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Isolation of some Biocontrol Fungi from Saudi soil and their Beneficial Role as Bio-Fertilizers

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Beneficial fungi found in the rhizosphere of cultivated plants next to some harmful microorganisms. These fungi which used in the vital biological control could be propagated and then put back again to the soil to get useful results for resisting plant diseases. The objective of this study was to isolate some biocontrol fungi to be used to reduce inhibitors phenols in olive press solid cake and convert it into a soil as bio-fertilizers. Twenty-five rhizosphere soil samples were collected from 25 locations in Khoaa village, Sakaka, Aljouf governorate, northern part of Saudi Arabia. A total of 3512 fungal isolates related to 11 genera were obtained from rhizosphere soil of alphalpha during November 2014. Four hundred and forty-two, 3 and 298 isolates of *Gliocladium roseum*, *Pythium oligandrum* and *Trichoderma harzianum* were isolated, respectively. *Gliocladium roseum* and *Pythium oligandrum* were capable to decompose phenols which is responsible for inhibiting cucumber seed germination. On the contrary, *Trichoderma harzianum* showed negative effect in reducing phenol content of the olive press cake, however, it showed its ability as a biocontrol. *Gliocladium roseum* and *Pythium oligandrum* can be used to convert the inhibitory olive press cake to media for the growth of cucumber seeds as well as a reservoir of biocontrol elements.

Key words: Biological control, *Gliocladium roseum*, Olive pressing solid cake, Phenolics; *Pythium oligandrum*, *Trichoderma harzianum*.

Recently, the green revolution jumped to intensify the world agricultural production to meet the ever increasing load for crops and food.

Fungi found in many places on the globe. They Present in air, water, soil and all the different surfaces of inanimate, plants, animals or human beings. These microorganisms flourish in soil, especially in the immediate vicinity of roots of plants in an area called rhizosphere. Plant roots secrete substances called root oxidates attract fungal spores to grow around the outer layer of the roots by taking advantage of these organic secretions. In many cases, those fungi protect roots of the cultivated plants by working as wall protection from invading pathogenic microorganisms. On the other hand, other microorganisms can invade the roots of plants, causing diseases to plant seedlings. One of the principals of biological control is the use of beneficial non-pathogenic organisms to control pathogenic organisms. Since many decades, fungi have been used in the vital resistance to other pathogenic fungi to cultivated plants. Fungi of *Gliocladium roseum*, *Pythium oligandrum* and *Trichoderma harzianum* were used to control many fungal diseases of plants[1-6]. It is worth mentioning that the market trade of agricultural materials is full of products that are used for resistance to fungal diseases of cultivated plants.

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Isolation of some biocontrol fungi is not the only goal of this research but in addition to increase of their numbers is to take advantage of them to get rid of the overload of phenols present in the solid waste squeezing olives. It is very important to isolate organisms from the same environment to be used as biocontrols[7].

This research was conducted in Aljouf governorate in the northern region of Saudi Arabia. Aljouf area is characterized by prosperity implant olive where the weather is similar to the Mediterranean climate[8,9]. There are approximately 16000000 olive trees and more than 16 presses for squeezing the fruits. Seasonally, due to the large production of olive in Aljouf region and squeezing coincided olives operations, huge amounts of solid residues of olives accumulate[9]. Tons of olive press solid cake that generated from pressing olive fruits represent a big environmental problem. These wastes are usually get rid from them in a wrong way. They are thrown to the desert or added to agricultural sandy land. Due to the high content of phenols in the wastes, they encourage the growth of harmful organisms and pollute the environment. Additionally, it has been found that the high content of phenolics prevent germination of plant seeds and retard plant growth[10].

The objective of this study was to study the prospect of using the isolated biocontrol fungi to reduce phenols in olive press solid cake. Conversion of the inhibitory waste of the olive press solid cake to organic fertilizers filled with propagates of three biocontrol elements is one of the present aims.

MATERIALS AND METHODS

Sampling sites

Twenty-five agriculture fields distributed in Khoaa village, Sakaka (29º 48' 6' N, 40º 26' 27''), Aljouf Governorate were chosen for rhizophere soil sampling of alphalpha during November, 2014.

Fungal isolation

Samples were collected by scarping adhered soil to the roots and packing each sample in sterile vinyl bag and carried to the laboratory.

