Conversion of the Solid Waste of Pressing Olives to Soil Fertilizers Using the Bio-control fungus (*Gliocladium roseum*) and the Edible Mushroom (*Pleurotus ostreatus*)

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Abstract

There are approximately 14 million olive trees in 15000 farms in Aljouf governorate, Saudi Arabia, and it is expected to increase the number of olive trees for up to 20 million trees in the next few years. The outcome from the squeeze and compress olives generate enormous amount of olive residues that are not utilized at all, and cause significant environmental problem of pollution. The objective of this study was to study the possibility of using olive pressing cake (OPC) for production of basidiomes of the mushroom of *Pleurotus ostreatus* to reduce inhibitors phenols in OPC. The possibility of using *Gliocladium roseum* (important in biological control to many fungal plant diseases) to grow on OPC, and convert it into a soil for planting tomato seeds and fertilizers is one of the present aims. *Pleurotus ostreatus* and *Gliocladium roseum* have proven to be particularly efficient at decomposing the phenolic substances which are mainly held responsible for retarding tomato seeds germination. The distinct properties of *Pleurotus ostreatus* and *Gliocladium roseum* could be exploited for the incorporated management at agricultural byproducts.

Key words: *Gliocladium roseum*; Olive; Olive pressing cake (OPC); Phenolics; *Pleurotus ostreatus*.

Introduction

Olive cultivation in Aljouf area of the northern part of Saudi Arabia, represents a modern agricultural boom in that region of the Arabian peninsula. Olive cultivation in Aljouf area began in the last nineties of the last century because of the suitable environment for growing of such plant. Subsequently, the number of olive plants reached more than 14 million fruitful tress (El-Khateeb *et al*., 2014).

The count and form of production of olives often, used for the production of oil which represents an enormous amount of usefulness on the level of agricultural and commercial values. The outcome from the squeeze and compress olives generate enormous amount of olive residues that are not utilized at all, and cause significant environmental problem of pollution (Roberta and Giuseppe, 2012; Maria *et al*., 2013). The process of squeezing olives produces polluted waters (Kotsou *et al*., 2004) and dry remains cake (Olive Press Cake, OPC) (Sampedro *et al*., 2004) of a big environmental problem because of their high organic load. Soil amendment with OPC increases the soil organic matter (OM) and the concentration of inorganic elements essential for plant growth (Paredes *et al*., 1999), however, addition of OPC is also known for its phytotoxic properities (Martin *et al*., 2002). Phytotoxic effects are likely related to the high content of phenolic compounds (Sampedro *et al*., 2004). Fungi belonging to the mushroom fungus *Pleurotus* possess a very efficient ability to grow on OPC and degrade lignocellulose and decompose lignin yield products of high nutritional value. It is reported that; at the first stages of mycelium growth, biomass was developed and concentration of phenolics decreased,
subsequently (Tomati et al., 1991). Additionally, some terrestrial fungi reported efficient ability to decrease phenolics (Aranda et al., 2006).

Liquid wastes, generated from pressing olives, can be eliminated easier than dry wastes via sanitation. Dry wastes have a great effect on the surrounding environments because of difficult disposal and its high toxicity to plant cultivation (Roig et al., 2006). OPC contain polyphenols, sugars, tannins, proteins, polysaccharides, lipids minerals and ash (Hafidi et al., 2005). The concentration of phenols reaches up to 10 g/L (Borja et al., 1992), which contributes to a high plant toxicity and antibacterial activity.

An idea grew out of here for using of OPC as a medium for the growth of the useful fungus of Gliocladium roseum and the mushroom of Pleurotus ostreatus. Those beneficial fungi are usually used for the purpose of biological control and production of edible protein, respectively.

The objective of this study was to study the possibility of using OPC for production of basidiomes of the mushroom of P. ostreatus to reduce inhibitors phenols in OPC. The possibility of using G. roseum (important in biological control to many fungal plant diseases) to grow on OPC, and convert it into a soil fertilizer is one of the present aims.

