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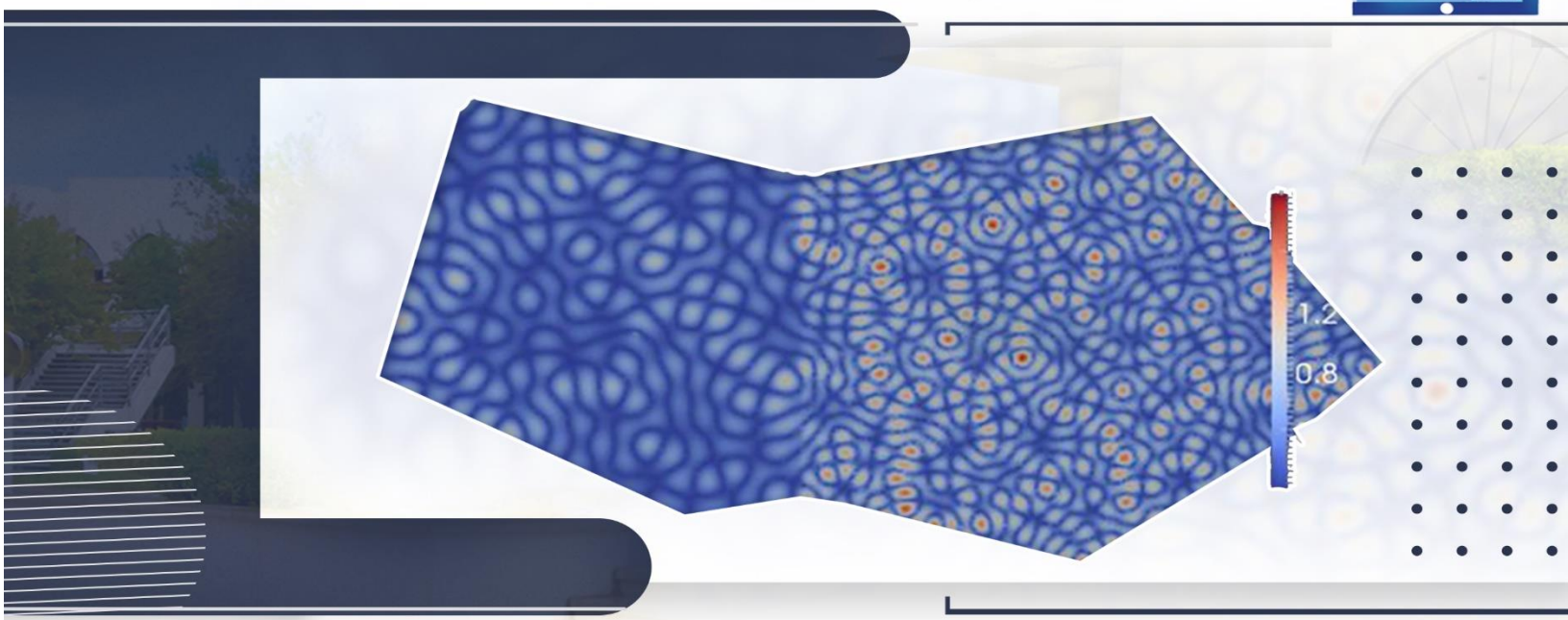

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The Role of Inoculation with Glomus macrocarpum and Saprophytic Fungi on Growth of Wheat Plant Grown in Addition with Olive Mill Residues

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A B S T R A C T

This study was carried out to test the influence of olive mill dry residue (DOR), Aqueous extraction (ADOR) and (SDOR) fraction treated with saprobe fungi on growth of Wheat (*Triticum aestivum* L.) plants colonized by *G. macrocarpum*.These fungal genera *Aspergillus niger* and *Penicillium crustaceum* were reported to possess the ability of detoxifying by degrading its phenolic compounds found in olive mill dry residue (ADOR and SDOR)) fraction . The percentage of mycorrhizal colonization by *G. macrocarpum* strongly decreased in presence of DOR, but the level of AMF colonization likewise increased in presence of ADOR or SDOR. Our study demonstrates that, in controlled conditions, The use of certain saprobe and AM fungi allows the possibility of using DOR as an organic fertilizer.

1 Introduction

Soils in the mediterranean region contain small quantities of organic matter that is important for plant growth (Brunetti *et al.*, 2005). The importance of the olive mill in Arabic countries is known and gaining importance in other countries outside the Mediterranean area.

