Estimate the Presence of Pathogenic Fungi in the Soil

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ABSTRACT: Ten random samples were collected from two sites in the city of Tobruk for the purpose of the study, the first site was from Al-Haj Ahmed Atallah grape farm at the east entrance of the city and the second site was from a Faculty of sciences belonging land and transported immediately to laboratory Botany department for analysis. inoculated on CZAPEK DOX AGAR and incubated at 25±2 C° and observed for 4-7 days after which the different colonies obtained were identified using the slide culture technique. The pathogenic fungal analysis shows that , *Mucor sp* has the highest frequency of occurrence (33.3 %) followed by *Aspergallius spp* (32.8 %) then *Penicilium spp* (22%) *Alternaria spp* with (20.8 %) *Rhizoctonia solani* (19.4) and *Fusarium spp* (14.9) frequency of occurrence.

Keywords: Pathogenic **,** Fungal analysis, frequency of occurrence.

Introduction

Soil is a most precious natural resource and contains the most diverse assemblages of living organisms. Indigenous microbial populations in soil are of fundamental importance for ecosystem functioning in both natural and managed agricultural soils (O'Donnell *et al*. 1994; Doran and Zeiss 2000) because of their involvement in such key processes as soil structure formation, organic matter decomposition, nutrient cycling and toxic removal (Van Elsas, 1997; Doran and Zeiss, 2000). The community of soil flora and fauna is influenced directly or indirectly by management practices, e.g. cultivation and the use and application of organic and inorganic fertilisers (Bloem *et al*. 1994; Matson *et al*., 1997). A growing number of studies show that organic farming leads to higher soil quality and more biological activity (microbial populations and microbial respiration rate) in soil than conventional farming (Droogers and Bouma, 1996; Mader *et al*. 2002; Girvan *et al.,* 2004). Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil (Pansombat *et al.,* 1997; Tokuda and Hayatsu, 2002). Further, considerable evidence indicates that changes in the composition of a microbial community can be used to predict and dictate alteration in soil quality (Van Brugen and Semenov, 2000; Breure, 2005) .

Microbial communities, particularly bacteria and fungi constitute an essential component of biological characteristics in soil ecosystems. It has been estimated that 1.5 million fungal species are present in natural ecosystems, but only 5 –10% have been described formally (Hawksworth 2001). Schmit and Mueller (2007) estimated that there is a minimum of 7, 12,000 fungal species worldwide. The actual number of fungi is still unknown; however, only 5-13 % of the total estimated global fungal species have been described (Wang et al. 2008). Research on fungal diversity provides a basis for estimating the functional role of fungi in ecosystems. Soil fungal population is favoured largely by organic

farming systems (Drinkwater et al., 1995; Girvan et al., 2004) .

Fungi are an important part of the microbial ecology. The majority of fungi decompose the lignin and the hard-to-digest soil organic matter, but some fungi consume simple sugars. Fungi dominate in low pH or slightly acidic soils where soils tend to be undisturbed (Lavelle

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& Spain, 2005). Fungi break down the organic residues so that many different types of microbes can start to decompose and process the residues into usable products.

Approximately 80 to 90 percent of all plants form symbiotic mycorrhizae fungi relationships by forming hyphae networks. The hyphae are about 1/60 the diameter of most plant root hairs and assist the plant in acquiring nitrogen, phosphorus, micronutrients and water in exchange for sugar produced by the plant. This mutually beneficial relationship is called a mycorrhizae network (Magdoff & Van Es, 2009).

fungi are small, generally microscopic, eukaryotic, usually filamentous, branched, spore-bearing organisms that lack chlorophyll. Fungi have cell walls that contain chitin and glucans (but no cellulose) as the skeletal components. These are embedded in a matrix of polysaccharides and glycoproteins.

Fungi constitute a highly versatile group of eukaryotic carbonheterotrophic organisms that have successfully occupied most natural habitats. The vast majority of fungi are strict saprophytes; <10% of the *lOO,OOO known fungal sp-ecies are able to colonize plants, and an even smaller fraction of these are capable of causing disease. Among the causal agents of infectious diseases of crop plants, however, phytopathogenic fungi play the dominant role not only by causing devastating epidemics, but also through the less spectacular although persistent and significant annual crop yield losses that have made fungal pathogens of plants a serious economic factor, attracting the attention of farmers, plant breeders, and scientists alike. All of the .v300,000s pecies of flowering plants are attacked by pathogenic fungi. However, a single plant species can be host to only a few fungal species, and similarly, most fungi usually have a limited host range. The evolution of fungal phytopathogens toward a high degree of specialization for individual plant species may be reflected in the different levels of specialization observed in extant plant-funga1 interactions (Scheffer, 1991).

The first level may be Seen in opportunistic parasites, which enter plants through wounds or require otherwise weakened plants for colonization. These fungal species are usually characterized by a broad host range but a relatively low virulence, that is, they cause only mild disease symptoms. The next level comprises true pathogens that rely on living plants to grow byt that under certain circumstances can survive outside of their hosts. Many of the more serious plant pathogens are found at this level; most are highly virulent on only a limited number of host species. Finally, the highest level of complexity is achieved by obligate pathogens, for which the living host plant is an absolute prerequisite to fulfill their complete life cycle (Keen, 1986).

