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PREVALENCE OF TRICHODERMA SPECIES IN AL JABAL AL AKHDAR REGION, LIBYA

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Abstract: *Trichoderma* occurs in moderate frequency in Al Jabal Al Akdar soil it was isolated from 5 out of 23 soil samples. *Trichoderma* selective medium, Martin's medium and potato dextrose agar were the superior media for isolating *Trichoderma* from the tested soil than the others media examined, while martin's medium and malt extract medium were the most valuable for isolating the greatest number of total fungal count. *Trichoderma* counts (by dilution plates method) were ranged from 0.5- 1×10^3 CFU/g over dry soil, Five *Trichoderma* were identified as *T. harzianum* isolates (T_5, T_7, T_8, T_{14} and T_{15}), according to their morphological and culture characteristics. Various *T. harzianum* isolates showed inhibitory effect against *F.solani* grown in dual culture on PDA, *T. harzianum* isolates (T_7) and (T_8) recorded maximum percentage of inhibition (32.5%) against *F. solani.*

Keywords: Trichoderma, Martin's medium, fungal count

INTRODUCTION

Trichoderma spp. are widely distributed all over the world Domsch, *et al*., (1980a, b and Attitalla *et al*., 2012) and occur in nearly all soils and other natural fabitat, especially in those containing organic matter. Individual aggregates may be restricted in their geographical distribution Danielson and Davey, (1973a). *T. virde* and *T. polysporum* for example, were reported to be restricted to areas where low temperature prevail ans *T. harzianum* were mostly found in warm climatic regions whereas, *T. harzianum* and *T. koningii* occur widely under diverse climatic conditions (Samuels, 1996). 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 is a secondary colonizer since it is usually isolated from well decomposed organic matter. (Samuels 1996). Provided detailed observation and comments on the utility of morphological characters to define species in *Trichoderma*. However, molecular techniques allow rapid and reliable identification of The *Trichoderma spp*. and strains (Moubasher, 1993, El-Naghy, *et al*., 1998, Gherbawy, *et al*., 2004).

There have been numerous attempts to study the occurrence, distribution and isolation of *Trichoderma* with natural habitats, few researches have attempted quantitative study of its population survival and proliferation in soil and other habitats. Quantitative estimate of *Trichoderma spp*. in soil is often difficult because of the relatively rapid growth of other fungi on conventional agar media such difficulties were faced to a large extent.

During the past few years and research on *Trichoderma* has entered a new phase with the introduction of new culture media for isolation and enumeration, the development of new techniques for studying survival and proliferation on soil and plant, the induction of new biotypes resistant to fingicides, increased emphasis on the production of metabolites and development of new biotechnological approaches to promote growth and develop delivery system (Lewis and Papavizas, 1987; 1991; Shaban and EL-Komy, 2000).

Mycoparasitism has been described as the main process involved in the antagonistic action of *T. harzianum* against fungal pathogens. Mycoparasitism is a complex process that includes the release of lytic enzymes, degradation of cell wall and further penetration in the host mycelium (El-katatny *et al*., 2003).

Due to the potential importance of *Trichoderma spp*. as an effective antagonists to certain pathogenic fungi in soil and the difficulties encountered in studying their distribution, survival and proliferation. This research was planned to through light on the following aspects; occurrence and distribution of *Trichoderma spp.* in Al Jabal Al Akhder soil collected from different localities, Evaluation of different media for isolation and enumerating *Trichoderma* from soil and studies the antagonistic effect of some *Trichoderma* isolates

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against some pathogenic fungi *in vitro*. *Trichoderma* sp. Are widely distributed all over the world (Attitalla and Salleh, 2010).

MATERIAL AND METHODS

Collection of Soil Samples: Twenty three soil samples were collected from different localities in Al Jabal Al Akhder, (Fig.1) which represented in cultivated and non-cultivated soils. For each soil sample the top surface soil was removed (about 3cm) and 5 sub samples were taken at random in to a depth of 15 cm for each site using a sterile auger.

Samples were collected in sterile polyethylene bags under aseptic conditions. The subsamples of each site were bulked to yield one composite samples representing the area. The samples allowed to dry and 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). The soil type was determined from the triangular diagram of Prescott *et al*., (1934) Fig. (2).

Soil Chemical Analysis:

Total Soluble Salts: A known weight of each soil sample was taken in a volume of distilled water for about 30 minutes and the mixture was left over night 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 salt per one gram over- dry soil was calculated.

Organic Matter Content: It was determined according to walky and Black method (Jackson, 1958).

PH value: ABeakman PH meter was used as described by (Jackson, 1958).

Determination of Soil Fungi By Dilution Plate Methods: A modified method of that described by (Johnson, *et al*., 1959) was used.

Isolation Media: Five different types of media were compared for Isolation soil fungi including *Trichoderma* from tested soil. The culture media include: Glucose-Czapek's agar medium (CZ) 2- Martin's medium (Martin, 1950) 3- Malt extract medium (ME). 4- *Trichoderma* selective medium (TSM) (Elad, *et al*., 1981) 5- Potato dextrose agar (PDA).

