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Molecular Identification of Rhizosphere *Trichoderma* spp. and Their Antagonistic Impact Against Some Plant Pathogenic Fungi

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Abstract:

The main aim of this study was to molecular identification and determine the antagonistic impact of rhizosphere *Trichoderma* spp. against some phytopathogenic fungi, including (*Magnaporthe grisea*) *pyricularia oryzae, Rhizoctonia solani* and *Macrophomina phasolina*. Four *Trichoderma* isolates were isolated from rhizosphere soils of the different host plants in different locations of Egyptian governorates. The morphological characterization of isolated *Trichoderma* as well as using of (ITS1-5.8S-ITS2) ribosomal gene sequence acquisition and data analyses. By comparing the results of DNA sequences of ITS region, the fungi represented one isolate were positively identified as *T. asperellum* (1 isolate T1) and one as *T. longibrachiatum* (1 isolate T2) and two as *Trichoderma harzianum* (2 isolates T3 and T4). The results showed similarity value of (5.8S-ITS) region sequence of the two isolates, T1 (*T. asperellum*) and T2 (*T. longibrachiatum*) of (99%, 99%), respectively. The similarity value of (5.8S-ITS) region sequence with isolates of T3, T4 (*T. harzianum*) of (99%). On the other side, the results of molecular identification of phytopathogenic fungi represented high similarity value of (5.8S-ITS) region sequence and were identified as *P.oryzae, R. solani* and *M. phasolina* (99, 96 and 99%) respectively. Variations and genetic relationships among 4 *Trichoderma* isolates were investigated by using the Rapid Amplification of Polymorphic DNA (RAPD) profiles using ten random primers. All *Trichoderma* isolates were assessed for their antagonistic impact on phytopathogens *P. oryzae, R. solani* and *M. phasolina*. Though *T. harzianum* isolates were more affects than *T. longibrachiatum* and *T. asperellum* isolates, the percent inhibitory effect among *T. harzianum* isolates were vary much (44.8 to 91.6%). The inhibitory effect of *T. asperellum* isolates ranged from 42.2 to (86.0%), while *T. longibrachiatum* exhibiting affect ranged between (47.5%) to (83.8%).

Key words: Biological Control, *Trichoderma*, RAPD, Rhizosphere Soil, Plant Pathogens

Introduction:

Biological control agents (BCAs) could be available alternative way over chemicals treatments in management of fungal crop diseases. All members of genus *Trichoderma* are free living and known as imperfect

fungi (Deuteromycetes), fast growing in culture and have the ability to produce several types of green spores. These occur worldwide and are commonly found in the soil environments and associated with roots of plant and debris [1]. The potential effect of *Trichoderma* species as biological control agents in plant disease control was introduced in the early 1930s by [2] who was first researcher elucidate the mycoparasitic activity of the members of *Trichoderma* genus against wide range of microorganisms like soil-born fungal and bacterial pathogens. Currently, commercial products of *Trichoderma* species are available in the market as biological control agents or can be used as amendments enhancers for plant growth [3]. Biological control mechanisms of *Trichoderma* species results either from competition activity for nutrients and area, on the other side *Trichoderma* species have ability to produce several compounds as a resist metabolites that either inhibit spore germination, kill the plant cells or decrease pH of the rizhospher soil, in the end all these mechanisms lead to kills the plant pathogens and preventing growth of these pathogens. Biological control agents may also result from parasitism/predation activity between the target pest (plant pathogens itself) and biological control agents such as *Trichoderma* Spp. thus through direct interaction with plant pathogens, which involves direct contacts and produce of some types of cell-wall-degrading enzymes, toxic compounds and secondary compounds/or antibiotics that act synergistically with some types of enzymes. From the other side *Trichoderma* products (BCAs) can play important role and clear positive effects on plants by increase the plant growth also can play role in the stimulation of plant defense mechanisms [4,5,6]. *Trichoderma* species are green-spored ascomycetes present in nearly all types of temperate and tropical soils, they can present in decaying plant material and in the rhizosphere habits of plants [7]. There are many approaches that can be used to identify and characterized the genus of *Trichoderma*, these included that mycelia growth rate, morphological characters (phialides and phialospores) as well as colony features, recently molecular technique has been characterized several type of fungi like *Trichoderma* spp. The internal transcribed spacer (ITS) region of the rDNA and random amplified polymorphic DNA (RAPD) most widely used in the characterization of these fungi [8,9]. *Trichoderma* species are usually found colonizing plant root ecosystems, establishing symbiotic relationship with plants. However, the colonization of the root tissues are only limited at the root cortex due to the deposition of callose which restrict the penetration of hyphae, the callose barriers made *Trichoderma* become harmless to the plants [10]. Since weinding [2] reported that antagonistic impact of *T. lingnorum* (*viride*) against soil borne fungal pathogen of *Rhizoctonia solani*, several scientists have been widely studied *Trichoderma* species as bio-agents against wide range of fungal species that causes disease for plants [11,12,13]. Moreover, *Trichoderma* species have been studied as bio-agents against plant fungal pathogens isolated from many types of soil [14]. Data obtained from many studies showed that some isolates of *Trichoderma* spp. had a significant reducing potential on plants diseases caused by pathogenic fungi, also some species of these bio-agents are known as biocontrol agents from increased localized and systemic resistance to plant diseases (inducing