Samples were cultured on Potato dextrose agar (PDA), which is most effective culture media in the isolation of fungi including biocontrol agents of *G. roseum* and *T. harzianum*[11] with Rose-bengal [1/15000 ml / media / l medium] by using dilution plate methods[12]. Plates were incubated at 27ºC for 7 days or until appearance of colonies and then examined. The number of the identified fungi was counted.

Pythium isolation:

Soil serial dilution method

Rhizosphere soils were diluted from 10-1 to 10-5 dilutions, and the diluted soil samples were placed on NARM selective medium in Petri-dishes [Nystatin (10 mg L-1), Ampicillin (250 mg L-1), Rifampicin (10 mg L-1) and Miconazole (1 mg L-1) in corn meal agar (CMA)]. Dishes were incubated at 27ºC for 3 days or until the appearance of colonies[5,7].

Identification of fungi

It was performed using morphological characteristics of taxonomic keys of[13-18].

Identification of *Pythium* spp.:

Morphological identification of *Pythium* species

Asexual and sexual structures were mainly used for *Pythium* identification. Zoospores formation, types of zoosporangia, antherial shape, size and mode of its attached to the oogonium, oogonial shape, size, wall type, and oospore wall thickness and number of oospores within the oogonium were used for identification. Identification was followed using two the keys of[19,20].

Pathogenicity of *Pythium ultimum* var. *ultimum*:

Pre-emergence damping-off trial: Two and half per cent inoculum concentration of *P. ultimum var. ultimum* was used. Five grams of maize grass blade leaf segments (0.5 cm x 1 cm), 2 gm glucose and 10 ml distilled water were introduced in each 250 ml Erlenmeyer flask. After sterilization, each flask was injected with three disks (7mm dam) of PDA containing growth of *P. ultimum var. ultimum* and kept at 25 ºC for a week. One gram of the previously colonized grass leaf segments was mixed in the Erlenmeyer flask with 50 g of oven-dried (70-80 ºC for 2 days) clay loam soil using a sterilized mortar and pestle. Two and half grams of the above mentioned mixture were mixed with sterilized 97.5 g of clay loamy. Pre-emergence damping-off was determined as difference in emergence between control soil and infested one.

Inoculated soil (100 g) each were distributed into 10 replicate pots (100 ml capacity and 7.5 diam) and 6 cucumber seeds were planted

in each pot. The experiment was carried out in the laboratory at 27ºC (adjusted by controlled units of the central air conditioner).

Seed coating with *G. roseum* (JU121), *P. oligandrum* (JU221) and *T. harzianum* (JU321)[21]

Pads of mycelia of *G. roseum*, *P. oligandrum* and *T. harzianum* were collected from cultures grown in Potato dextrose liquid medium. Twenty ml of the medium were distributed in 9 cm Petri-dishes and each was inoculated with a mycelial disc from the PDA fungal colony. All cultures were incubated at 27°C for 7 days in darkness. Mycelial growth were removed, washed in sterile water and split in distilled water using a mixer and then stored at 5°C until use. Cucumber seeds were coated by this preparation. Hundred ml of each of 3% solution of carboxymethylcellulose (CMC) and the preparation were mixed and 100 seeds of surface sterilized cucumber seeds were added. After soaking for 2 min in the preparation, the seeds were detached, spread in sterile open Petri-dish, and allowed to dry overnight in a laminar flow cabinet at 27ºC and were then used immediately in the damping-off test.

Testing the ability of biocontrol fungi to produce toxins that affect the germination of cucumber seeds

In order to use fungi as biocontrol agents, *G. roseum*, *P. oligandrum* and *T. harzianum* must not produce any toxins that hinder seed germination and plant growth. Sterilized filter paper 9 cm humidified with 10 ml filter sterilized cultural filtrates of each of the tested fungi were placed in each dish and were incubated at 25ºC until seed germination. Ten ml sterilized distilled water was used as control.

Effect of tested fungi on infection of cucumber germinating seeds by *Pythium* species

Testing biocontrol activity of each of *G. roseum*, *P. oligandrum* and *T. harzianum* on infection of cucumber germinating seeds by *Pythium ultimum* var. *ultimum* was done using cucumber seeds coated with each one of these fungi.