Identification of Fungi: *Trichoderma* species were identified using the key of (Rifai, 1969).

Culture characteristics of *Trichoderma* isolates:

- This includes: Microscopic characters, measurement of growth at 28C and odour observation.
- Antagonistic activities of *Trichoderma harzianum* isolates against some phytopathogenic fungi.

Dual Culture Experiments: The antagonist (*Trichoderma* spp.) and phytopathogen *Fusarium oxysporum* shelecht) were growth in dual culture in PDA plates. Each plate was mycelial discs (5mm) cut from actively growing colonies of the antagonist and the phytopathogen. A disc from phytopathogen was placed at the center of the plate, whereas discs of antagonist were placed 1cm distance from the edge of the plate. Four discs were placed in each PDA plate representing two isolates of *Trichoderma* spp. Two discs for each isolates placed perpendicular to each other.

Control plates were also set up with either the antagonist or the pathogen alone. It is suitable to inoculate *Trichoderma* spp. discs after 72 hrs of *Fusarium solani* discs inoculation because of slow growth rate of *Fusarium solani*. Growth of fungi was measured as the average diameter of radius of colony (in four directions at right angles) often 24, 48 hrs of the inoculations.

The percentage of inhibition which occurred in growth rates of both the antagonist and the pathogen in dual cultures were calculated using the equation as reported by (Johnson, *et al*., 1959) % of inhibition:

$$= \frac{C - T}{CX} \times 100$$

Where C: daily increase in growth of control (pathogen grown separately). T: daily increase in growth of pathogen in dual cultures Hyphal interaction: The area of interaction between Trichoderma spp. isolates (as antagonist) and the phytopathogen was examined under a light microscope after, 2, 3, 4, days of incubation together in dual culture experiment.

RESULTS

Soil Characteristics:

The result in (Table1) show that the soil organic matter content was ranging between 0.8% (cultivated sandy soil in AL-Hania region) and 4.3 (cultivated sandy loam soil in AL-Bayda region). Total soluble salts was ranging between (E.C.) 0.13-0.51 ds/m with exception of sample No. 23 collected from EL-Hania region which recorded 0.92ds/m. The PH values of the soil samples were mostly alkaline, ranging between 7.6-8.4.

- Quantitative and qualitative estimation of *Trichoderma spp*. in soil.

- Five different types of media were compared for their suitability for isolation of *Trichoderma* from soil. Comparing the *Trichoderma* selective medium with 40 other media, results presented in (Table2) indicated that both of Martin's medium and potato dextrose agar were superior to the others for isolating, and counting of *Trichoderma* gave the highest number (1×10^3CFU/g dry soil) in soils numbers 5 and 8.
- The *Trichoderma* isolates were recovered on 3 out of 5 media (MT, TSM and PDA). *Trichoderma* isolates were identified according to the key of (Refai, 1969) as *T. harzianum*. *Trichoderma* was isolated only from 5 out of 23 soil samples Table (3).

Results of Antagonism:

Testing the antagonism of *T. harzianum* isolates against *F. solani* grown in dual cultivate on PDA at 28C showed that of the incubation period, the fungal isolates of *Trichoderma* grew towards the host hyphase (*F. solani*) to inhibit its growth. *T. harzianum* isolates showed various inhibitory effect and maximum percentages of inhibition were obtained by *Trichoderma* isolates (T_7) and (T_{15}) which recorded 32.5% inhibitory effect. Plate (1).

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 their research to find the most favourable medium for isolation and enumeration of *Trichoderma* from the collected soil samples showed that, besides *Trichoderma* selective medium (TSM), Martin's medium (MT) and potato dextrose agar (PDA) were suitable for isolation of *Trichoderma*. These results agree with those reported by Papavizas and Lumsden (1988) and El Naghy, *et al*., (1998).

Results of the survey of *Trichoderma* in different localities in Al Jabal Al Akhder region indicated that *Trichoderma* was isolated from 5 out 23 soil samples, and its number per gram soil was ranging from 0.5-1×10^3 CFU/g. soil. The natural levels of *Trichoderma spp*. in soil are between 10^2 and 10^4 CFU per gram of soil, with the lower level being most common (Green, 2003). The population level of *Trichoderma* in soil depends on the biotic and biotic factors of the environment. Generally, high levels of organic matter and clay and low PH values make higher population Level (Alabouvette and Steiribery, 1995).

Concerning literatures on the survey of *Trichoderma* Libyan soil are very rare, Youssef (1974) reported that that sixty three fungal species in twenty genera were isolated from sixteen different localities in Libya. of their species four were phycomycetes, ten were Ascomycetes, and forty nine were Deutromycetes 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 favorable for the development of *Trichoderma*.