the defensive mechanisms of host plant) as well as overall plant growth, such as several soil-borne fungal plant pathogens including (*Magnaporthe grisea) pyricularia oryzae, Rhizoctonia solani and Macrophomina phasolina* [15,16,17]. Therefore, the goal of this study was to molecular identification and determine an antagonistic impact of using different *Trichoderma* species as biocontrol agents to diminish and control of some fungal plant pathogens including *M. Phaseolina, R. solani* and *P. oryza*.

Materials and Methods:

Fungal strains

In the present study, fungal biocontrol agents belonging to the genus *Trichoderma* sp. were collected from rhizosphere soils of different host plants in various locations of Egyptian governorates. Thirty-one soil samples collected from different agricultural fields and forests in Egypt. The soil particles tightly were adhered with root surface were removed separately and suspended in 10 ml of sterile distilled water. After serial dilution, one ml suspension from 10^6 dilutions was transferred to sterile Petri dishes containing *Trichoderma* specific media (TSM) that included different types of cultural media: (1) Potato-Dextrose-Agar (PDA, Biolife), (2) Malt Extract Agar (MEA; Biolife), (3) Rose Bengal Agar (Sigma Aldrich), (4) Oat Flour Agar (Sigma Aldrich) and incubated at (28)°C for 5 days. After incubation, the colonies were determined to be *Trichoderma* spp., according to [18] were purified.

Isolation of phytopathogenic fungi from Egypt plants:

Infected plants were collected; some pieces that showed symptoms of the infected plant parts were disinfested in 5% sodium hypochloride (NaOCl) for five minutes. Then, the samples were washed extensively with sterile distilled water and placed on Petri dishes containing potato-dextrose-agar (PDA-Biolife) Sulfate streptomycin antibiotic (30 mg/L) was used to remove bacterial contamination and rose bengal (3.3 mL of 1% (w/v)), fungal isolates incubated at (25)°C for 72 hour according to [19,20,21]. The Fungal isolates were obtained from infected plants were purified and identified, according to [22, 23, 24, 25, 26, 27].

Genomic DNA Extraction from *Trichoderma* species and phytopathogenic Isolates:

For DNA extraction, mycelia were transferred from PDA to 250 mL Erlenmeyer flasks containing potato-dextrose broth (PDB-Biolife). After 5 days growth at (28±2) °C the mycelia were firstly collected by filtering through muslin cloth, after that washed with distilled water, then frozen in liquid nitrogen and ground in a mortar according to the procedure recommended for DNA extraction and purification of genomic DNA purification kit (Gene JET™). After that we are obtained DNA in concentration (50 to 100 ng/µl) and determined by using spectrophotometer at 260 nm, finally stored at (–20) °C for further use [28].