Culturing *Gliocladium roseum*, *Pythium oligandrum* and *Trichoderma harzianum* on olive press solid cake

Samples of olive press solid cake were obtained from an olive mill located in Sakaka city, Aljouf, Saudi Arabia in December, 2014. In each 1 L Erlenmeyer flasks, 200 g of olive press solid cake moisture with 150 ml distilled water was added and then sterilized by autoclaving at 121°C for 30 min. Inoculation was performed as static case at 27°C for 1-4 weeks.

Total Phenols in the olive press solid cake before and after growth of the tested fungi (within 1-4 weeks) were analyzed according to[22], using tannic acid as a standard, and expressed as grams per kilogram of the olive press solid cake.

The pre-cultured olive press solid cake with the tested fungus was analyzed for their suitability for growing seeds of cucumber (*Cucumis sativus* L.) then added to plastic pots (each 100 g). Six seeds were seeded on the surface of each pot containing tested olive press solid cake and inoculated pots were incubated in the laboratory (adjusted to 25ºC). Emergence seedlings were counted through 5-20 days.

Statistical analysis

ANOVA was used to assess data using Minitab statistical software (version 12) unless somewhere else mentioned.

RESULTS

Data in Table (1) show that 24 species belonging to 11 genera were isolated from 25 locations in Khoaa village, Sakaka, Aljouf governorate, Saudi Arabia. *Aspergillus* was consistently the most frequent genus (100% of places) and contributed the broadest spectrum of species (7 species) of which *A. flavus* and *A. niger* were of high frequency (25 places out of 25) contributing 4.4% and 9.7% of the total count respectively by dilution plate method. *Penicillum* was recovered from 24 samples matching 25.6% of total count fungal isolates. It was represented by 6 species of which *P. chrysogenum* and *P. italicum* were common sharing with 9.4% and 9.8% by dilution plate method. *Cldosporium* and *Chaetomium* gave 3.3% and 2.7% of the total count, respectively. *Cldosporium* was represented by *C. cladosporioides* which appeared in 18 places contributing 3.4% of total count. *Gliocladium* (*roseum*) and *Trichoderma* (*T. harzianum* and *T. longibrachyata*) appeared in high contributing 12.7 % and 11.5% of the total count. *Alternaria*, *Mucor*, *Paecilomyces*, *Stachybotrys* and *Ulocladium* were less frequently isolated from rhizosphere soil of

alphalpha plants cultivated in Khoaa village, Sakaka, Saudi Arabia.

Data in Table (2) reveal that 4 species and one group of *Pythium* species were isolated from 15 places out of 25 places, in Khoaa village, Sakaka Aljouf governorate. *Pythium* "group HS" was the most frequent fungus (50% of places) contributing 30.6% of total count of pythia by dilution plate method. *P. ultimum* var. *ultimum* came in second with 25% of total count. *Pythium catenulatum and P. diclinum* were recovered from 3 samples matching 19.4% and 16.6 of the total count, respectively. The biocontrol *Pythium oligandrum* appeared in 3 places contributing 8.3% of the total count of *Pythium* isolates.

Table 1. Total counts of fungal genera and species recovered from 25 samples of rhizosphere soil of alphalpha collected from Khoaa, Sakaka, Aljouf, Saudi Arabia by dilution plate, number of cases of isolation (NCl; out of 25 cases), occurrence remarks (OR), and percentage of total counts (TC%) on PDA agar at 27°C

Genera and species	Rhizosphere		
	NCl	OR	TC (%)
Alternaria	84	2R	2.4
A. alternata	72	1R	2.1
A. teunissima	12	1R	0.3
Aspergillus	899	15H	25.9
A. clavatus	99	13H	2.8
A. flavus	154	15H	4.4
A. fumigatus	150	15H	6.0
A. niger	204	15H	9.7
A. ochraceus	95	13H	2.7
A. sulphureus	98	4L	2.8
A. terreus	99	8M	2.8
Cladosporiumcladosporioides	114	7M	3.3
Chaetomium	93	5M	2.7
C. globosum	56	4L	1.6
C. indicum	37	6M	1.1
Gliocladiumroseum	442	11H	12.7
Mucor	286	4L	8.2
M. circinelloides	187	4L	5.4
M. racemosus	99	4L	2.8
Paecilomycesvariotii	87	3L	2.5
Penicillium	890	12H	25.6
P. oxalicum	34	10H	1.0
P. chrysogenum	328	14H	9.4
P. citrinum	58	13H	1.7
P. italicum	339	14H	9.8
P. islandicum	104	13H	3.0
P. purpurogenum	27	8M	0.8
Trichoderma	400	14H	11.5
T. harzianum	298	14H	8.6
T. longibranchyata	102	13H	2.9
Stachybotryschartarum	38	2R	1.1
Ulocladiumchartarum	143	3L	4.1
Gross total counts	3476		
No. of genera	11		
No. of species	24		

