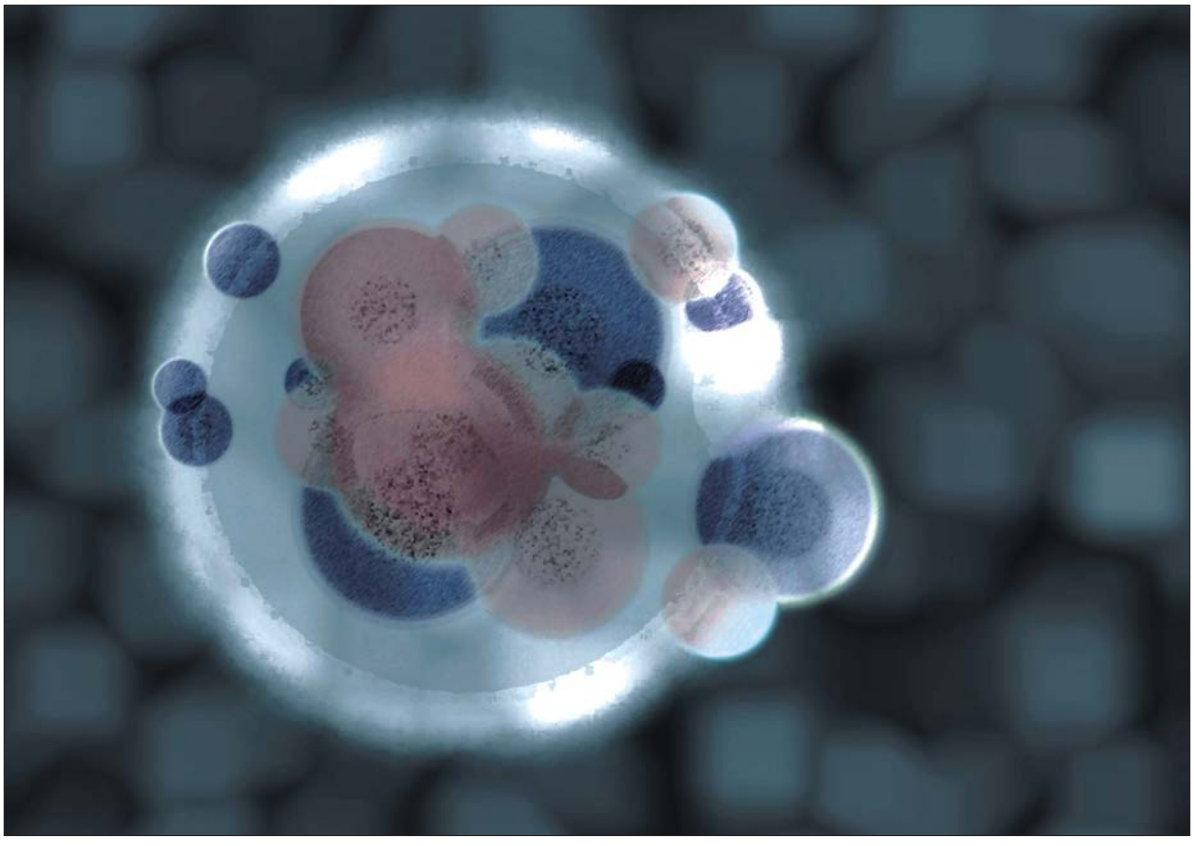

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Occurrence and Microbiological Characteristics of *Trichoderma* in Al-Jabal Al-Akhdar Region, Libya

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Abstract: The fungal flora with special attention to *Trichoderma* in 23 soil samples collected from Al-Jabal Al-Akhdar Region, Libya was studied using different culture media. *Trichoderma* Selective Medium (TSM), Martin's medium (MT) and Potato Dextrose Agar (PDA) were the superior media for isolating *Trichoderma*. Martin's medium (MT) and Malt Extract medium (ME) were the most valuable for isolating the greatest number of total fungal count. *Trichoderma* Selective Medium (TSM) supplemented with 100 μg mL^{-1} PCNB was the most effective medium for counting *Trichoderma* and recorded 120-140% efficacy of re-isolation. *Trichoderma* occurred in moderate frequency in the tested soil and was isolated from 5 soil samples. *Trichoderma* counted 0.5-1×10^3 CFU g^{-1} dry soil and five *Trichoderma* isolates were identified as *Trichoderma harzianum*. *Aspergillus* and *Penicillium* sp. were the most frequent fungi isolated from the tested soil and were averaged 8.3-5.5 CFU mg^{-1} soil, respectively. Further studies are needed to clarify the distribution of fungal flora especially *Trichoderma* sp. in the Libyan soil.