The cultivation and production of olive oil (Olea europea L.) is of great importance in the Mediterranean region (Sampedro *et al* 2008). Dermeche *et al.* (2013) assessed and compared products of olive oil, in which phenolic substances, carbohydrates, organic acids and mineral elements were distributed differently, depending on the cultivation methods, were compared in terms of the use of olive dry residue (DOR) for cultivation (Parides *et al.*, 1999; Bonanomy *et al.*, 2006).And it was found from previous studies conducted using DOR that it contains toxic substances for microbes due to its phenolic content (Perez *et al.*,1992; Martin *et al.*, 2002). By using saprobe fungi, the toxicity of DOR on the plant can be reduced. The saprophytes break down cellulose materials into simple sugars as a source of energy for microbes that include the mycorrhizal (AM) (Radford *et al.*, 1996). In addition to that DOR toxicity can be reduced by inoculation with saprophytes around 20 weeks (Sampedro *et al.*, 2004). DOR can improve plant resistance to attacking pathogens to stimulate root growth and plant growth due to its high content of phenolic compounds Sasanelli *et al* 2011. The AM root fungus helps host plants grow in a polluted environment (Shetty *et al.*, 1994) by supplying phosphorous to the plant (Querejeta *et al.*, 1998).

Previous studies confirm the role of AM fungi in the resistance of host plants to growth in a polluted environment (Shetty *et al.*, 1994) by increasing the plant's ability to absorb phosphodiester (Querejeta *et al.*, 1998). And the effects of DOR had an effect on soil

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microbes and texture (Karpouzas *et al.,* 2010). These effects were negative in sandy clay soils and were not observed in clay soils due to the absorption of polyphenols in these soils (Rousidou *et al.,* 2010). It is possible to reduce the phytotoxicity of toxic residues dependent on intracellular AM (Volante *et al.,* 2005). It appears that the rhizosphere AM increases the effect of toxicants in olive residues (Martín *et al.,* 2002). The symbiotic effects of saprophytes and AM in plant roots have the potential to increase the host's tolerance to heavy metals in soil (Fracchia *et al.,* 2004; Martínez *et al.,* 2004).

This study is to determine the role of AM *Glomus macrocarpum* and Saprobe fungi in resisting the effects of phenols present in DOR, through their effect on the dry weight of wheat plants.

2 Materials and Methods

DOR was collected from the olive oil plant (Al-Marj area), sieved and sterilized by autoclave at 120 ℃ and stored in a refrigerator before use. The main characteristics of DOR was as follows: total organic carbon, 52.7%; total nitrogen, 1.15%; total phosphorous, 0.17%; lignin, 18.3%; cellulose 21%; hemicellulose 14.3%; total phenols, 3.15%; fat, 0.4%; Ash is 11.5%.

The most abundant elements, the concentration of which is reported in g.kg-1 DOR were: potassium, 27.4; calcium, 17.3; magnesium, 2.7; iron, 1.4; sodium, 0.19; copper, 0.09; zinc, 0.07 and manganese, 0.08.

An aqueous extract was obtained from the dry and by shaking this residue DOR with distilled water in a ratio of 1:2 (w/v) for 8 hours at room temperature and filtered with layers of Fabric obtained with (ADOR) and solid residues (SDOR), these were incubated with ADOR and SDOR or with saprophytes.

The saprobe fungi used are: *Aspergillus niger* and *Penicillium crustaceum* obtained from Omar Mukhtar University (Department of Plant Protection Laboratory). They were propagated and stored on PDA agar and 2% malt extract agar (MEA). Done

Supplementation with ADOR and shaking at 125 rpm at room temperature with Chapek in the presence of 50% ADOR extract for two weeks. The fungi were collected by filtration and washed with distilled water. Fungi were grown in an Erlenmeyer flask (250 mL) containing 70 mL of ADOR extract for 15 days and shaken at 125 rpm. Each vial was inoculated with 0.60 g^{-1}, then perform SDOR incubation in 500 ml Erlenmeyer flasks containing 125 g of SDOR, the flasks were covered with sterile cotton and incubated under at 28 ℃ for 15 weeks. Sterile, incubated ADOR and SDOR were prepared under the same conditions.

Experiments were carried out in pots containing 500 g of soil that had been autoclaved for one hour (110℃). The soil used is a clay texture collected from the Al-Marj region (Northeast of Libya). In addition, sift the soil 2 mm thick, mixed well to remove the original AM. Properties of soil analysis were described (Page. *et al* 1982) 29% sand, 30% silt, 41% clay, pH 7.8, (ECe) 2.1 d Siemens/m at room temperature; 3.2 mg/kg P ,2.1% organic matter, and C.E.C 22.62 cm mol/kg (Mg, Ca, Na and K were 0.5, 2.2, 21.6 and 3.02 cm mol/kg respectively).