OR = Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0% , L = 20 - 32%, and R = 7 – 19%

Pathogenicity of *P. ultimum* var. *ultimum* and its possible control capability by *G. roseum, P. oligandrum* and *T. harzianum* isolates in soil pots assay is presented in table (3). The three biocontrol agents of *G. roseum, P. oligandrum* and *T. harzianum* were proven to be non-virulent to cucumber germinating seeds. In contrast, *P. ultimum* var. *ultimum* was highly pathogenic causing 100% pre-emergence damping-off. Application of each of *G. roseum, P. oligandrum* or *T. harzianum* as seed dressing resulted in significant improvement in seedlings emergency of cucumber from 0 – 86.7% in soil treatments.

Total phenols (g/kg) content of olive press solid cake were measured before and after growth of *G. roseum P. oligandrum* and *T. harzianum*. The amount of total phenols highly significantly declined when *G. roseum* and *P. oligandrum* were previously cultivated on olive

Table 2. Total counts of *Pythium* species recovered from 25 samples of rhizosphere soil of alphalpha collected from Khoaa, Sakaka, Aljouf, Saudi Arabia by dilution plate, number of cases of isolation (NCl; out of 25 cases), occurrence remarks (OR), and percentage of total counts (TC%) on NARM medium at 27°C

Pythium spp.	Rhizosphere		
	NCl	OR	TC (%)
P. catenulatum	7	4L	19.4
P. diclinum	6	3L	16.6
Pythium "group HS"	11	5M	30.6
P. oligandrum	3	3L	8.3
P. ultimum var. *ultimum*	9	5M	25
Gross total counts	36		
No. of genera	1		
No. of species	3 + one group		

OR = Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0% , L = 20 - 32%, and R = 7 – 19%

press solid cake starting from the first week and reached a peak decrease after 3 weeks of the growth for *P. oligandrum* and 4 weeks in case of *G. roseum*. On the other hand, growth of *T. harzianum* did not offer any change of total phenols in comparison to the control (Fig. 2).

Germination of cucumber seeds on olive press solid cake previously cultured with *G. roseum*, *P. oligandrum* and *T. harzianum*

The application of olive press solid cake previously incubated with either of *G. roseum* or *P. oligandrum* during 1-4 weeks significantly increased cucumber seed germination compare with cucumber grown in crude olive press dry cake. On the other hand, the application of olive press dry cake previously incubated with *T. harzianum* inhibited cucumber seed germination (Table 4).

Table 3. Effect of dressing cucumber seeds with *G. roseum* (JU121), *P. oligandrum* (JU221) and *T. harzianum*(JU321) isolates on pre-emergence damping-off disease caused by *P. ultimum* var. *ultimum* grown in pots containing clay loamy soil at 27ºC

Treatment	Cucumber Soil pots		
	No. of survival	Inhibition (%)	Survival (%)
Control (without*Pythium*)	30[c]	0.0	100
P. ultimum var. *ultimum*	3[a]	100	0.0
P. oligandrum	30[c]	0.0	100
G. roseum	30[c]	0.0	100
T. harzianum	30[c]	0.0	100
P. ultimum var. *ultimum* + *G. roseum*	26[b]	13.3	86.7
P. ultimum var. *ultimum* + *P. oligandrum*	21[b]	30	70
P. ultimum var. *ultimum* + *T. harzianum*	20[b]	36.7	63.3

*Value within a column followed by the same latter are not significantly different according to Dancun,s multiple range test[23] (P e" 0.05)

Table 4. Emergency of 60 cucumber (*Cucumissativus* L.) seeds inoculated or not with *G. roseum*, *P. oligandrum* and *T. harzianum* in presence or absence of olive press solid cake incubated at different times