Key words: *Trichoderma* isolation, identification, characterization, pentachloronitrobenzene (PCNB), *Trichoderma* selective medium, Martin's medium and potato dextrose agar

INTRODUCTION

Trichoderma is a genus of the hypomycetes which gained specific attention during the past few years and some excellent reviews were published on this subject (Papavizas, 1985; Samuels, 1996; Chet, 1987; Lumsden, 1992). The genus *Trichoderma* was introduced into mycological literature by Papavizas (1985) to accommodate four species of fungi now commonly considered to be unrelated to one another, namely *Trichoderma viride* Pers. Ex S. F. Gray, *Xylohypha nigrescens* (Pres. Ex Fr) Mason, *Sporotrichum aureum* Pers. Ex Fr. and *Trichothecium roseum* (Pers.) Link ex S. F. Gray. The most frequently recorded green *Trichoderma* species found on fallen branches and other substrate has been regarded as the most typical representative of this genus. Therefore, it is not surprising that *Trichoderma* had been classified in the Gasteromycetes or the Myxomycetes and that many totally unrelated forms have been assigned to it. However, the hyphomycetous nature and the concept of this genus have been well established.

Trichoderma sp. are widely distributed all over the world (Domsch *et al*., 1980a, b; Attitalla and Salleh, 2010) and occur in nearly all soils and other natural habitats, especially in those containing organic matter. Individual aggregates may be restricted in their geographical distribution (Danielson and Davey, 1973a). *Trichoderma* is a secondary colonizer since it is usually isolated from well decomposed organic matter. Studies with *Trichoderma* prior to the work of Rifai (1969) were hampered by taxonomic uncertainties and thereafter also by the lack of precise techniques for culturing, isolation and enumeration of these fungi. Samuels (1996) provided detailed observations and comments on the utility of morphological characters to define species in However, molecular techniques allow rapid and reliable identification of *Trichoderma* sp. and strains (Moubasher, 1993; El-Naghy *et al*., 1998; Gherbawy *et al*., 2004).

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Trichoderma species were isolated from forest humus layer (Wardle *et al*., 1993). Individual species were reported to exhibit some restriction in their geographic distribution and were also formed to show preference to certain soil temperature and moisture content (Danielson and Davey, 1973a). *T. viride* and *T. polysporum* for example, were reported to be restricted to areas where low temperature prevail and *T. harzianum* were mostly found in warm climatic regions whereas, *T. hamatum* and *T. koningii* occur widely under diverse climatic conditions (Samuels, 1996). *T. hamatum* and *T. pseudokoningii* were reported to be adapted to conditions of excessive soil moisture (Danielson and Davey, 1973b). Additional factors which were reported to influence the distribution of *Trichoderma* members in different soils include; soil pH, soil chemical properties, salt and organic matter content and presence or absence of microorganisms in soil (Samuels, 1996; Kredics *et al*., 2003).

Trichoderma sp. have been reported from Congo, New Zealand, Australia, Germany, Norway, Italy, Spain, Turkey, Chad, Pakistan, Nepal, China, Peru, Canada, UK, India and the USA (Domsch *et al*., 1980a, b; Barooah and Borthakur, 1994). In Arabic-countries, *Trichoderma* spp. were recovered also from Libya (Youssef, 1974), Kuwait (Halwagy *et al*., 1982), Saudi Arabia (Abdel-Hafez, 1982) and Syria (Abdel-Kader *et al*., 1983). The aim of the this study was to identify and characterized the fungal flora in Al-Jabal Al-Akhdar soil, Libya and focusing on the distribution and recognition of the genus *Trichoderma*. This study is the first that demonstrated the occurrence and the distribution of fungal flora in Al-Jabal Al-Akhdar region, Libya.

MATERIALS AND METHODS

Collection of soil samples: Twenty three soil samples were collected from different localities, during 2008 and 2009, collections have been made in all seasons (autumn, winter, spring and summer times) (Fig. 1) which represented both cultivated and non cultivated soils in Al-Jabal Al-Akhdar. For each soil sample the top surface soil was removed (about 3 cm) and 5 subsamples were taken at random to a depth of 15 cm for each site using a sterile auger. The soil was transferred to the laboratory in sterile polyethylene bags under aseptic conditions the subsamples of each site were bulked to yield one composite sample representing the area. The soil was allowed to dry by exposure to ambient temperature. When adequate moisture content was reached the samples were sieved through 2 mm mesh and soil characters were determined.

