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Occurrence and Characterisation of *Trichoderma* in Some Libyan Soils

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Abstract:

Trichoderma occurs in some tested Libyan soil. Seven *Trichoderma* strains were isolated out of 13 soil samples. *Trichoderma* selective medium (TSM), Glucose Czapek's agar medium (CZ) according to our results were the preferable for isolating *Trichoderma* from the tested soil than the others tested media. *Trichoderma* isolates were identified as *Trichoderma ghanense* (isolate number T3) and *Trichoderma harzianum* (isolate number T5), *Trichoderma koningii* (isolate numbers T6, T8, T9, T10, T11) and *Trichoderma* counts were ranged from 0.5 to 7.0x10^3 CFU / mg of soil.

Key words: *Isolation, Trichoderma, Soil, Media and Libyan.*

Introduction:

Fungi are distributed worldwide in all terrestrial ecosystems. *Trichoderma* spp. is one of the most diverse groups of the fungi on the soils. They colonise wide range of habitats, especially those containing organic matter. Their abundance is dame to their ability to decompose various organic substrates, *Trichoderma* spp. often occur at levels of 100 to 10 000 propagules per gram of soil [1]. *Trichoderma* spp. were isolated from soil samples at depth ap 100 cm soil samples a depth and *T. viride, T. hamatum* and *T. polysporum* were the most common species ، 369 strains of *Trichoderma* were isolated and identified into eleven species. *Trichoderma* species have been described as excellent biological control agent [2, 3].

Trichoderma were easily isolated from their environment, soli and plant organic matter. They are known as imperfect fungi. *Trichoderma* spp. are characterized by rapid growth and the production of numerous spores (conidia) [4 and 5]. Species of the genus produce conidial pigmentation varies from colorless to various green shades and sometimes also gray or brown [4].

Materials and Methods:

- Collection of soil samples

For studying the distribution of *Trichoderma* in some Libyian soils, 13 soil samples were collected from different localities which represented both cultivated and non cultivated soils presented in **Table (1).**

- Isolation of *Trichoderma*:

- Isolation media

Five different types of media were used for isolation of *Trichoderma* from the tested soil. These culture media include:

A. **Glucose – Czapek's agar medium (CZ)** containing the following components (g/l): Glucose, 20; $NaNO_3$, 2; KH_2PO_4, 1; Kc1, 0.5; $MgSO_4$. $7H_20$, 0.5; Fe_2SO_4, 0.01; agar, 20.
B. **Martinn's medium (MT)** [6] containing the following components (g/l): Glucose, 10; Peptone, 5; K_2HPO_4, 1.0; $MgSO_4$. $7H_20$, 0.5; agar, 20, Rose – Bengal, 0.15 were added after autoclaving.
C. **Malt Extract medium (ME)** with the following composition (g/l): malt extract, 20; agar, 20.
D. ***Trichoderma* selective medium (TSM)** [3] containing the following (g/l): $MgSO_4$. $7H_20$, 0.2; KH_2PO_4, 0.9; KCl, 0.15; NH_4NO_3, 1.0; Glucose, 3.0; agar, 20, Rose – Bengal, 0.15 were added after autoclaving.
E. **Potato dextrose agar (PDA)** contained the following (g/l): Potato, 25 g; Glucose, 20; agar, 20.

- Medium efficiency

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. The medium was incubated for 24 hours at 28°C. After that the *Trichoderma* count was determined on different – media by the plate count method.

- Growth characteristics of *Trichoderma* isolates on different media

- Microscopic characters

Each *Trichoderma* isolate was grown on PDA slants for 4 days at 28 °C. Cultures were examined under a light microscope (Olympus, Cx21, Japan). Mode of branching Conidiophores, shape of Conidia, phialides characters were

observed for each isolate (Identification of *Trichoderma* isolates were checked in Mycological Center, Faculty of science Assiut University, Egypt).

- Colony color

Agar plates of each medium for each isolate were prepared from fresh culture on PDA and incubated for 3 days at 28^{o}C to sporulate and the colony color as well as the reverse side was recorded.