PCR Amplification and sequencing of ITS Region of *Trichoderma* Isolates:

PCR was performed by using Maxima Hot Start PCR master mix (Thermo) in 50 µl of mixture containing polymerase reaction buffer, deoxy nucleotide triphosphates (dNTPs), mix primers and (Taq) enzyme DNA polymerase. PCR reaction was accomplished in a total volume of (100 µl, containing 78 µl Deionized water, 10 µl 10 X Taq pol buffer, 1 µl of 1 U Taq Polymerase enzyme, 6 µl 2mM each of the four (dNTPs), 1.5 µl of 100 mM reveres and forward primers and 2 µl of 50 ng

of template DNA. The PCR program was setting as an initial denaturing at 95 °C for 5 min. Followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec extension at 70 °C for 2 min and final extension at 72°C for 10 min. Internal Transcribed Spacer (ITS)-1 Region of rDNA: Two universal primers were used in this study, ITS1 and ITS2 regions together with (5.8S) gene in rDNA were amplified using specific primer pair ITS1 (5′-TCCGTA GGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTAGTATGC-3`) according to [29]. The sequences were commercially designed by Medigene Company. A nuclear rDNA region, containing the internal transcribed spacer region-1 was amplified by polymerase chain reactions using the primer combinations ITS1 and ITS2. Sequences were specified by blasting the sequence with the available Genbank resources using NCBI-BLAST search [30, 31].

RAPD-PCR Reactions

A set of ten RAPD primers in (Table 2) were used in the detection of polymorphism. The amplification reaction was performed in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1µM primer, 1U *Taq* DNA polymerase and 30 ng template DNA.

Antagonistic activity of *Trichoderma* isolates by using Dual cultures method

The *invitro* antagonistic activity test was accomplished using method described by [32]. Dual cultures were performed by using 7 days old cultures of test pathogens and *Trichoderma* spp. on PDA medium. The agar medium was inoculated with a 5-mm in diameter disc of antagonist positioned diametrically opposite a 5 mm in diameter disc of the pathogen, after that placed at a distance of 2 cm away from perimeter of petri dish. The experiment was conducted in triplicate and repeated twice for each antagonist. The plates were incubated at (28 ± 3) °C temperatures and the results are observed after six days for growth of *Trichoderma* isolates and test fungus. Data were obtained for percent inhibition of radial growth (PIRG) = (R1–R2)/R1 x 100. Where R1 = radial growth of pathogen in control. R2 = radial growth of pathogen in dual culture experiments with antagonists. The degree of antagonism between each of the *Trichodema* spp. and test pathogens in dual culture was scored on scale of (R1 - R5) that is, R1 = *Trichoderma* spp. completely overgrow the pathogen and covered the whole cultural medium surface (100% over growth). (R2) = *Trichoderma* spp. overgrew at least two-third of the cultural medium surface (75% over growth); (R3) = *Trichoderma* spp. and the pathogen each colonized one-half of the medium surface (more than one-third and less than two-third). The organism does not appeared to be dominant over the others. (50% over growth); (R4) = *Trichoderma* spp. and the pathogens contact point after inoculation; R5= Pathogens overgrow *Trichoderma* spp. [33].

Results:

Molecular identification of *Trichoderma* species and phytophathogenic fungi.

In this study, four different isolates of *Trichoderma* were isolated in pure cultures from the rhizosphere zones of soil of different host plants in different locations of Egyptian governorates. All of the *Trichoderma* isolates were identified and characterized to species level with homology percentage of the nucleotide sequences of ITS region of rDNA at