Determination of soil texture: The soil type was determined by the hydrometer method, as described by Piper (1955). Bouyoucos hydrometer was calibrated to read directly in percentage of soil remaining in suspension.

Soil chemical analysis

Total soluble salts: For the determination of total soluble salts, a known weight of each soil sample was shaken in a volume of distilled water for about 30 min and the mixture was left overnight to settle. The soil extract was then filtered and a known volume was evaporated in an oven at 105°C. The dry residue was then weighed and the amount of total soluble salts per one g oven-dry soil was calculated.

Organic matter content: It was determined according to Walky and Black method (Jackson, 1958).

pH value: A Beckman pH meter was used for the determination of soil pH. The electrodes were immersed in the soil paste made with water to a ratio of 1:1 to avoid the error arising through higher dilutions (Jackson, 1958).

Determination of soil fungi: Using the dilution plating method modified by Johnson *et al*. (1959). After incubation at 28°C, usually from five to seven days, the resulting colonies were counted. The average number of colonies per dish was multiplied by the dilution factor to obtain the number per gram in the original soil sample.

Isolation media: Five different types of media were compared for isolation soil fungi including *Trichoderma* from the tested soil. These culture media include: (1) glucose-Czapek's agar medium (CZ) (2) Martin's medium (Martin, 1950), Rose Bengal was added at a concentration of 1/15000 and streptomycin 50 ppm after autoclaving. (3) Malt Extract medium (ME); (4) *Trichoderma* Selective Medium (TSM) (Elad *et al*., 1981), Chloromycetin, 0.25 and Rose-Bengal, 0.15 were added after autoclaving.

Potato Dextrose Agar (PDA): For determination of the medium efficiency for isolation of *Trichoderma* from soil, conidial suspension was prepared from *Trichoderma* slants growing on PDA medium and added to the soil to give a concentration of 10^4 Conidia/g soil and inoculated soil was incubated for 24 h at 28°C. After which *Trichoderma* count was determined on different-media by the plate count method.

Identification of fungi: The identification of fungal genera other than *Trichoderma* was made through the help of the following references: Ainsworth (1971), as dictionary of the fungi; Bernett (1960), for the genera of

imperfect fungi; Domsch and Gams (1972), for fungi in general; *Trichoderma* species were identified using the key of Rifai (1969).

Some culture characteristics of *Trichoderma* isolates

Microscopic characters: *Trichoderma* isolates of grown on malt extract from PDA slants for 4 days at 28°C in the dark then exposed to light before examination were obtained. Cultures were examined under a light microscope (Olympus, C×21, Japan). Mode of branching Conidiophores, shape of Conidia, phialides characters were observed for each isolate.

Measurement of growth at 28°C: Czapek's agar medium was inoculated in the center with a mycelial disc (0.5 cm diameter) of each isolate obtained from fresh culture of the fungus grown on PDA and incubated at 28°C for 4 days. The radial growth (colony diameter) of the fungus colony was measured daily from the reverse side (three replicates were used and the results were averaged).

Colony colour and reverse side: Three agar plates of each medium for each isolate were prepared from fresh culture on PDA and incubated for 2 days in the dark and allowed 2 days in the light at 28°C to sporulate and the colony colour as well as the reverse side was recorded.

Odour observation: The growth experiment was also used to record the fungus of specific odour by the fungus particularly the aromatic or the coconut odour of each isolate on each media.

RESULTS

For studying the distribution of *Trichoderma* in different areas on Al-Jabal Al-Akhdar region, 23 soil samples were collected from different localities which represented both cultivated and non cultivated soils.

Table 1 show that the soil organic matter content was ranging between 0.8% (cultivated sandy soil in El-Hania region) and 4.3 (cultivated sandy loam soil in Al-Byda region). Total soluble salts were ranging between (E.C.) 0.13-0.51 dS m^{-1} with exception of sample No. 23 collected form El-Hania region which recorded 0.92 dS m^{-1}. The pH values of the soil samples were mostly alkaline, ranging between 7.6-8.4.

Quantitative and qualitative estimation of *Trichoderma* sp. in soil is often difficult due to the relatively rapid growth of some soil fungi in conventional agar media. Five different types of media were compared for their suitability for isolation of *Trichoderma* from soil. These include: 1: glucose-Czapek's agar medium (CZ), 2: Martin's medium (MT), 3: Malt extract medium (ME), 4: *Trichoderma* selective medium (TSM), 5: Potato dextrose agar (PDA), Comparing the *Trichoderma* selective medium with 4 other media, results presented in Table 3, 4 indicated that both of Martin's medium and Potato dextrose agar were superior to the others for isolating and counting of *Trichoderma* and gave the highest number (1.0×10^3 CFU g^{-1} dry soil) in soils numbers 5 and 8.