Material and methods

The organisms used here were G. roseum (MU121, Minia University, Egypt) P. ostreatus (MUAGRI 1102, Egypt, this strain was obtained as ready spawn grown on sorghum grains, afterward the spawn was prepared from a pure culture of the strain which was isolated on Malt Extract Agar media). OPC was obtained from a local olive oil producing press (Sakaka, Aljouf, Saudi Arabia) and used immediately (after autoclaving) for the preparation of the substrates.

Cultivation of P. ostreatus

Experiments were done in a glass house and two treatments were used including the control treatment with five replicates. Data were statistically analyzed and treatments were compared using Duncan multiple range test. The control growth medium was composed of 95% vermiculite and 5% gypsum. The conducted treatments was prepared on a dry weight basis, of 95% olive press cake and 5% gypsum. The olive cake that was used in this study was the olive press cake deriving directly from the olive – oil mill.

Sterilization of the substrate medium of P. ostreatus

Each treatment was mixed with the gypsum and placed in a cloth bag then autoclaved for two successive days at 121°C for 1 hour and left three days prior usage. The glasshouse was disinfected using NaOCl solution (commercial, Clorox). The substrate medium was then placed in big plastic bags in order to allow the manipulation of mixing the spawn with the substrate by shaking manually, afterward it was inoculated with P. ostreatus spawn at a rate of 5% on the dry weight basis. Bags were then tied at the top by a nylon thread and punctured by a sterilized fork.

Adjustment of culture circumstances

The substrates were positioned inside plastic bags and inoculated with the spawn at a rate of 5 % of their dry weight and were incubated at 20-25°C, under humid conditions between

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80-95% (R.H.) in the dark during the first days up to the appearance of the white fungal hyphae. Afterward, the colonized substrates were subjected to a cold shock in the refrigerator (5°C) for 2 days to encourage emergence of the first flush. During fruiting period, ventilation was very important, so upper parts of the bags were opened and air was allowed to enter. Temperature must be around 25°C on an average, and relative humidity adjusted between 80–90% by watering the bags twice daily, and putting vast water containers on the floor.

**Harvesting and Measurement of Parameters**

Fruiting structures (Basidiocarps) of *P. ostreatus* were harvested when the pilei were fully mature and before they started to curl up. Adhered substrate residues on stipes were detached before weighing. After harvesting mushroom incubation periods, average weight of individual basidiomata determined as quotient of the total weight of fresh mushrooms harvested by their total number, the average yield for each treatment and diameter of the pilei and average diameter were measured.

**Culturing *G. roseum* on OPC**

Olive press cake (OPC) was collected from an olive mill (Aljouba, Sakaka Aljouf, Saudi Arabia). *G. roseum* was obtained from Department of Botany and Microbiology, Faculty of Science, Minia University, Egypt and transferred to tubes of potato dextrose agar (PDA). Agar discs containing the growth of the fungus were used for OPC inoculation and subculturing. The incubation process was carried out in 1 L Erlenmeyer flasks, each containing 200 g of OPC and 150 ml distilled water and sterilized twice, inoculated or not with *G. roseum*. Static incubation was performed at 28°C for 1-4 weeks.

Phenol content of OPC before and after growth of *G. roseum* and *P. ostreatus*

Total phenolic contents of OPC were analyzed according to Ribereau-Gayon (1968), using tannic acid as a standard, and expressed as grams per kilogram of OPC. Chemical analyses were performed for treatments before and after growth of *G. roseum* and *P. ostreatus* for 1-4 weeks.

Germination of tomato seeds on OPC previously cultured with *G. roseum* and *P. ostreatus*

The OPC that previously cultured with each of *G. roseum* and *P. ostreatus* was tested for their suitability for growing seeds of tomato (*Lycopersicum esculentum* L) was then added to plastic pots (each 100 g). Fifty tomato seeds were seeded on the surface of each pot containing tested OPC and inoculated pots were incubated in a growth cabinet at 25°C with 12 h photoperiod (91 µmol m⁻² s⁻¹). Emergence seedlings were counted through 5-20 days.