least (99%) (Table 1). According to the blast search results among of these isolates, the ITS region of four isolates (T1,T2,T3 and T4) submitted to National Center for Biotechnology Information (NCBI) and representing the first isolate identified as *T. asperellum* (T1), the second isolate identified as *T. longibrachiatum* (T2), while the isolates of (T3 and T4) identified as *T. harzianum*. On the other hand, isolates of plant pathogens were used in this study identified according to the ITS regions of ribosomal genes and have high similarity percentage of (5.8S-ITS) according to the blast results, these isolate were identified as (*P.oryzae*, *R. solani* and *M. phasolina*) and the percentage of homology were (99, 96 and 99%) respectively (Table 1). The ITS region of rDNA was amplified using genus specific ITS-1 and ITS-2 universal primers, amplified products of size in the range of (609-649 base pair) was produced by the primers. In our study, results of rDNA sequencing were in agreement with results documented by [31] who reported the identification and genetic diversity of the *Trichoderma* isolates. These results were in agreement with several investigators who tested that the amplified rDNA fragment of an approximately (510 to 610 base pair) by using of ITS-PCR in *Trichoder*ma spp. [9, 34, 35].

Table 1. Molecular identification and homology of *Trichoderma* isolates and test pathogens.

Isolates No.	Source of *Trichoderma* spp	Species Identified	Percentage of Homology (%)	Types of Sample soil
1	Qualybia	*T. asperellum* (T1)	99	Rhizosphere soil
2	Minufya	*T. longibrachiatum*(T2)	99	Rhizosphere soil
3	Cairo	*T. harzianum* (T3)	99	Rhizosphere soil
4	Giza	*T. harzianum*(T4)	99	Rhizosphere soil
5	Cairo	*P. oryzae*	99	Rice leaves
6	Giza	*R. solani*	96	Tubers of potato
7	Qualybia	*M. phasolina*	99	Roots of strawberry

Polymorphism as detected by Randomly Amplified Polymorphic DNA (RAPD) analysis:

RAPD markers were developed by [36]. RAPD technique utilizes arbitrary primed Polymerase chain reactions (PCR) involve the use of a single short (10 pb) oligonucleotides to amplify several discrete fragments of DNA products in low-stringency. RAPD markers require no prior knowledge of the DNA sequence, which makes them very suitable to investigate species that are not well known. The method is fast and easy to perform but it is not reproducibile, since small changes in the PCR conditions may lead to changes in the amplified fragments.

Ten RAPD primer pairs were employed to investigate the genetic polymorphism among four *Trichoderma* isolates. RAPD primers give better reproducible and scorable patterns and the amplification profiles were screened for the presence of polymorphism (Fig. 1). As shown in (Table 3), a total of 95 fragments were generated by the ten primers with an average of 9.5 fragments, the number of fragment per primer ranged from 7 to 16, while the number of polymorphic fragment varied from 2 to 9 and the average level of polymorphism was (38.2%). Primer 7 yielded the highest number of products (16 amplicons), while primer 3 yielded the lowest number of products (7

amplicons). The average number of polymorphic fragment/primer among *Trichoderma* isolates was 3.8. Furthermore, the size of the amplified alleles varies with different primers and it is ranging from 300 to 6000 base pair.

Table 2. Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by RAPD markers among *Trichoderma* isolates

Primer	Total No. of amplicons	Monomorphic amplicons	Polymorphic amplicons	% of polymorphism
1	9	7	2	22.2
2	8	6	2	25
3	7	5	2	28.7
4	9	7	2	22.2
5	8	5	3	37.5
6	10	6	4	40
7	16	7	9	56.3
8	10	5	5	50
9	9	4	5	55.5
10	9	5	4	44.4
Total	**95**	**57**	**38**	**38.2**
average	**9.5**	**5.7**	**3.8**	