Table 2 shows that Martin's medium and Malt extract medium were the most efficient in isolation. They gave the highest fungal numbers for soil numbers: 2, 6, 9, 10, 12, 20, 22 and 23 for Martins medium and soil number: 1, 3, 4, 7, 8, 12, 13 and 21 for Malt extract medium. However, Glucose Czapek's and Potato Dextrose Agar and *Trichoderma* selective medium recorded the lowest total fungal population except in case of soil numbers: 11, 16, 18 and 19 for CZ medium and soil numbers 5 and 17 for PDA and soil number 15 for *Trichoderma* selective medium. Results also indicate the *Trichoderma* were

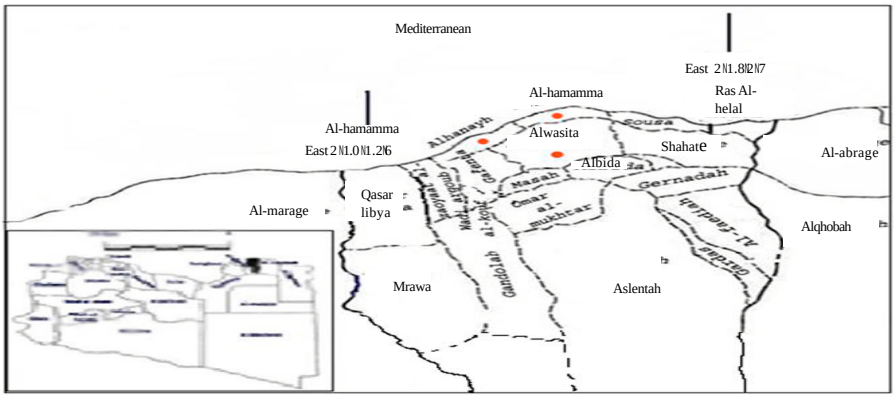

Fig. 1: Map of Al-Jabal Al-Akhdar region shown different places of collection of soil samples (1-23 place)

Table 1: Characteristics of the soil samples and plant used for isolation of *Trichoderma*

Sample No.	Place	Particle size distribution (%) Sand	Silt	Clay	Texture	pH	EC (dS m^{-1})	Organic matter (%)	Plant under cultivation
1	Grenada	64.70	26.8	08.05	Sandy	7.7	0.2	3.3	*Pimpinella anisum*
2	Al-Beida	66.68	20.72	12.59	Sandy	8.2	0.3	4.3	*Triticum vulgaris*
3	El-Kharika	70.6	26.72	2.59	Sandy	7.9	0.4	4.1	*Phagnalon rupestre*
4	Oma	64.69	20.35	14.95	Sandy	8.2	0.47	2.4	*Thapsia garganica*
5	El-Faidia	60.68	23.72	15.59	Sandy	7.9	0.33	2.2	*Marrubium vulgare*
6	El-Mansora	63.04	30.45	6.51	Sandy	7.7	0.43	1.9	*Portulaca oleracea*
7	Shahat	70.68	20.72	8.59	Sandy	7.8	0.41	3.1	*Thymus serpyllum*
8	Wardama	70.68	24.72	4.59	Sandy	7.6	0.30	3.0	*Marrubium vulgare*
9	Masa	64.68	24.72	10.59	Sandy	7.7	0.38	2.7	*Glycine max*
10	Belhaded	66.68	24.72	8.59	Sandy	7.8	0.27	2.0	*Ceratonia siliqua*
11	El-Waseta	68.68	18.72	12.59	Sandy	8.1	0.22	3.2	*Zea mays*
12	Eslenta	68.68	19.72	11.59	Sandy	7.8	0.62	3.8	*Cucumis sativus*
13	Gandola	68.4	19.0	12.59	Sandy	7.7	0.51	1.3	*Brassica oleracea*
14	Gardas	68.68	16.72	14.59	Sandy	7.8	0.32	1.0	*Hordeum vulgaris*
15	El-Khwimat	84.68	10.08	5.23	Sandy	8.3	0.26	0.9	*Artemisia herba-alba*
16	Marawa	66.68	26.72	6.59	Sandy	8.3	0.13	1.4	*Thapsia garganica*
17	Eljehad	72.68	18.72	8.59	Sandy	7.9	0.34	3.6	*Thapsia garganica*
18	Kaser Libya	73.04	26.36	0.59	Sandy	8.4	0.25	1.4	*Hordeum vulgaris*
19	Zawiat	78.68	08.72	12.59	Sandy	8.4	0.27	1.6	*Triticum vulgare*
20	Ekfenta	56.68	34.00	9.31	Sandy	8.0	0.18	1.5	*Thapsia garganica*
21	El-Hamama	69.68	21.72	8.59	Sandy	8.0	0.26	1.2	*Artemisia* sp.
22	El-Koof	73.04	18.36	8.59	Sandy	8.3	0.31	1.8	*Paronychia argentina*
23	El-Haneia	90.68	05.44	3.87	Sandy	7.7	0.92	0.8	*Lycopersicum* sp.