Wheat (*Triticum aestivum* L.) plants were used. The seeds were sterilized and picked before sowing. Plants were grown in a greenhouse. The pots were regularly watered with distilled water to maintain 70% of the field capacity by regular weighing of the pots, and they were fed with a nutrient solution (Hewitt, 1966).

A species of fungi (AM) (*Glomus macrocarpum*)

Obtained and prepared by the Faculty of Agriculture, University of Cairo, Egypt.5 g of mycorrhiza inoculum was added to each pot. In our experiment DOR, ADOR and SDOR were applied to the 500 g soil at concentrations of 0.15 and 30 g/kg^{-1}. Plants were harvested after 8 weeks and measured dry weight, roots were sampled and determination of root colonization with mycorrhizal fungi as described by (Phillips and Hayman 1970), and AM colonization by determining percentage of using a method by (Giovannetti and Mosse, 1980).

The percentage of AM colonization was calculated from the following equation: Percentage of AM colonization = (Root length colonized / Root length observed) ×100.

The experiment was designed in a randomized, whole-plant design, in which two experiments were used: (1) plants in sterile soil and (2) plants grown in sterile soil inoculated with 5 g of *G. macrocarpum* inoculum. These experiments were performed with three repetitions of each treatment.

Determination of phenol content: 0.5 g of DOR, ADOR and SDO was incubated for 24 h in 10 ml of distilled water/acetone to extract phenols under orbital shaking from DOR, ADOR and SDO according to (Ribereau-Gayon 1968).

Statistical analysis: using the ANOVA procedures according (Snedecor and Cochran 1972), treatments were compared by Duncan's method.

3 Results and Discussion

Root colonization by fungi was noted in roots of wheat plants inoculated with *G. macrocarpum* in presence of 15 and 30 $g.kg^{-1}$ of DOR, ADOR or SDOR (Table 1). The percentage of AMF colonization likewise decreased. Specifically, the AM colonization of plants

at 30 g. kg^{-1} of DOR (19.7%) (Table 1). The symbiotic relationship with the mycorrhizal fungus depends on the concentration of phenolic compounds in the soil (Leadir et al., 1997). Moreover, the decrease in the phenol concentration of ADOR And SDOR by saprobe fungi is one of the factors beneficial effect of these fungi on the formation of AM colonization reached by the roots of wheat plants (Table 4).But AM colonization was higher in presence of30g.kg^{-1} of ADOR or SDOR than in presence of 30 g.kg^{-1} of DOR (Table 1),and this was indicated by (Scervino *et al.*, 2005; Sampedro *et al*; 2008) that the effect of phenols on AM fungi depends on the type of plant and fungus.

Table (1). Shoot dry weight and (AMF) colonized root of wheat plants (*Triticum aestivum* L.) inoculated or not by *G. macrocarpium* with (DOR), (ADOR) and (SDOR).

Treats.	Addition level of olive residue (g.kg^{-1})	Shoot dry weight (mg)		Root coloniza-tion rate (%)
		with AM	Without AM	
Control	0	550[b]	304[a]	52.6[b]
with DOR	15	95.8[e]	106.7[d]	34.2[d]
	30	20.5[f]	43.9[g]	19.7[f]
A DOR	15	679.5[a]	252.6[b]	65.4[a]
	30	510.8[c]	174[c]	41.6[c]
S DOR	15	502.4[c]	89.5[e]	67.8[a]
	30	156.3[d]	62.7[f]	28.4[e]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

The application of olive mill residues reduce the shoot dry weight related to that of plants grown in absence of DOR (Table 1). The phytotoxic effect of DOR was higher than that of ADOR or SDOR (Table 1). This is due to the presence of toxic substances in DOR that are soluble in water, although other fatty acids and aldehydes also have a negative effect on plant growth (Komilis et al., 2005)

Table (2). Shoot dry weight and (AMF) colonized root of wheat plants (*Triticum aestivum* L.) inoculated or not with *G. macrocarpium* in presence of (ADOR) inoculated with *Aspergillus niger* (ADORAN) and *Penicillium crustaceum* (A DORPC)

Treats.	Addition level of olive residue (g.kg^{-1})	Shoot dry weight (mg)		Root coloniz-ation rate (%)
		with AM	Withou t AM	
Control	0	211.8[b]	146.3[b]	29.7[a]
ADOR	15	276.5[a]	124.4[c]	26.8[b]
	30	188.7[c]	78.6[e]	13.4[c]
A DORAN	15	269.3[a]	187.6[a]	34.7[a]
	30	127.8[e]	89.7[d]	26.3[b]
A DORPC	15	217.8[b]	179.5[a]	36.2[a]
	30	139.7[d]	180.6[a]	24.7[b]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