Treatments	Time of incubation (days)			
	5	10	15	20
	Cucumber seed germination			
Vermiculite	52*	54**	54	54
Olive press dry cake	0[a]	0	0	0
Olive press dry cake previously incubated with *G. roseum* for 1 week	36[c]	40[c]	41[c]	41[c]
Olive press dry cake previously incubated with *G. roseum* for 2 week	50[c]	50[c]	50c	50c
Olive press dry cake previously incubated with *G. roseum* for 3 week	51[c]	52[c]	52[c]	52[c]
OPC previously incubated with *G. roseum* for 4 week	51[c]	51[c]	51[c]	51[c]
Olive press dry cake previously incubated with*P. oligandrum* for 1 week	49[c]	50[c]	50[c]	51[c]
Olive press dry cake previously incubated with *P. oligandrum* for 2 week	48[c]	52[c]	52[c]	52[c]
Olive press dry cake previously incubated with *P. oligandrum* for 3 week	42[c]	42[c]	42[c]	42[c]
Olive press dry cake previously incubated with*P. oligandrum* for 4 week	35[c]	36[c]	37[c]	37[c]
Olive press dry cake previously incubated with *T. harzianum* for 1 week	0	0	0	0
Olive press dry cake previously incubated with *T. harzianum* for 2 week	0	0	0	0
Olive press dry cake previously incubated with*T. harzianum* for 3 week	0	0	0	0
Olive press dry cake previously incubated with *T. harzianum* for 4 week	0	0	0	0

*Germination of cucumber seeds from a total of 60 ones.
**Means within each column followed by the same letter were not significantly different (compared with the control in Olive press solid cake) according to Duncan's Multiple range test[23] (P= 0.05)

DISCUSSION

The role of fungi in the rhizosphere is too complex and those microorganisms are essential components in the soil ecosystem. They are an essential constituent of the soil microbiota characteristically contributing more of the soil biomass than bacteria[24]. Functionally, fungi play an important role in the recycling of organic compounds in the soil and break down toxins, contributing to the continuation of carbon cycle as well as stability of the soil ecosystem[25]. Subsequently, fungi are present in abundance and density in rhizosphere area of different plants grown in the soil because of food detachment from those roots[7].

In the present investigation the fungal species of the genus *Aspergillus* was consistently the most frequent (100% of places) and contributed the broadest spectrum of species (7 species) of *A. clavatus, A. flavus, A. fumigates, A. niger, A. ochraceus, A. sulphureus and A. terreus*. *Penicillum* was recovered from 24 out of 25 samples matching 25.6% of total count fungal isolates. It was represented by 6 species of *P. oxalicum, P. chrysogenum, P. citrinum, P. italicum, P. islandicum* and *P. purpurogenum*. *Cldosporium cladosporioides, Chaetomium, Gliocladium roseum, Trichoderma* (*T. harzianum* and *T. longibrachyata*) *Alternaria, Mucor, Paecilomyces, Pythium, Stachybotrys* and *Ulocladium* were also isolated from rhizosphere soil of alphalpha plants cultivated in Khoaa village, Sakaka, Saudi Arabia which were also previously reported from Saudi Arabian soil[26,27]. This indicates the prevalence of these fungi in Saudi Arabia. It is

Fig. 1. Growth of *Gliocladium roseum, Pythium oligandrum* and *Trichoderma harzianum* (from left to right, respectively) on olive press solid cake after 14 days at 27°C in the dark

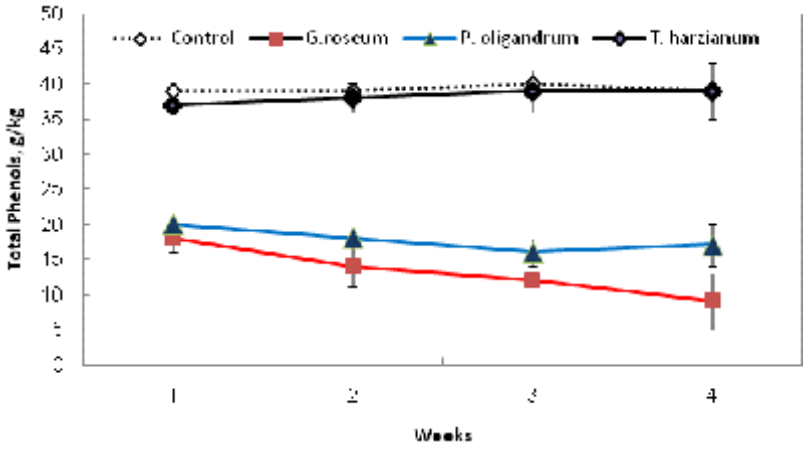

Fig. 2. Phenol content (g/kg) of olive press solid cake treated with each of *G. roseum, P. oligandrum*and *T. harzianum*during different treatment times (1, 2, 3 and 4 weeks). Data are averages (± S.E.) of 5 replicates and significant values against control represent: ** = highly significant at p Â 0.01, *** = very significant at p Â 0.001