Table 2: Total fungal counts (colonies /mg dry soil) isolated from different soil samples on different media

Media	Sample No. 1	2	3	4	5*	6	7*	8*	9	10	11	12	13	14*	15*	16	17	18	19	20	21	22	23
Czapek's glucose	5.5	15	6.0	8.0	12.5	7.5	9.5	10.5	9.5	2.5	13.0	15.0	11.0	12.5	06.5	8.5	2.0	14.0	10.5	7.5	11.5	14.0	9.5
Martin's (MT)	5.5	15.5	14.0	12.5	14.5	16.0	18.5	11.5	11.0	13.0	12.5	9.5	11.5	11.5	11.0	7.0	2.5	13.0	9.4	13.5	11.5	15.0	15.5
Malt extract (ME)	5.5	15.5	13.0	13.5	8.5	13.0	14.1	15.5	6.5	12.5	7.5	7.5	12.5	13.5	12.5	1.5	6.5	9.5	9.1	9.5	14.0	5.0	12.5
Trichoderma selective medium (TSM)	1.0	6.0	02.5	8.0	14.5	2.0	4.0	4	7.5	1.5	6.5	1.5	4.0	2.5	13.5	1.5	1.5	5.5	8.5	12.0	13.0	12.0	13.5
Potato dextrose agar (PDA)	3.5	9.0	8.3	12.5	16	13	9.0	12.0	3.0	2.5	2.5	2.5	5.5	9.0	9.5	1.5	7.5	7.5	6.0	9.5	8.5	11.5	7.5

**Trichoderma* was slated

Table 3: Counts of *Trichoderma* (colonies/mg dry soil) isolated from different soil samples* on different media

Medium	Sample No. 5	7	8	14	15
Czapek's glucose (CZ)	0.0	0.0	0.0	0.0	0.0
Martin's (MT)	1.0	0.5	0.0	0.0	0.0
Malt extract (ME)	0.0	0.0	0.0	0.0	0.0
Trichoderma selective medium (TSM)	0.0	0.0	0.0	0.5	0.0
Potato dextrose agar (PDA)	0.0	0.0	1.0	0.0	0.5

*The total soil samples were 23 and *Trichoderma* was only present in 5 soil samples

Table 4: Evaluation of medium efficiency for isolation of *Trichoderma harzianum* (T7 and T8) from soil

Medium	*T. harzianum* isolate	Total count (colony/ mg soil)	Medium efficiency (%)
Czapek's glucose (CZ)	T7	8	80
	T8	6	60
Martin's (MT)	T7	10	100
	T8	11	110
Malt extract (ME)	T7	9	90
	T8	8	80
Trichoderma selective medium	T7	12	120
	T8	14	140
Potato Dextrose Agar (PDA)	T7	5	50
	T8	6	60

recovered on 3 out of 5 media (MT, TSM and PDA). The growth of *Trichoderma* isolates on different culture media (Fig. 3, 4) were studied and were identified according to the key of Rifai (1969) as *T. harzianum*. *Trichoderma* was isolated only from 5 out of 23 soil samples (Table 2).

In an attempt to evaluate the efficiency of the five media for isolation of *Trichoderma* added to the soil, Table 4 shows that both *Trichoderma* Selective Medium (TSM) and Martin's medium (MT) were efficient for isolation of *Trichoderma* from the tested soil. TSM was superior compared with other media and this medium recorded an efficiency ranging from 120-140%, indicating the favorability of TSM for further studies with *Trichoderma* to determine its survival and proliferation in Al-Jabal Al-Akhdar soils.