Material and methods :

- **Samples Collection .**

Ten random samples were collected from two sites in the city of Tobruk for the purpose of the study, the first site was from Al-Haj Ahmed Atallah grape farm at the east entrance of the city and the second site was from a Faculty of sciences belonging land . All the samples collected were placed in a sterile polythene bags separately and labeled appropriately and transported to Botany department laboratory. Faculty of Sciences, University of Tobruk.

- **Isolation of fungi .**

1000 ml of CZAPEK DOX AGAR medium was prepared and after sterilization in the autoclaving and the temperature of the mixture was reduced to 45 °C, an antibiotic (Amoxicillin 250 mg) was added to the medium and distributed in Petri dishes with 18 plates distributed in 6 plates for each of the first and second sites in addition to the control, the soil was added after solidification of the medium at a rate of 1 g for each dish and then placed in the incubator at a temperature of 25 ± 2° C for 4-7 days to observe the fungal growth.

From the incubated plates the different fungal isolates with different colorations observed includes; (1) Brown (2) Black (3) Red (4) Green and (5) White which signified the occurrence of different fungal colonies. The fungal colonies that emerged were continuously sub-cultured in order to obtain a pure culture of the fungal isolates.

- **Identification of the Fungal Isolates :**

The one to four weeks pure cultures of the fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation (Sparrow, 1976), by slide culture techniques (Oyeleke and Manga, 2008). A small portion of the aerial mycelia from the representative culture was picked using a sterile inoculating needle and inoculated on a slide containing a fraction of a prepared solidified Potato Dextrose agar and incubated for 24-48hours, after which it was viewed under the light microscope first with (x10) and then with (x40) objective lens to detect spore, hyphae and other special structures.

The Morphological characteristics and appearance of the fungal isolated from the soil used in this study were confirmed and authenticated with the help of Pictorial Atlas of Soil borne Fungal Plant Pathogens and Diseases (Taylor & Francis Group 2018) .

Results and Discussion :

The isolated fungi from the Soil and their frequencies of occurrence are shown in Table 1. This study shows that Out of the fungi isolated, *Mucor sp* has the highest frequency of occurrence (33.3 %) followed by *Aspergallius spp* (32.8 %) then *Penicilium spp* (22%) *Alternaria spp* with (20.8 %) *Rhizoctonia solani* (19.4) and *Fusarium spp* (14.9) frequency of occurrence.

Table 1: Fungi isolated from the Soil .

Fungi	Frequency (%)	
	Site A	Site B
Mucor sp	10.4	33.3
Rhizoctonia solani	19.4	7.4
Penicilium spp	31.3	23.6
Alternaria spp	2.9	20.8
Aspergallius spp	32.8	2.7
Fusarium spp	14.9	0

It is clear from the table that there is a observe difference between the two sites. that the present *Fusarium spp* is found in site A in the rhizosphere and is not found in the second site.

In the Pacific Northwest and other geographical areas with low annual rainfall (20–40 cm) and dryland farming practices, Fusarium foot rot of wheat caused by *F. culmorum* can limit crop production. The pathogen survives as chlamydospores in the crop debris mulch layer and infects roots 2–3 cm below the soil surface

where secondary roots emerge. Under adequate moisture conditions, no further disease development occurs, but under water stress conditions (very low water potentials), the pathogen colonizes the crown roots and moves up the stem one to three internodes causing a chocolate brown discoloration of the inner tissues while the leaf sheath wrapped around the internodes remains apparently healthy (Taylor & Francis Group 2017).

the fungus *Mucor sp* and *Alternaria spp* with the highest percentage in the second site of the study . however the *Rhizoctonia solani*, *Penicillium spp* and *Aspergillus spp* were found in the first site with the highest percentage compared to the second site. The presence abundant organic matter in the first site This is however in agreement with , Taylor & Francis Group 2017 .

R. solani is a common soilborne fungal pathogen with a very large host range that causes pre- and post-emergence damping-off, root rot, and stem and crown rots in plants Most agricultural soils contain inoculums of *R. solani* in the form of hyphae associated with colonized organic matter and sclerotia. (Taylor & Francis Group 2017).

Table 2: Between Comparison Mean .

		N	Mean	Std. Error
Fungi	Site A	6	1.8167	±0.57470
	Site B	6	2.4000	±0.75366
	CONTROL	6	.0000	±0.00000

Using spss statistical analysis, no significant differences were observed between sites.

تقدير نسبة تواجد الفطريات الممرضة في التربة

المستخلص:أجريت الدراسة في مدينة طبرق من خلال تحديد موقعين للدراسة الموقع الأول مزرعة الحاج احمد عطية الله والموقع الثاني قطعة ارض خاصة بقسم علم النبات كلية العلوم جامعة طبرق . جمعت العينات بواقع عشر عينات عشوائية ونقلة للمعمل للتحليل . تم تنمية فطريات التربة علي وسط غذائي (CZAPEK DOX AGAR) ووضعت في الحضانة علي درجة حرارة 2 ± 25 درجة مئوية لمدة 4 – 7 أيام لملاحظة نمو الفطريات . لوحظ من خلال التعريف وجود ست أجناس فطرية النامية علي الوسط الغذائي وكانت نسبة تكرار وتواجد الفطريات ، فطر *Mucor sp* (33.3 %)وفطر *Aspergallius spp* (32.8 %) وفطر *Penicilium spp* (22%) وفطر *Alternaria spp* (20.8 %) وفطر *Rhizoctonia solani* (19.4) وفطر *Fusarium spp* (14.9) .

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