2015; Colas et al., 2016; Liu et al., 2017). For instance, tillage practices may disrupt fungal network, fertilization alter nutrient balance and pesticides can affect their community directly or indirectly (Willekens et al., 2014; Wu et al., 2015; van Bruggen et al., 2016). In our study, the conventional farming system in Oman has been found to harbor differential level of fungal diversity. Richness estimates revealed that the level of fungal diversity in soil from organically grown cucumbers/tomatoes was higher compared to cucumbers/tomatoes grown inorganically. This is the first 454-pyrosequencing-based study, which addressed fungal diversity in CN under arid conditions.

Previous studies have shown that microbial diversity increases significantly in farms, which depend on organic manure as compared to chemical fertilization (Qiu et al., 2012; Zhang et al., 2012). Organic manures are known to harbor high fungal diversity (Al-Mazroui and Al-Sadi, 2015; Al-Sadi et al., 2015b; Al-Mazroui and Al-Sadi, 2016). The application of organic animal manures instead of mineral fertilizers in the organic farm could have contributed to increasing the diversity of fungi in organic soil samples (Al-Yahyai and Khan, 2015). In addition, organic composts, which are applied frequently to these farms are usually treated with some fungal species for biocontrol purposes, which may explain the higher level of fungal diversity in these farms (Al-Sadi et al., 2015b; Kohler et al., 2015; On et al., 2015).

The lower level of fungal diversity in the rhizosphere of CN cucumbers and tomatoes could be related to the use of fungicides in this farming system. Cucumbers and tomatoes are affected by wilt and other root diseases, which necessitate frequent applications of mefenoxam, thiophanate-methyl, hymexazol and other fungicides (Al-

Sadi, 2012; Al-Sadi et al., 2012a; Al-Sadi et al., 2015a). Applications of these has been found in several cases to exceed 10 applications in a season. In addition, pesticides are applied for the management of insect pests, and their application rates can be once every three days (Al-Kiyumi, 2006). It is therefore likely that fungicide applications could have resulted in lowering fungal populations in the rhizosphere of cucumbers. Fungicide applications have been shown to contribute to the reduction of fungal diversity in soils (Esmaeili Taheri et al., 2015).

Unweighted UniFrac analyses of fungal diversity showed clustering of the different soil samples into different groups as influenced by the farming systems, i.e. different farming systems made a qualitative difference in fungal diversity. This may suggest that associated cultural practices determine the fungal community structure as well as the quantity of fungal taxa in a given farm. In another study on *Agave* species, Coleman-Coleman-Derr et al. (2016) suggested that biogeography of the host species is the major determinant of fungal microbiome.

*Ascomycota* is the largest and widespread phylum of fungi and is abundant in soil and composts (Abed et al., 2013; De Gannes et al., 2013; Kazeeroni and Al-Sadi, 2016). Differences in the species composition between the different soils could be related to the different growing systems, cultural practices and crops. *Ascomycota* phylum was the most dominant phylum in most of the samples from OR and CN. This phylum is widespread in different soils around the world (Qiu et al., 2012; Abed et al., 2013; Acosta-Martínez et al., 2014; Huang et al., 2015; Chen et al., 2017). This is mainly because *Ascomycota* contains several pigmented species which usually tolerate higher temperatures and because many species of this phylum produce abundant spores (Abed et al., 2013).

*Microsporidia* phylum was also common in some farm soils. *Microsporidia* are obligate, spore-forming, fungi-related, intracellular parasites that infect many vertebrates and invertebrates. Several species of microsporidia have been described as biocontrol agents and pathogens of several beneficial insects (Bjornson and Oi, 2014). For example, *Nosema pyrausta* is effective in controlling European corn borer (*Ostrinia nubilalis*) (Lewis et al., 2009). A formulation of *Paranosema locustae* is commercially available for biological control of rangeland grasshoppers (Bjornson

and Oi, 2014). Several studies reported that *Microsporidia* are natural intracellular parasites of the nematodes including *Caenorhabditis* (Kaya et al., 1988; Troemel et al., 2008; Zhang et al., 2016). The *Microsporidia*, *Nematocida parisii* was reported as a natural intracellular pathogen of *C. elegans* (Troemel et al., 2008). Zhang *et al.* (2016) described six new species in the *Nematocida* genus that are capable of infecting *C. elegans*.

Physicochemical analysis of soils showed variations in soil pH, carbon and nutrients from one soil and farm to the other. The levels of organic carbon were the same or lower in the organic soils compared to soils from conventional farms. Previous studies have shown that organic systems do not always increase organic matter in the soil compared to conventional systems (Li et al., 2012). The slightly lower soil pH in CN compared to OR is in agreement with previous studies (Li et al., 2012). Soil pH and nutrients are usually affected by vegetation, soil type, carbon and nitrogen (Barak et al., 1997; Kuramae et al., 2012).

Previous studies have shown that fungal communities present in each system vary with the soil physiochemical properties and the cropping systems (Huang et al., 2015; Thomson et al., 2015). The presence of relatively high percentage of TOC and TIC in farms may be favorable for the growth of most fungi. The application of fertilizers by growers could have contributed to creating differences in the available minerals in soils between farms and soil types (Grüter et al., 2017; Wang et al., 2017). Grantina *et al.* (2011) reported a positive impact of the available potassium in soil on the total number of cultivable filamentous fungi (CFF) and on fungal diversity. In another investigation, a negative impact of phosphorus was observed on species richness of fungi in soil (Huang et al., 2005). As suggested by Gyaneshwar *et al.* (2002), this could be due to variations in the number of phosphate solubilizing microorganisms in soil.

The current investigation has shown that most of the detected fungal taxa in soils belong to genera which are saprophytic or used as biocontrol agents. A very limited number of potentially pathogenic taxa were observed in farm soils (e.g. *Fusarium*). This indicates the health status of soils, especially organic soils.