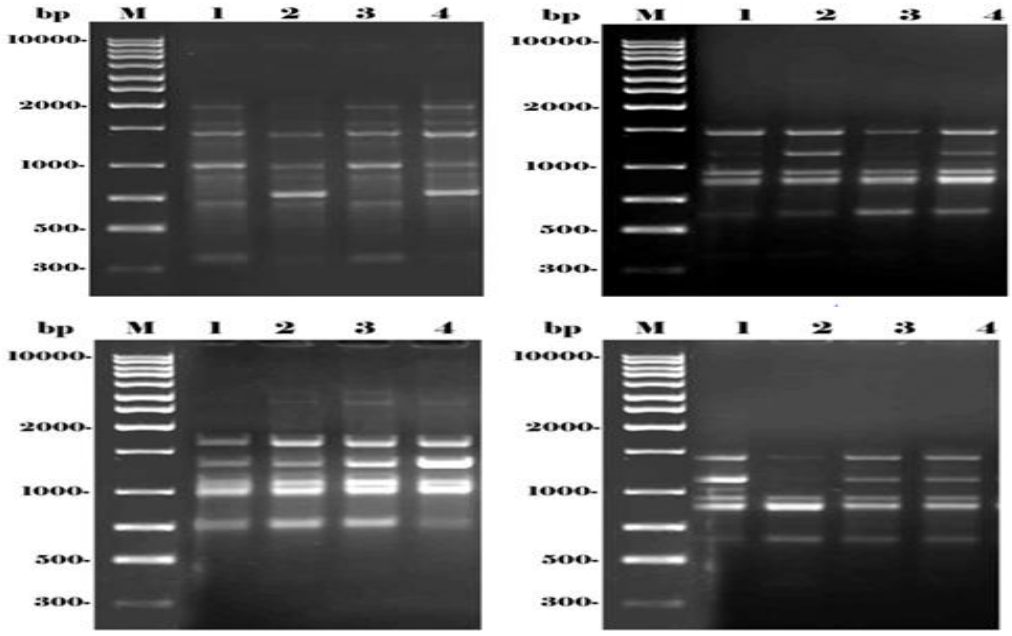

Fig. 1: RAPD profiles for *Trichoderma* isolates as detected with primers 1, 2, 3 and 4 Lanes 1 to 4. M: 1 Kb ladder DNA marker.

Genetic relationships among *Trichoderma* isolates.

To determine the genetic relationships among the isolated *Trichoderma*, the obtained data (1 for presence and 0 for absence) resulting from the ten RAPD primers were used to compute the similarity matrices according to [37]. These similar matrices were used to generate a dendrogram using the UPGMA technique, as it shown in Table (3). The estimated genetic similarity is ranged from (83%) to (89%). This revealed moderate levels of genetic similarity among the *Trichoderma* isolates. The highest genetic similarity (89%) was found between the isolates

(T3 and T4), while the lowest genetic similarity (83%) was noted between *Trichoderma* isolates (T1 and T2).

Table 3: Genetic similarity matrices among *Trichoderms* isolates as computed according to Dice coefficient from RAPDs.

	1	2	3	4
1	100			
2	83	100		
3	86	86	100	
4	84	85	89	100

Cluster Analysis as Revealed by RAPDs:

The UPGMA cluster analysis was performed to represent the genetic distances among *Trichoderma* isolates by drawing graph represent these (Fig. 2). The obtained dendrogram was divided into two main clusters; one cluster included (isolate T2). The other main cluster included two subclusters. One of these two subclusters contained the (isolate T1), the other subcluster included the two samples of (isolates T1, T3).

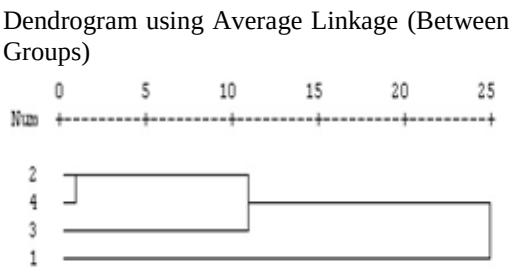

Fig. 2: Dendrogram for the *Trichoderma* isolates constructed from the RAPDs data using Unweighed Pair-group Arithmetic (UPGMA) and similarity matrices computed according to Dice coefficient.