- Odor Observation

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

Results & Discussion:

Quantitative and qualitative estimation of *Trichoderma* spp 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.

Data presented in Table (2) and Figure (1) showed that the *Trichoderma* was isolated from 7 out of 13 soil samples. Maximum *Trichoderma* counts were (7 CFU / mg soil), and were isolated on *Trichoderma* selective medium (TSM) from Benghazi soil sample. The least *Trichoderma* counts were (0.5 CFU / mg soil), and were isolated on Potato dextrose agar (PDA) from Ajdabiya soil sample. In an attempt to evaluate the efficiency of the five tested media for isolation of *Trichoderma* added to the soil, Table (3) and Fig.(2) shows that both *Trichoderma* selective medium (TSM), and Martin's medium (MT) were efficient for isolation of *Trichoderma*. TSM was superior compared with other media, and recorded 130 – 110 % efficiency of isolation for the tested isolates (T8 & T9), respectively. Martins medium (MT) recorded 90 – 100 % efficiency of isolation for T8 & T9, respectively. Counting and estimation of *Trichoderma* in soil is difficult because of the relatively rapid growth of other fungi on agar medium [5] Results of this study to find the most favourable medium for isolation and enumeration of *Trichoderma* from the tested soil samples showed that *Trichoderma* selective medium (TSM) and Glucose Czapek's agar medium (CZ) were suitable for isolation of *Trichoderma*. Results also indicated that TSM recorded the highest efficiency value for isolation of *Trichoderma*, which ranged from 130-110% as compared with other media. These results are in accordance with [1] who recorded that TSM gave the highest efficiency for isolation of *Trichoderma*, which ranged from 120-140% compare with other tested media. TSM also contains a low concentration of glucose which still allows relatively rapid growth and

sporulation of *Trichoderma*, enabling convenient and rapid identification of *Trichoderma* colonies [7]. Literatures concerning the survival studies of *Trichoderma* in Libyan soil are very rare, it was reported that 63 fungal species in twenty genera were isolated from 16 different localities in Libya. Four of these species were Phycomycetes, ten were Ascomycetes, and forty nine were Deutromycetes [11-12]. Recently, reported that *Trichoderma* was recovered in 5 out of 23 soil samples from different areas in El-Jabal – El-Akhdar regions [1]. Moreover, these authors also reported that *Trichoderma* occurrences in moderate frequency of numbers ranging from 0.5-1 × 10^3 CFU / g soil. In Egypt, *Trichoderma* fungi were isolated from 13 soil samples out of 20, and non-cultivated soils with high content of soluble salts and very low organic matter were not favorable for the development of *Trichoderma*. Generally, previous studies on mycoflora of Egyption soil revealed that *Trichoderma* occur in moderate or low frequencies [13- 15].

Results of this investigation showed that one isolate of *Trichoderma* was identified as *Trichoderma ghanense* (isolate number T3), one as *Trichoderma harzianum* (isolate number T5) and five isolates were identified as *Trichoderma koningii* (isolate numbers T6, T8, T9, T10, T11) according to the key of [11]. The eardery, characters used to characterise and differentiate species of *Trichoderma*.

Table (1): The location of soil samples and plant used for isolation of *Trichoderma.*

Soil No.	*Place*	*Plant under cultivation*
1	**Al Bayda**	***Agropyron repens L.***
2	**Al Marj**	***Solanum lycopersicum***
3	**Benghazi**	***Euphorbia***
4	**Derna**	***Zea mays***
5	**Tobruk**	***Phaseolus vulgaris* L.**
6	**Ajdabyia**	***Pmpinella anisum***
7	**Tazirbu**	***Mangifera***
8	**Sabha**	***Olea europaea.L***
9	**Tripoli**	**Non cultivated soil**
10	**Sirte**	***Triticum aestivum***
11	**Misrata**	***Cucumis sativus***
12	**Al Kufrah**	***Phoenix dactylifera***
13	**Murzuq**	**Non cultivated soil**

Table (2): *Trichoderma* Counts (colonies/mg soil) isolated from different soil samples on different media.