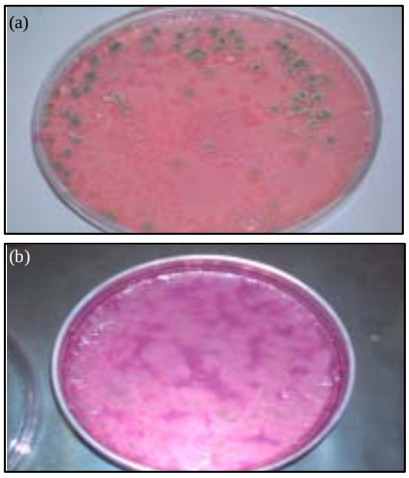

Fig. 2 (a, b): Growth of *Trichoderma harzianum* (T7) on (a) TSM+PCNB and (b) without PCNB

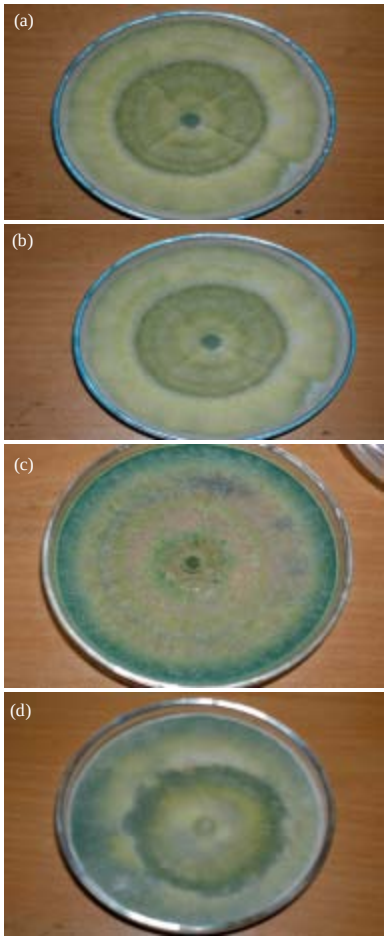

Fig. 3 (a-d): Growth of *Trichoderma harzianum* (T7) on different culture media, (a) T7-PDA, (b) T7-CZ, (c) T7-TSM and (d) T7-ME

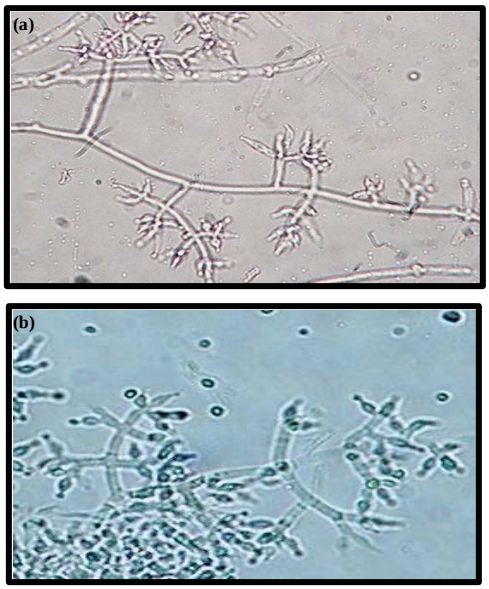

Fig. 4 (a-b): Conidia, conidiophore branching and phialospores in *Trichoderma harzianum* isolates, (a) T8 and (b) T15

Results presented in Fig. 2a and b also show the effect of addition of the fungicide pentachloronitro benzene (PCNB) at the concentration of 100 μg mL^{-1} on total fugal counts on different culture media. Results showed that the addition of the PCNB clearly reduced the total fugal counts isolated on all the five culture media. Moreover, the addition of PCNB restricted the colony size (especially with *Trichoderma*) to be easily for counting.

Results presented in Table 5 shows that *Aspergillus* was the most frequent fungus. It was collected from 20 samples out of 23 on Martin's medium with average total count 8.3 colony mg dry soil and it was isolated from 19 soil samples out of 23 on Malt extract medium with count 13.3 colony mg dry soil. The second most common genus was *Penicillium* which was collected from 20 soil samples out of 23 on Martin's medium with total count 22.3 colony mg dry soil and it was isolated from 19 soil samples out of 23 on malt extract medium with average total count 5-4 colony mg dry soil.

Members of mucorales (such as *Mucor* and *Rhizopus* sp.) and class deuteromycetes; (*Fusarium*, *Alternaria*, *Curvularia*, *Humicola* and *Cephalosporium* sp.) were also recorded in moderate to low frequency of occurrence.