The dry weight of the buds was lower in the presence of SDOR than in ADOR, especially clearly in inoculated with *G. macrocarpum* as Compare with non-AM inoculated soil (Table 1). The results also indicated in previous studies that the detrimental effect of applying 25 g/kg of DOR in soil on the root system and dry weight of tomatoes and alfalfa (Martin *et al.*, 2002). The application of 15 g.kg–1 of ADOR to soil inoculated with *G. macrocarpum* increased the shoot dry weight to that of plants grown in control without any residues (Table 1).

Table (3). Shoot dry weight and (AMF) colonized root of wheat plants (*Triticum aestivum* L.) inoculated or not with *G. macrocarpium* in presence of (SDORAN) inoculated with *Aspergillus niger* and *P. crustaceum* (SDORPC).

Treats.	Addition level of olive residue (g.kg^{-1})	Shoot dry weight (mg)		Root coloniz-ation rate (%)
		with AM	Without AM	
Control	0	501.6[a]	309.7[b]	44.6[b]
SDOR	15	214.8[d]	112.3[e]	38.4[c]
	30	118.5[f]	58.7[g]	29.7[d]
SDORAN	15	312.2[b]	189.4[c]	52.4[a]
	30	188.6[e]	77.8[f]	49.7[a]
SDORPC	15	295.4[c]	345.3[a]	20.8[e]
	30	189.6[e]	163.2[d]	18.6[e]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

AM fungi seems to facilitate the action or transfer of toxic substances to wheat plants, increasing the sensitivity of plant to the toxicity of ADOR and SDOR, Opposite trend was noticed for the shoot dry weight with DOR. The shoot dry weight of plants grown in presence of 15 g.kg^{-1} of ADOR incubated with *Aspergillus niger* was higher than that of plants grown in absence of ADOR (Table 2). However, all doses of ADOR incubated whether *Aspergillus niger* or with *Penicillium crustaceum* increased the shoot dry weight relative to that of plants grown in absence of saprophytic fungi. In the present study both tested saprophytic fungi eliminated similar quantity of phenols from both ADOR and SDOR.

Table 4. Phenol content (g.kg^{-1}) of (ADOR) and (SDOR) incubated with *A. niger and P. crustaceum*

Treatments	ADOR	SDOR
Control	22.6[a]	17.5[a]
Aspergillus niger	9.3[b]	7.1[b]
P. crustaceum	8.7[b]	6.8[b]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

This study has revealed that saprobe fungi decreased the toxicity of ADOR and SDOR on wheat plants of incubation. Phenols appear to be the main substances responsible for the harmful effect of DOR on the dry

weight and the level of AM mycorrhization of plants, hence the valuable effect of saprobe fungi on both parameters seems to be related to the reduction that these fungi cause in the phenol concentration of DOR (Fiestas Ros de Ursinos ,1986). In the present study, the fact that The phenol content of ADOR was reduced by *Aspergillus niger* and *Penicillium crustaceum* 9.3 and 8.7g.kg–1, respectively and 7.1 and 6.8 g.kg–1, respectively with SDOR (Table 4). Incubation of ADOR and SDOR with saprobe fungi decreased its phenol content, these result are agreement with different plants were observed by (Aranda *et al.*, 2009) who found that the saprophytic fungi *Trametes versicolor* and *Pycnoporus cinnabarinus*.

Table (5). N and P content (g/kg^{-1}) of DOR after incubation with saprobe fungi for 8weeks.

Treatments	N	P
Control	14.8[a]	2.2[a]
Aspergillus niger	16.3[a]	2.4[a]
P. crustaceum	15.2[a]	2.7[a]

Similar letters in the same column are not significantly different at 0.05 according to Duncan's multiple range test.

After 8 weeks of incubation N and P content of DOR did not differ significantly from those of the control (Table 5). However, saprobe fungi did not decrease the N and P content of DOR, these results did not explain the decrease of the toxicity of DOR incubated with saprobe fungi for AM fungal colonized plants.

The application of ADOR and SDOR incubated with the saprophytic fungi during eight weeks increased the shoot dry weight of Wheat Plant inoculated with *G. macrocarpum* in comparison to AM colonized plants cultivated in absence of DOR, and, in conclusion, this olive mill residues allows the possibility to use as an organic fertilizer.

Conflict of Interest: The authors declare that there are no conflicts of interest

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