Soil No. / Media	3	5	6	8	9	10	11
Czapek's glucose (CZ)	3.5	1.0	0.0	0.0	0.0	1.0	0.0
Martin's (MT)	0.0	0.0	2.0	0.0	0.0	0.0	1.0
Malt Extract (ME)	0.0	1.5	0.0	1.0	0.0	0.0	0.0
Trichoderma selective medium (TSM)	7.0	1.0	4.5	1.0	3.5	6.0	2.0
Potato Dextrose Agar (PDA)	0.0	0.0	0.5	0.0	0.0	0.0	0.0

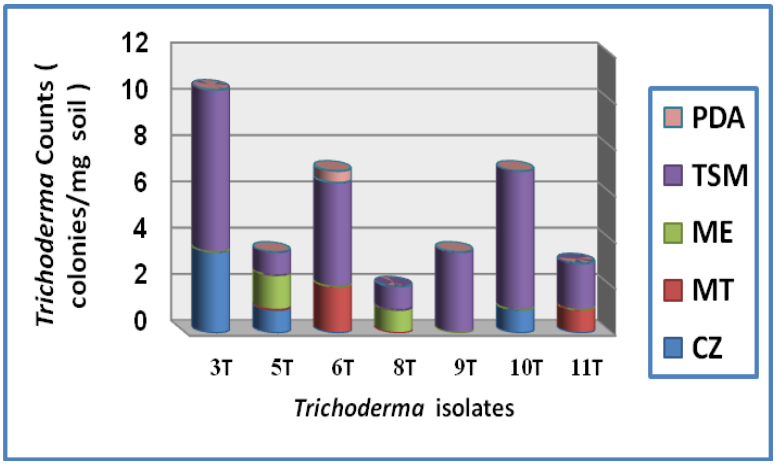


Figure (1): *Trichoderma* Counts (colonies/mg soil) isolated from different soil samples on different media.

Table (3): Evaluation of medium efficiency of *Trichoderma Koningii* (T8 & T9).

Media	*T.* isolate	Total count (colony/mg soil)	Medium efficiency (%)
Czapek's glucose (CZ)	T8	5.0	50
	T9	6.0	60
Martin's (MT)	T8	9.0	90
	T9	10	100
Malt Extract (ME)	T8	6.0	60
	T9	7.0	70
Trichoderma selective medium (TSM)	T8	13.0	130
	T9	11.0	110
Potato Dextrose Agar (PDA)	T8	4.0	40
	T9	7.0	70

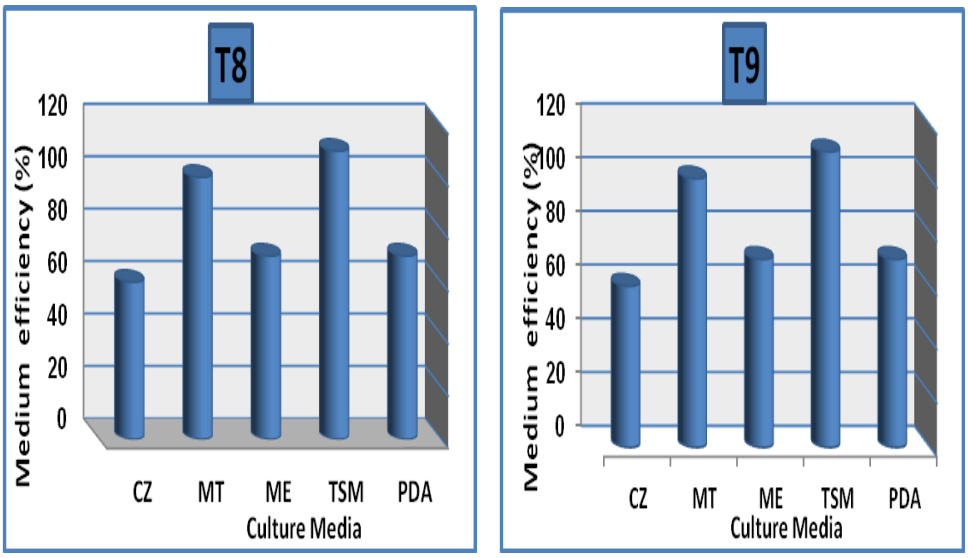
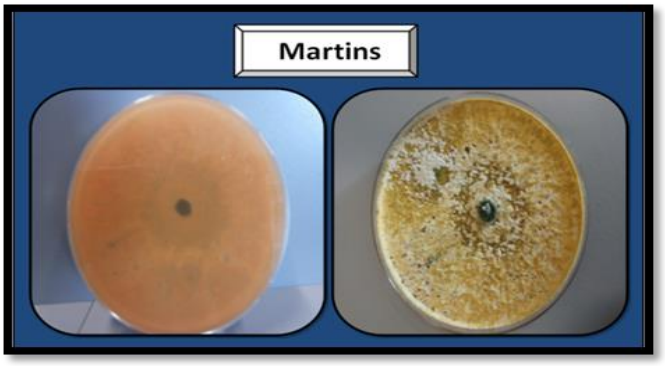