worth to mention that some of the fungi isolated in this study were able to control pre-emergence damping-off disease of cucumber caused by the isolated species of *Pythium ultimum* var. *ultimum*. Fungi of *Gliocladium roseum*, *P. oligandrum* and *T. harzianum* were proven to have the capacity to control damping-off of cucumber caused by *P. ultimum* var. *ultimum*. Results here are in consistent with the previous studies concerning the role of *G. roseum*, *P. oligandrum* and *T. harzianum* in control of damping-off disease caused by *Pythium* species[5, 28-30].

A huge amount of olive press solid cake accumulate as a result of pressing approximately 16 million fruitful olive press of Aljouf area, northern part of Saudi Arabia. These large amount of waste is disposed to the land in wrong ways however it contains the remains of valuable nutrients. One of the problems generates from these wastes is the large amounts of polyphenols that hinder the growth of plants and inhibit the germination of seeds of many plants as well as encourage a lot of contaminaing bacteria.

It is worth mentioning that the soil in the area of Aljouf district is poor calcareous that harden with serial irrigation to form a center which is not suitable for plant growth. Subsequently, many farmers believe that addition of olive press solid residues can assist to improve mechanical properties of the soil.

The results of this study demonstrate that the solid residues of pressing olive fruits have hampered the growth of the seeds of cucumber because these remains have a large amount of phenols. Results here are consistent with many previous studies[31,32]. The inhibitory effect of phenols in wastes appears in high concentration[33]. Crude olive press solid cake showed 39 g Kg-1 of total phenolic compounds, therefore, addition of olive press solid cake to the soil resulted in the inhibition of seed and plant germination.

According to the previous studies, it was found that fungi can grow on high levels of phenols. Those fungi can absorb and break down these phenolic compounds and use them in their metabolic interactions[31,34]. Another study reported that some species of the genus *Aspergillus* especially *Aspergillus niger* can grow heavily on olive press solid cake and degrade phenols[35].

Earlier results showed that phenolics decreased drastically as a result of growing *Coriolopsis rigida* on olive press solid cake[36]. As a result, *C. rigida* improved tomato yield as compared with the submission of olive press solid cake to soil without incubation with the fungus[37].

Results in Fig. 2 support the possibility of using *G. roseum* and *P. oligandrum* to significantly reduce the phenols of olive press dry cake to be used as medium for cucumber seed germination. The two fungi were capable to be very significant in reducing total phenols from 39 g / kg of olive press solid cake to 10-20 g total phenols/kg olive press solid cake within 4 weeks. In contrast, *T. harzianum* has not been able to reduce phenols in olive press solid cake however developing heavily fungal mycelia on olive residue. This may be due to the inability of this fungus to use and breakdown phenols in olive residue, but the high content of nutrients in olive residue is the main reason for such heavy fungal growth. As a result, seeds of cucumber strongly germinated on olive residue, which has already used as media for growing each of *G. roseum* and *P. oligandrum*. Therefore, *G. roseum* and *P. oligandrum* showed biocontrol ability against damping-off disease of cucumber as well as they detached toxicity of olive press solid cake as decreased phenols.

And for whole, learned from growing *G. roseum* and *P. oligandrum* on solid olive residues in being the sources of vital biocontrol agents as well as getting rid of phenols causing inhibition of seed germination and suppression of plant growth.

Further studies are needed to show the role of beneficial biocontrol fungi in bioremediation of olive press solid residues to be used as biofertilizers.

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REFERENCES

1. Shoulkamy, M. A., El-Katatny, G. M., Shaban, G. M. and Abdelzaher, H. M. A. Antagonism of *Trichoderma* or *Gliocladium* species on two

phytopathogenic species of *Fusarium*., *Journal of Natural Fibers* 2006; **3**(2/3): 1-17.