Healthy soils are harboring microbes that many of them are capable of suppressing plant diseases. Disease suppression can be attributed to the production of antimicrobial compounds, emitting plant growth promoting compounds, induction of defense mechanisms in plants, and nutrient competition with pathogens (Ting et al., 2014; Arroyave-Toro et al., 2017; Collazo et al., 2017; Culebro-Ricaldi et al., 2017; Zhang et al., 2017). All of these activities may occur simultaneously through the activity of one or more beneficial microbes. Applying beneficial microorganisms as biocontrol agents is an environmentally friendly method to protect plants against diseases and reduce the use of harmful chemicals (Prasanna et al., 2015; Zhang et al., 2017). Litterick et al. (2004) reported that beneficial microbes such as *Penicillium* spp., *Trichoderma* spp., *Bacillus* spp., etc. in compost can act as biological control agents. Antagonistic microbes can flourish due to the organic material or pre-inoculation of composts with beneficial microbes (Kohler et al., 2015; Nicol and Burlakoti, 2015; On et al., 2015; Al-Mazroui and Al-Sadi, 2016).

The isolation and identification of antagonistic microorganisms as potential and suppressive biocontrol strains from different sources such as soil and plant is becoming more popular (Huang et al., 2016; Siala et al., 2016; Raza et al., 2017). In this research work, *Talaromyces pinophilus* (TT266) significantly reduced cucumber damping-off caused by *Pythium aphanidermatum* and *Rhizoctonia solani* by 31% and 54%, respectively. The efficiency of *T. flavus* as biocontrol agent against various soil borne disease such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Verticillium dahliae* has been reported (Ojaghian, 2011; Kakvan et al., 2013; Naraghi et al., 2014; Yuan et al., 2017). In addition, the antagonistic activity of *T. flavus* against *Verticillium* wilt has been reported on various crops such as cotton, eggplant, potato and tomato (Naraghi et al., 2010; Naraghi et al., 2012). Our study appears to be the first record of the use of *T. pinophilus* to control *Pythium* and *Rhizoctonia* damping-off of cucumber.

Application of *T. pinophilus* (TT266) to cucumber led to the improvement in dry weight of seedlings. Sembiring et al. (2017) reported the positive effect of *T. pinophilus* on phosphorus uptake that leads to the increase in growth and yield of potato. In addition, novel bioactive compounds have been isolated from *T. pinophilus* that may have promising effect to protect against pathogens and offer significant

benefits to promote plant growth as well as for industrial uses (Xian et al., 2015; Krishnapura and Belur, 2016; Sathiyabama and Parthasarathy, 2017; Sembiring et al., 2017; Vinale et al., 2017a; Vinale et al., 2017b).

Our results regarding the biocontrol of *Pythium* and *Rhizoctonia* by *Trichoderma asperellum* isolate TO144 support previous findings that demonstrated the ability of *T. asperellum* to reduce Pythium and Rhizoctonia diseases in several crops (Mbarga et al., 2012; Kakvan et al., 2013; Kipngeno et al., 2015; Vongphachanh et al., 2016; Manjunath et al., 2017; Vasumathi et al., 2017). Several species of *Trichoderma* including *T. harzianum* and *T. viride* are known as very interactive biocontrol agents in soil and roots and able to reproduce and multiply rapidly (Ozbay et al., 2004; Blaya et al., 2013; Yu et al., 2014; Velmourougane et al., 2017). *T. asperellum* isolates have the ability to elicit plant immune responses against several plant diseases (Fernández et al., 2014; Patel et al., 2017). In addition, the fast growth rate is another advantage of several *Trichoderma* species including *T. asperellum*, which enables them to compete for space and nutrients with target pathogens (Simon and Sivasithaparam, 1988; Benítez et al., 2004).

Management of damping-off disease should take into account the use of integrated disease management strategies. Disease resistance in plants is one of the most cost-effective ways to control diseases. At present, no cucumber cultivars resistant to Pythium damping-off disease are known to exist. However, variability in resistance and tolerance between varieties and accessions has been reported in cucumber and other crops (Karmakar et al., 2003; Higginbotham et al., 2004; Lucas and Griffiths, 2004).

Defence responses deployed by host plants in reaction to invasion by pathogens are an important component of integrated management of plant diseases. It can reduce or eliminate the need for using chemicals and biological formulations and therefore reduce the costs and environmental hazards associated with increased applications of chemicals.

Increasing the defence response in plants to infection has been attempted through the use of a number of techniques including the use of chemicals such as salicylic acid and benzothiadiazole (Benhamou and Bélanger, 1998; Karmakar et al., 2003), non-



pathogenic microorganisms such as *Fusarium oxysporum* Schltdl. strain Fo47 to induce an increased defence response to *Pythium ultimum* infection in cucumber (Benhamou et al., 2002) and silicon (Chérif et al., 1992; Chérif et al., 1994; El-Samman, 2000).

Cultural practices are an important tool in the management of damping-off disease of cucumber. They include the use of soil solarization, crop rotation, replacement of old soil with new soil and irrigation management. Al-Samarria *et al.* (1988) found soil solarization to be the best in the control of *P. aphanidermatum* and some other soil pathogens in Iraq. Crop rotation has also been found to be effective in management of root disease caused by *Pythium* species (Sumner et al., 1999). Irrigation management represents another control tactic for reducing incidences of damping-off in greenhouse cucumbers (Al-Kiyumi, 2006). Soil replacement is another cultural practice employed by growers in Oman to manage *Pythium* inoculum in greenhouse systems (Al-Sa'di et al., 2008). Thus, the use of a combination of these methods may help reduce severity of damping-off disease.