Four isolates of *Trichoderma* that were collected from rhizosphere soil of different host plants were tested for their antagonistic impact against the plant pathogens *P. oryzae*, *R. solani* and *M. phasolina*. Through the dual culture technique we observed that all four isolates had varying levels of inhibition against growth of phytopathogenic fungi. The rate of inhibition was calculated as percentage of overgrowth of *Trichoderma* in petri dish, the result of antagonistic activity by using Dual culture assay showed that isolates *T. harzianum* (T3 and T4) exhibited better inhibition of radial mycelial growth of *P. oryzae* were (91.6 and 87.5%) respectively followed by *T. asperellum* (T3) (83.8%) and *T. longibrachiatum* (T4) (86.0%), (Table 4 and Fig. 3). The results showed moderate inhibition against radial mycelial growth of *M. phasolina* (59.3, 63.6, 50.0 and 56.2%) for the isolates T3, T4, T1 and T2 respectively (Table 5 and Fig.3). Whereas the isolates of *Trichoderma* exhibited weak inhibition against mycelial growth of the *R. solani* and the results or RGM appears (50, 44.8, 42.2 and 47.5 %) for the isolates T3, T4, T2 and T1 respectively, (Table 6 and Fig. 3) showed the Dual culture plates and showing interaction between biological control agent *T. harzianum* T3 and phytopathogenic fungi *P. oryzae*, T3 covers the growth of *P. oryzae* > 90 %.

Table 4. *In vitro* antagonistic activity of *Trichoderma* spp. against *P. oryzae* by using dual assay culture

S. No	Test mycoflora	Geographic origin	Growth of antagonist (cm)	Growth of pathogen (cm)	PIMG (%)
1	T1	Qualybia	7.75	1.25	83.8
2	T2	Minufya	7.90	1.10	86.0
3	T3	Cairo	8.14	0.86	91.6
4	T4	Giza	8.0	1.00	87.5

Table 5. *In vitro* antagonistic activity of *Trichoderma* spp. against *M. phasolina* by using dual assay culture

S. No	Test mycoflora	Geographic origin	Growth of antagonist(cm)	Growth of pathogen (cm)	PIMG (%)
1	T1	Qualybia	6.0	3.0	50.0
2	T2	Minufya	6.1	2.9	56.2
3	T3	Cairo	6.4	2.6	59.3
4	T4	Giza	6.6	2.4	63.6

Table 6. *In vitro* antagonistic activity of *Trichoderma* spp. against *R. solani* by using dual assay culture

S. No	Test mycoflora	Geographic origin	Growth of antagonist (cm)	Growth of pathogen (cm)	PIMG (%)
1	T1	Qualybia	5.9	3.1	42.2
2	T2	Minufya	5.7	3.3	47.5
3	T3	Cairo	6.0	3.0	50.0
4	T4	Giza	5.8	3.2	44.8

Table 7. Antagonistic activity of *Trichoderma* spp. against phytopathogenic fungi by dual culture, using Bell's scale (R).

***Trichoderma* spp**	**Test pathogen**		
	p. oryzae	***R. solani***	***M. phasolina***
T. asperellum	R_2	R_4	R_3
T. longibrachiatum	R_2	R_4	R_3
T. harzianum	R_2	R_3	R_3
T. harzianum	R_2	R_4	R_3