2. El-Katatny, M. H., Abdelzaher, H. M. A. and Shoulkamy, M. A. Antagonistic actions of *Pythium oligandrum* and *Trichoderma harzianum* against phytopathogenic fungi (*Fusarium oxysporum* and *Pythium ultimum* var. *ultimum*)., *Archives of Phytopathology and Plant Protection* 2006; **39** (4): 289-301.
3. Dubey, S.C, Bhavani, R. and Singh, B. Integration of soil application and seed treatment formulations of *Trichoderma* species for management of wet root rot of mungbean caused by *Rhizoctonia solani*. *Pest Manag Sci* 2011; **67**: 1163-1168.
4. Bhandari, P.C. and Vishunavat, K. Screening of different isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Fusarium moniliforme*. *Pantnagar Res* 2013; **11**(2): 243-247.
5. Elnaghy, M. A., Abdelzaher, H. M. A., Shoulkamy, M. A. and Sayed, S. R. Bean (*Phaseolus vulgaris* L.) damping-off caused by *Pythium ultimum* var. *ultimum* and its possible control by *Pythium oligandrum*. *Journal of Pure and Applied Microbiology* 2014; **8** (SPL. EDN.): 161-169.
6. Sharma, P. , Sharma, M., Raja, M. and V. Shanmugam, 2014. Status of *Trichoderma* research in India: A review. *Indian Phytopath.* **67** (1) : 1-19.
7. Elnaghy, M. A., Abdelzaher, H. M. A., Shoulkamy, M. A. and Sayed, S. R. Ecological Studies on *Pythium* Species Associated with Some Plants Rhizosphere in El-Minia, Egypt. *Journal of Pure and Applied Microbiology* 2014; **8**(1): 195-204.
8. Aranda, A., Sampedro, I., Ocampo, J. A. and Garcia-Romera, I. Phenolic removal of olive-mill dry residues by laccase activity of white-rot fungi and its impact on tomato plant growth. *International Biodeterioration & Biodegradation* 2006; **58**: 176-179.
9. El-Khateeb, M. A., Tantry, B. A., Mohammed, A. H. and Rahiman, S. Phenotypic characterization of phenol degrading microorganisms isolated from olive mill waste. *African Journal of Microbiology Research* 2014; **8**(21): 2131-2136.
10. Maria, C. C., Anna, M. G. and Angela, V. Soil amendment with olive mill wastes: Impact on groundwater. *J Environ Manage* 2013; **131**: 216-221.
11. Vargas Gil, S., Pastor, S. and March, G. J. Quantitative isolation of biocontrol agents *Trichoderma* spp., *Gliocladium* spp. and actinomycetes from soil with culture media. *Microbiological Research* 2009; **164**(2): 196-205.
12. Abdel-Hafez, A. I. I., Abdel-Hafez, S. I. I., Mohawed, S. M. and Ahmed, T. A. M. Fungus flora of air, root and leaf surface of lentil plant cultivated in upper Egypt. *Bull Fac Sci Assiut Univ* 1990; **15**(2), 140-147.
13. Raper, K. B. and Fennell, D. J. The genus *Aspergillus*. *Williams and Wilkins* 1965; Baltimore, USA.
14. Pitt, J. I. The genus *Penicillium* and its telemorphic states *Eupenicillium* and *Talaromyces*. Commonwealth Scientific and Industrial Research Organisation Division of Food Research, North Ryde, N. S. W. 2113 *Australia Academic Press* 1979; A subsidiary of Harcourt Brace Javanovich, Publishers London. New York. Toronto. Sydney.San Fransisco.
15. Sivaneasan, A. Graminicolous species of Bipolaris, Curvularia, Derechslera, Exserohilu and their teleomorphs. *Mycological Papers*; 1987; 158: 1-261, CAB International Mycological Institute, Ferry Lane, Kew, Surrey, UK.
16. Moubasher, A. H. Soil fungi in Qatar and other Arab Countries. *Environmental studies center* 1993; University of Qatar.
17. Leslie, J. and Summerll, B.A. *Fusarium* Laboratory Manual. *Blackwell publishing professional* 2006; 1st edition, Iowa USA. pp.388.
18. Domsch, K. H., Gams, W., and Anderson, T. H. Compendium of soil fungi. 2007; London. New york Academic press.
19. Plaats-Niterink, A. J. van der. Monograph of the genus *Pythium*. Studies in Mycology,no.21.*CentraalbureauVoorSchimmelcultures: Baarn*, the Netherlands 1981; 242 pp.
20. Dick, M. W. Keys to *Pythium*. Reading: *College of Estate Management*, 1990; 64 pp.
21. Al-Sheikh, H.and Abdelzaher, H. M. A. Isolation of *Aspergillus sulphureus*, *Penicillium islandicum* and *Paecilomyces variotti* from agricultural soil and their biological activity against *Pythium spinosum*, the damping-off organism of soybean. *Journal of Biological Sciences* 2010; **10**(3): 178-189.
22. Ribereau-Gayon, P. Les Composes Phenoliques des Vegetaux. Dumond, 1968; Paris.
23. Duncan, D. B. Multiple range and multiple F tests. *Biometrics* 1955; **11**: 1–42,.
24. Ainsworth, G. C. and Bisby, G. R. Dictionary of the fungi. *Commonwealth Mycological Institute Kew* 1995; Surrey, pp 445.