Our results showed that the isolated 5 *Trichoderma* spp. were identifiedes *T. harzianum*. According to the morphological characters described by Rifai (1969). which includes, rapid growth, bright green or with conidial pigment, poorly defined conidophores structure. Reverse side uncolored or -variously buff yellow, amberduall, reddish, or yellow green. Characteristic aromatic odours resembling coconut are produced by some strains. Condition effect or tufted or forming compact pustules typically in green shades or otherwise colorless chlamydospores usually present and often abundant especially in submerged mycelium. Vegetative hyphae usually hyline, smooth walled (Samules, 1996).

The direct mycoparasitic activity of *Trichoderma spp*. has been proposed as one of the major mechanisms for antagonistic activity against phytopathogenic fungi (Chet, 1990).

The results of antagonistic of *Trichoderma harzianum* isolates against *Fusarium solani* in dual culture of PDA revealed that, *Trichoderma* isolates grew towards the host hyphae and inhibited its growth. Results also showed that maximum inhibition percentages recorded by *T. harzianum* isolates (T_7) and (T_{15}) were 32.5%. Benhamou and Chet (1993) reported that *T. harzianum* was capable to inhibit *R. solani* and *F. solani* by lysing the host mycelium after ceiling around the hyhpae. Elad, *et al*., (1988_3) demonstrated hyhpae penetration of pythium ultimum by *Trichoderma spp*. mediated by enzyme. Activity specially chitinase, and B-1,3 glucanase.

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

Soil No	Place	Partical size distribution				pH	E. C. Dsm	Organic matter %	Plant under cultivation
		Sand %	Silt %	Clay %	Texture				
1	Gernada	64.70	26.8	8.5	Sandy Loam	7.7	0.27	3.3	*Pmpinella anisum*
2	Al-Bieda	66.68	20.72	12.59	Sandy Loam	8.2	0.38	4.3	*Triticum vulgaris*
3	ElKharika	70..68	26.72	2.59	Sandy Loam	7.9	0.41	4.1	*Phagnallon rupestre*
4	Oma Al–Mukhtar	64.69	20.35	14.95	Sandy Loam	8.2	0.47	2.4	*Thapsia gargnica*
5	El–Faidia	60.68	23.72	15.59	Sandy Loam	7.9	0.33	2.2	*Marrubium vulgare*
6	El–Mansora	63.04	30.45	6.51	Sandy Loam	7.7	0.43	1.9	*Portulaca oleracea*
7	Shahat	70.68	20.72	8.59	Sandy Loam	7.8	0.41	3.1	*Thymus serpyllum*
8	Wardama	70.68	24.72	4.59	Sandy Loam	7.6	0.30	3.0	*Marrubum vulgare*
9	Masa	64.68	24.72	10.59	Sandy Loam	7.7	0.38	2.7	*Glycin max*
10	Belhaded	66.68	24.72	8.59	Sandy Loam	7.8	0.27	2.0	*Certonia siligua*
11	El–Waseta	68.68	18.72	12.59	Sandy Loam	8.1	0.22	3.2	*Zea mays*
12	Eslenta	68.68	19.72	11.59	Sandy Loam	7.8	0.62	3.8	*Cucumis sativus*
13	Gandola	68.4	19.0	12.59	Sandy Loam	7.7	0.51	1.3	*Brassic oleracea*
14	Gardas	68.68	16.72	14.59	Sandy Loam	7.8	0.32	1.0	*Hordium vulgaris*
15	El–Khwimat	84.68	10.08	5.23	Sandy Loam	8.3	0.26	0.9	*Artemesa herba–alba*
16	Marawa	66.68	26.72	6.59	Sandy Loam	8.3	0.13	1.4	*Thapsia garganica*
17	Eljehad	72.68	18.72	8.59	Sandy Loam	7.9	0.34	3.6	*Thapsia garganica*
18	Kaser Libya	73.04	26.36	0.59	Sandy Loam	8.4	0.25	1.4	*Hordium vulgaris*
19	Zawiat Elarkob	78.68	8.72	12.59	Sandy Loam	8.4	0.27	1.6	*Triticum vulgar*
20	Ekfenta	56.68	34.0	9.31	Sandy Loam	8.0	0.18	1.5	*Thapsia garganica*
21	El–Hamama	69.68	21.72	8.59	Sandy Loam	8.0	0.26	1.2	*Artemesia sp.*
22	El–Koof	73.04	18.36	8.59	Sandy Loam	8.3	0.31	1.8	*Paronychia argentina*
23	El–Haneia	90.68	5.44	3.87	Sandy	7.7	0.92	0.8	*Lycopersicum sp.*

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

Media \ Soil 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 (3): Total fungal counts (colonies /mg dry soil) isolated from different soil samples on different media.

Media \ Soil 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 (CZ)	5.5	15	6.0	8.0	12.5	7.5	9.5	10.5	9.5	2.5	13.0	15	11.0	12.5	6.5	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	2.5	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

* *Trichodema* was slated

Plate (1): Antagonistic effect of *T. harzianum* (T15 & T24) against *F. solani* (A), and lyses of *F. solni* by *Trichoderma* sp. (B).

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