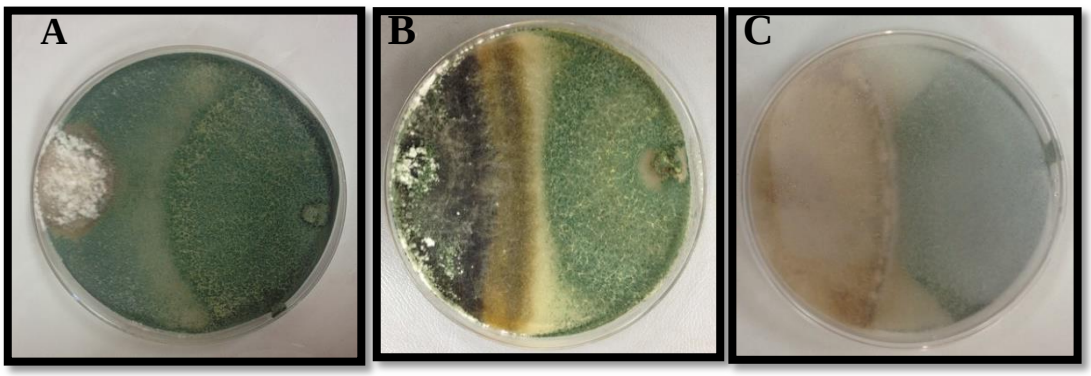

Fig.3. Dual culture technique of antagonist *T. harizainum* (isolateT1) against mycelial growth of test pathogens: (A) *P. oryzae*, (B) *R. solani* and (C) *M. phasolina* were cultured on PDA medium. Plates were incubated at (28 ± 3) °C for 6 days.

The right side of the plate has *Trichoderma*, and on the left has *P. oryzae*, whereas, T3 covers the growth of *M. phasolina* >50 % and *R. solani* > 40 respectively. During the confrontation period in dual culture method, the *Trichoderma* spp. colonized a wide area of the culture medium in the plates, which due to the speed of their mycelial growth, and thus was higher than that of the test phytopathogenic fungi. On the hand, pathogenic fungi strains were normally have slower growth rates than the pathogens. In addition, it was also noted that several *Trichoderma* strains sporulate large amount, when growing over the pathogenic colony, thereby indicating that they can be highly competitive for space and nutrients (Fig.3). Thus caused morphological deformations and disorganization in the structure of their cell wall, so that its appearance becomes rough, probably is due to the secretion of antifungal substances (enzymes and antibiotics) by *T. harzianum* (Fig.3 b and c). The disintegration of mycelial walls resulted in the total destructions of the colony of tested pathogen.

Discussion:

In this work, Morphological and Molecular identification (DNA sequencing of the 5.8S-ITS region) were carried out to identify the *Trichoderma* isolates that were obtained from rhizospere soil. Initially, the morphological identification suggests that these isolates were morphologically similar and phylogenetically very closely related to *T. asperellum* (T1), *T. longibrachiatum* (T2) and *T. harzianum* (T3 and 4). DNA sequencing of the ITS1-5.8S-ITS2 region may be the most widely reliable loci that can be used for the molecular identification of strains at the species level [38]. This method was used by comparing the results of sequences of the 5.8S-ITS 1 and 2 region to the sequences deposited in (National Center for Biotechnology Information NCBI "Genbank, http://www.ncbi.nlm.nih.Gov/ BLAST"), results obtained from present study showed all isolates of *Trichoderma*, that can be identified to species level with the homology percentage of at least (99%) (Table 1). Many research teams focused their works on the investigation of biological control activity of *Trichoderma* species against fungal plant pathogens, and has been carried out mainly in controlling pathogens isolated from roots, while there are few reports on their application in controlling foliar diseases [39]. In the separated studies high antagonistic potential was observed by using *Trichoderma* spp. and used as biocontrol agents against many soils plant pathogen isolated from soil samples [13, 39, 40]. According to the experimental results, *Trichoderma* isolates had a potential biological control activity in a dual culture studies against the phytopathogenic fungi of *P.oryzae, R. solani* and *M. phasolina*. The present work of *in vitro* plate assays showed that *T. harzianum* is more effective in suppressing the growth of test pathogens followed by *T. asperellum* and *T. longibrachiatum*. The obtained results were in agreement with findings of [41], who reported that the bioagents *T. Koningi* and *T. harzianum* have reducing the radial growth of *M. phaseolina*, on the other hand Shalini and Kotasthans [42] evaluated the antagonistic activity of seventeen *Trichoderma* strains against plant pathogens of *R. solani* (*in vitro*), several *Trichoderma* strains including *T. harzianum, T. viride* and *T. aureoviride* were isolated from soil and investigated against the growth of plant pathogens *R. solani*, results showed good inhibition to pathogen. In