25. Christensen, M. A view of fungal ecology. *Mycologia* 1989; **81**: 1-19.
26. Bahkali, A. H. Cellulose-Decomposing fungi from Saudi Arabia soil. *Qatar University Science Journal* 1996; **16**(1): 77-80.
27. Al-Sheikh, H. and Abdelzaher, H. M. A. Materials for *Pythium* flora of Saudi Arabia (I) Occurrence, pathogenicity and physiology of reproduction of *Pythium aphanidermatum* (Edson) Fitzp. isolated from north and east regions of Saudi Arabia. Research Journal of Microbiology 2012; 1-19, ISSN 1816 – 4935/ DOI: 10,3923/jim, 2012.
28. Abdelzaher, H. M. A. Occurrence of damping-off of wheat caused by *Pythium diclinum* Tokunaga in El-Minia, Egypt and its possible control by *Gliocladium roseum* and *Trichoderma harzianum*., *Archives of phytopathology and plant protection* 2004; **37**(2): 147-159.
29. Abdelzaher, H. M. A. Biological Control of Damping-off and Root Rot Diseases of Soybean Caused by *Pythium spinosum* Sawada var. *spinosum* using Rhizosphere Species of *Aspergillus sulphureus*, *Penicillium islandicum* and *Paecilomyces variotii*, *Annual Meeting of Phytopathological Society of Japan*, Phytopathological Society of Japan, *Japanese Journal of Phytopathology* 2006; 247-248.
30. Maghazy, S, M. N., Abdelzaher, H. M. A., Haridi, M. S. and Moustafa, S. M. N. Biological control of damping-off disease of *Trifolium alexandrinum* L. caused by *Pythium spinosum* Sawada var. *spinosum* using some soil fungi., *Archives of Phytopathology and Plant Protection* 2008; **41**(6): 431-450.
31. Wang, X., Yu, J., Wang, Y. and Wang, L. Mechanism-based quantitative structure-activity relationships for the inhibition of substituted phenols on germination rate of *Cucumis sativus*. *Chemosphere* 2002; **46**: 241-250.
32. Abdelzaher, H. M. A. and Albahrawi, A. Z. Conversion of the Solid Waste of Pressing Olives to Soil Fertilizers Using the Bio-control fungus (*Gliocladium roseum*) and the Edible Mushroom (*Pleurotus ostreatus*). *International Journal of Environment & Water* 2014; **3** (5): 57-65.
33. Wang, T.S.C., Yang, T. and Chuang, T. Soil phenolic acids as plant growth inhibitors. *Soil Sci*. 1967; **103**: 239-246.
34. Moreno, E., Quevedo-Sarmiento, J., Ramos-Cormenzana, A. Antibacterial activity of waste waters from olive oil mills. In Cheremisinoff P.N. (ed.): *Encyclopedia of Environmental Control Technology, Gulf Publishing Co., Houston*, 1990; pp. 731-757.
35. Hamdi, M. and Ellouz, R. Use of *Aspergillus niger* to improve filtration of olive mill waste-waters. *J. Chem. Technol. Biotechnol*. 1992; **53**: 195-200.
36. Sampedro, I., Aranda, E., Martin, J., Garcia-Garrido, J. M., Garcia Romera, I. and Ocampo, J. A. Saprobic fungi decrease plant toxicity caused by olive mill residues. *Applied Soil Ecology* 2004; **26**: 149–156.
37. Sampedro, I., Romero, C., Ocampo, J. A., Brenes, M., Garcia-Romera, I. Removal of monomeric phenols in dry olive residue by saprobic fungi. *Journal of Agricultural and Food Chemistry* 2004; **52**: 4487–4492.