Table 5: Percentage of tatal *Aspergillus, Penicillium* and other genera counts in soil-samples (1-23) collected from Al-Jabal Al-Akhdar region

	Sample No. 1			2			3			4			5			6			7			8			9		
Media	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other
Czapek's glucose (CZ)	10	-	-	-	10	-	-	10	-	-	88.9	11.1	2.	98.0	-	13.3	66.7	20	10.6	77.3	12.1	85.2	14.8	-	84.2	15.8	-
Martin's (MT)	4.5	92.8	2.7	41.9	51.6	6.5	4.5	93.9	1.7	21.0	70.0	9.0	-	95.7	4.3	3.1	93.7	3.2	17.2	77.6	5.2	3.0	11.2	5.8	90.9	-	9.1
Malt Extract (ME)	10.8	78.4	9.8	36.4	36.4	27.2	-	100	-	2.9	91.1	6.0	-	100	-	53.8	46.2	-	27.0	68.0	5.00	89	11	-	100	-	-
Trichoderma selective medium (TSM)	10	-	-	66.7	25.0	8.	80.0	20	-	-	10	-	7.	90.2	-	10	-	-	-	10	-	75	25	-	97.1	-	2.9
Potato Dextrose Agar (PDA)	3.	83.9	12.9	5.	77.8	16.7	11.8	88.2	-	6.	62.5	31.1	43.7	56.3	-	15.3	6.	15.7	9.	86.3	3.	60.65	29.5	9.8	100	-	

	Sample No. 10			11			12			13			14			15			16			17		
Media	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other
Czapek's glucose (CZ)	-	100	-	1.07	96.7	2.23	40	40	20	31.25	18.75	50	82.1	7.4	10.5	-	53.8	46.	27	54	18.9	-	100	-
Martin's (M	96.1	3.9	-	2.5	91.6	5.9	57.0	21.05	3.15	81.9	-	18.1	65.4	27.2	7.4	84.2	-	15.	14.2	70.5	15.3	-	60	40
Malt Extract (ME)	100	-	-	81.	-	18.7	53.3	33.3	13.4	77.7	14.8	7.5	55.6	37.6	6.8	84.6	12.9	-	-	100	-	77	23	-
Trichoderma selective medium (TSM)	-	100	-	9.4	84.25	6.35	100	-	-	100	-	-	80	-	20	68.2	17.4	14.	16.4	65.5	18.1	-	100	-
Potato Dextrose Agar (PDA)	-	-	100	-	0.9	96.7	2.4	-	-	100	-	72.7	27.3	-	100	-	40.6	54.9	4.5	12.3	79.1	8.7	80	20

	Sample No. 18			19			20			21			22			23		
Media	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other	Asp.	Pen.	Other
Czapek's glucose (CZ)	19.6	80.4	-	66.3	30.7	3	41.9	49.5	8.6	9.3	76.7	14	-	85.7	14.3	5.4	54	40.6
Martin's (MT)	73.8	22.7	3.5	54.8	45.2	-	40.5	54.5	5	-	90.9	9.1	3.8	57.6	38.6	9.4	75.3	15.3
Malt Extract (ME)	31.2	60	8.8	72.5	27.5	-	39.2	56.4	4.4	-	73.1	26.9	14.2	-	85.8	-	100	-
Trichoderma selective medium (TSM)	64.3	29.7	6	19.1	76.4	4.5	55	37.5	7.5	-	78.1	21.9	5.3	94.7	-	-	88.5	11.5
Potato Dextrose Agar (PDA)	22.8	68.5	8.7	36.5	62	1.5	49.4	45.9	4.7	-	76.5	23.5	1.4	87.7	10.9	8.7	87.7	3.6

Asp.: *Aspergillus*, Pen.: *Penicillum*

DISCUSSION

Counting and estimation of *Trichoderma* in soil is difficult because of the relatively rapid growth of other fungi on agar medium. The results of the experiments which carried out in this study to find the most favourable medium for isolation and enumeration of *Trichoderma* from the test soil samples showed that, besides *Trichoderma* Selective Medium (TSM), Martin's medium (MT) and Potato Dextrose Agar (PDA) were suitable for isolation of *Trichoderma*. Results also indicated that despite of the negative effect of the addition of the fungicide, pentachloronitro benzene (PCNB), at the concentration of 100 μg mL^{-1} on total fungal counts, the fungicide restricts the colony size and making it easy to be count. Results also indicated that TSM recorded higher efficiency for isolation of *Trichoderma*, which ranged from 120-140% compare with other tested media.

The suitability of the addition of PCNB to the culture media for counting of *Trichoderma* sp. was noticed and recommended by Papavizas and Lumsden (1982), El-Naghy *et al.* (1998) and El-Komy (2001).