previous study [16], achieved control of *P. oryzae* by 22 of *Trichoderma* isolates used to investigated antagonistic activity, from this study *Trichoderma* isolate number two (T2) was the most potential against plant pathogen (*p. oryzae*) when used as in dual culture with *Bacillus substili*. The using of microbes as a biological control agents is dependent upon the complex interactions that these helpful microbes establish with pathogens and plants in the soil ecosystem. *Trichoderma* spp. have several mechanisms for fungal antagonisms, some strains produce antibiotics (Antibiosis), myco-parasitism has been attributed to producing several degrading (lytic) enzyme produced by *Trichoderma* spp. such as chitinases, glucanases… *ect.* That attack and lysing cell wall of pathogens, nutrients competition are the well-known mechanisms involved in biological control of fungal pathogens by *Trichoderma* spp. space and dominance being equally important and mutually inclusive phenomenon [17,43,44,45]. In addition, many *Trichodrema* spp. can stimulate systemic and localized resistance to several plant pathogens Moreover, some isolates may increase the plant growth and development.

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التشخيص الجزيئي للترايكوديرما المعزولة من التربة المحيطة بالجذور وفعلها التضادي تجاه بعض الفطريات الممرضة للنبات

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الخلاصة:

الهدف الرئيسي من هذه الدراسة هو التعريف على المستوى الجزيئي وتحديد الفعالية التضادية لفطر *Trichoderma* spp. المعزولة من التربة المحيطة لجذور النباتات تجاه بعض الممرضات الفطرية للنبات والتي تضمنت (*Magnaporthe grisea*), *Macrophomina phisolina*, *Rhizoctonia solani* *Pyricularia oryzae*.عزلت أربعة عزلات من *Trichoderma* spp. من التربة المحيطة لجذور بعض النباتات المضيفة من مواقع مختلفة من محافظات مختلفة في مصر. تم تشخيص عزلات *Trichoderma* spp. اعتمادا على المظهر الخارجي فضلا عن تعريفها على المستوى الجزيئي من خلال مقارنة نتائج تسلسل الحمض النووي على اساس تعريف المنطقة (ITS1-5.8S-ITS2) ومقارنة نتائج تسلسل الحامض النووي (DNA) لمنطقة ITS مع نتائج عزلات اصلية. اوضحت نتائج التعريف ان عزلة واحدة من بين العزلات الاربعة تعود الى (*T. asperellum* T1) ، عزلة واحدة تعود الى(*T. longibrachiatum* T2) وعزلتين تم تعريفها على انها (*T. harzianum* T3,T4) اوضحت التعريف الجزيئي نسبة شبه 99% لتسلسل المنطقة S-ITS5.8 للعزلتين (*T. harizanum* T3, T4) وكذلك نسبة شبه (99 %) لكل من (*T. asperellum* T1 و *T. longibrachiatum* T2) من جهة اخرى نتيجة التعريف الجزيئي للعزلات الفطرية للمرضة للنبات اوضحت نسبة شبه عالية وتم تعريفها على انها (*P. oryzae*, *M. phisolina*, *R. solani*) وبنسبة شبه (99، 96، 99%) على التوالي. بالنسبة للعلاقة الوراثية بين عزلات الترايكوديرما الاربعة تم بأستخدام تقنية (RAPD) ولعشرة بادئات تم اختيارها بصورة عشوائية. تم اختبار الفعل التضادي والمقاوم للعزلات الاربعة لفطر *Trichoderma* تجاه الفطريات الممرضة قيد الدراسة وبينت النتائج ان *T. harizanum* كانت اكثرة قوة وفعالية في تثبيط الفطريات الممرضة عنه بالنسبة لفطر *T. asperellum* و*T. longibrachiatum*. نسبة التثبيط بالنسبة لفطر *T. harzianum* كانت متغايرة كثيرا حيث بلغت (44.8 الى 91.6%) . التاثير التثبيطي لفطر *T. asperellum* تراوح بين (42.2 الى 86.0%)، بينما اظهر الفطر *T. longibrachiatum* فعلا تثبيطيا تراوح بين (47.5 الى 83.8%) .

الكلمات المفتاحية: السيطرة الحيوية، الترايكوديرما، الربد، التربة المحيطة لجذور النبات، ممرضات النبات.