Figure (2): Evaluation of medium efficiency of *Trichoderma koningii* (T8 & T9).



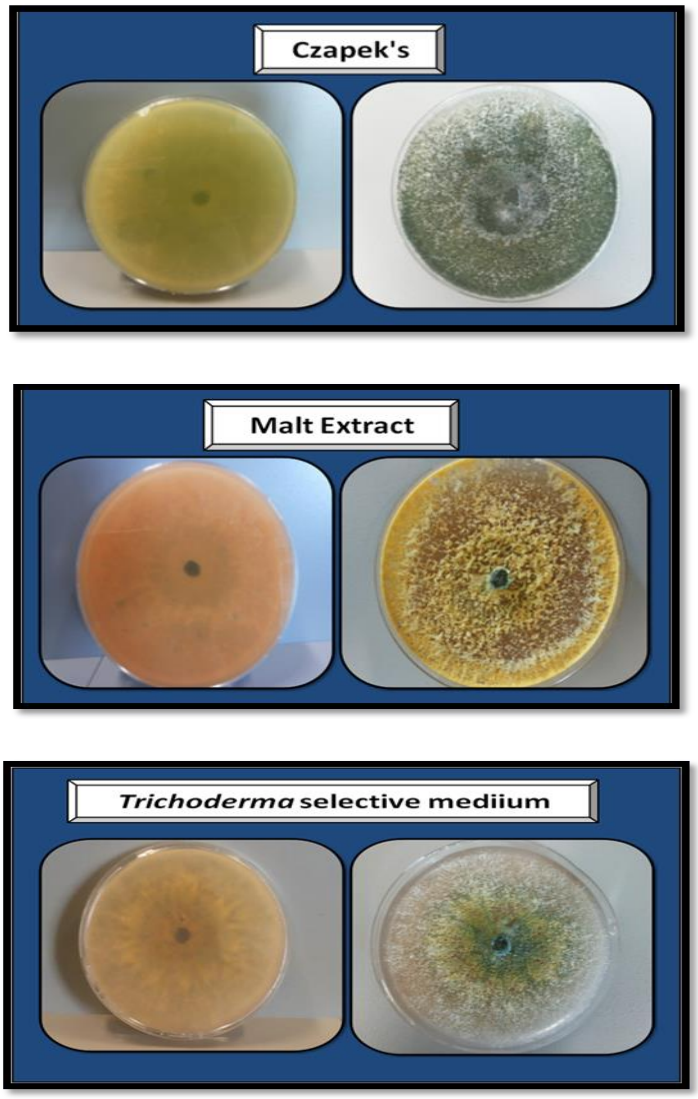


Figure (3): Growth and pigmentation of *Trichoderma Koningii* (T8) on different media.

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