PCNB is an effective agent in restricting the colony size of fast spreading fungi such as Mucorales as well as *Trichoderma* thus, facilitating the enumeration of the soil fungi (El-Katatny *et al*., 2004). The suitability of TSM with PCNB for counting *Trichoderma* is related to the fact that *Trichoderma* sp. are relatively tolerant to high levels of PCNB and rose Bengal (Papavizas, 1981; Elad *et al*., 1981) and to the capacity of *Trichoderma* to grow and sporulate on media containing low levels of glucose (Elad *et al*., 1983; El-Naghy *et al*., 1998). PCNB also reduced the number-forming units (CFU) of soil-fungi (Papavizas, 1981; Papavizas and Lumsden, 1982).

Thus, PCNB has been previously recommended to be included as an ingredient in the culture media used for isolation and counting *Trichoderma* sp. from soil (Elad *et al*., 1981) for example, the performance of V-8 medium containing PCNB as a fungal inhibitory agent (referred to as TME medium) which was suggested for the direct isolation and enumeration *Trichoderma* sp. from soil by Papavizas (1981) has been found among the various media tested to be the most satisfactory for inhibiting the rapidly growing fungi such as mucorales and encouraging and allowing the enumeration of *Trichoderma* from soil.

Results of the survey of *Trichoderma* in different localities in Al-Jabal Al-Akhdar region indicated that *Trichoderma* was isolated from 5 out 23 soil samples and its numbers per gram soil was ranging from $0.5\text{-}1\times10^3$ CFU g^{-1} soil. The natural levels of *Trichoderma* sp. in soil are between 10^2 and 10^4 CFU g^{-1} of soil, with the lower level being most common (Green, 2003). The population level of *Trichoderma* in soil depends on the abiotic and biotic factors of the environment. Generally, high levels of organic matter and clay and low pH values make higher population levels (Alabouvette and Steinberg, 1995; El-Naghy *et al*., 1998).

Literatures concerning the survey of *Trichoderma* in Libyan soil are very rare, Youssef (1974) reported that sixty three fungal species in twenty genera were isolated from sixteen different localities in Libya. Of these species four were Phycomycetes, ten were Ascomycetes and forty nine were Deuteromycetes. In Egypt, Shaban (1986) reported that *Trichoderma* fungi were isolated from 13 soil samples out of 20, pointed out that non-cultivated soils with high content of soluble salts and very low organic matter are not favourable for the development of *Trichoderma*. Generally, previous studies on mycoflora of Egyptian soil revealed that *Trichoderma* occur in moderate or low frequencies (Mazen and Shaban, 1983).

Results of this investigation showed that the 5 isolates of *Trichoderma* were identified as *T. harzianum* according to the key of Rifai (1969). The latter author have discussed the morphological characters used to characterise and differentiate species of *Trichoderma*.

Trichoderma can be identified by distinctive morphological characters such as, rapid growth, bright green or white conidial pigments and a repetitively branched, but otherwise, poorly defined condiophores structure. Reverse side uncoloured or variously buff, yellow, amber dull reddish, or yellow green. Characteristic aromatic odours resembling coconut are produced by some strains of *T. viride*, *T. atroviride* and *T. harzianum*. Conidation effuse or tufted or forming compact pustules typically in green shades or less often white, grey or brown conidia one celled, typically green, or otherwise colourless, greyish or brownish, smooth walled, to distinctly roughened or with sinuate, bullate, or wing-like projections, from outer wall, subglobose, obovoid, ellipsoid, oblong, or short cylindrical. Chlamydospores usually present and often abundant especially in submerged mycelium. Vegetative hyphae usually hyaline, smooth walled (Samuels, 1996). Recently molecular techniques allow a rapid and reliable identification of *Trichoderma* sp. and strains. Moreover, the molecular data confirm the morphological classification of *Trichoderma* (Papavizas, 1985; Gherbawy *et al*., 2004).

Results of this study also indicated that *Aspergillus* and *Penicillium* sp. were the most frequent fungi isolated from the studied soil-samples. Several other genera (*Mucor*, *Fusarium*, *Alternaria*, *Curvularia*; etc.) were also isolated in moderate, low or rare frequencies on the studied culture media. These results are in accordance

with many investigations reported everywhere. In Egypt, Abdel-Hafez (1974), Moubasher *et al.* (1977), Mazen and Shaban (1983), Shaban (1986) and Yaser (1999) reported that *Aspergillus*. *Penicillium* and *Fusarium* species were the most common genera recorded in their studies in Egyptian cultivation and desert soils.

CONCLUSION

Such *Trichoderma* species can be found in different regions in Libya, where the importance of such species as biocontrol agent should be tested for its capacity to control plant diseases. However, many research needed using molecular techniques to record and to identify fungal flora in Libya, this report is considered to the first which showed the distribution and identification of *Trichoderma* sp. in Al-Jabal Al-Akhdar, Libya.

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