**Statistical analysis**

Data was assessed using one-way analysis of variance (ANOVA) through Minitab statistical software (version 12) unless elsewhere indicated.
Results

Effect of different rates of olive cake on growth parameters of *P. ostreatus* including incubation period, yield, average weight and average diameter of pilei

Table (1) shows that days required for incubation period of olive cake substrate were about 13 days compared with the control treatment which needed 5 extra days. The highest yield was recorded in control but in OPC it showed significant differences between them and the yield fell by almost half. The average weight was 25.26 (g/cap) in control whereas it decreased to 17.99 (g/cap) in case of OPC. There were no significant differences between the control and OPC in their average pileus diameter.

Table (1): Effect of adding of olive press cake (OPC) on incubation period, yield, average weight and average diameter of *P. ostreatus*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Incubation period (days)</th>
<th>Yield (g/0.5 kg)</th>
<th>Average weight (g/cap)</th>
<th>Average diameter (cm/cap)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18a (1)</td>
<td>588.69a</td>
<td>25.26a</td>
<td>8.31a</td>
</tr>
<tr>
<td>OPC</td>
<td>13b</td>
<td>270.16c</td>
<td>17.99c</td>
<td>7.28a</td>
</tr>
</tbody>
</table>

(1) Means within each column followed by the same letter were not significantly different according to Duncan’s Multiple range test (P= 0.05).

Culturing *G. roseum* on OPC

*G. roseum* showed excellent growth on OPC, starting from the first week and appeared more intense growth between the second and the third week of incubation (Fig. 1).

![Fig. 1. Inoculation of OPC with *G. roseum* (a), and growth of the fungus on OPC after 2 weeks at 28ºC in the dark.](image)
Phenol content of OPC before and after growth of *G. roseum* and *P. ostreatus*.
The amount of total phenols strongly decreased when *G. roseum* and *P. ostreatus* grew on OPC starting from the first week and reached a peak decrease after 4 weeks of the growth (Fig. 2).

![Fig. 2](Image)

**Fig. 2** Phenol content (g/kg OPC) of OPC treated with each of *G. roseum* and *P. ostreatus* 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 < 0.01, *** = very significant at p < 0.001.

Germination of tomato seeds on OPC previously cultured with *G. roseum* and *P. ostreatus*
The application of OPC previously incubated with either of *G. roseum* or *P. ostreatus* during 1-4 weeks increased the emergency of tomato seedling compared with tomato grown in crude OPC.

Table 2. Emergency of 50 tomato (*Lycopersicum esculentum* L.) seeds inoculated or not with *G. roseum* and *P. ostreatus* in presence or absence of olive press cake (OPC) incubated at different times.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time of incubation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>39*</td>
</tr>
<tr>
<td>OPC</td>
<td>0</td>
</tr>
<tr>
<td>OPC previously incubated with <em>G. roseum</em> for 1 week</td>
<td>29c</td>
</tr>
<tr>
<td>OPC previously incubated with <em>G. roseum</em> for 2 week</td>
<td>38c</td>
</tr>
<tr>
<td>OPC previously incubated with <em>G. roseum</em> for 3 week</td>
<td>37c</td>
</tr>
<tr>
<td>OPC previously incubated with <em>G. roseum</em> for 4 week</td>
<td>39c</td>
</tr>
<tr>
<td>OPC previously incubated with <em>P. ostreatus</em> for 1 week</td>
<td>22c</td>
</tr>
<tr>
<td>OPC previously incubated with <em>P. ostreatus</em> for 2 week</td>
<td>32c</td>
</tr>
<tr>
<td>OPC previously incubated with <em>P. ostreatus</em> for 3 week</td>
<td>35c</td>
</tr>
<tr>
<td>OPC previously incubated with <em>P. ostreatus</em> for 4 week</td>
<td>35c</td>
</tr>
</tbody>
</table>

*Germination of tomato seeds from a total of 50 ones.*
Means within each column followed by the same letter were not significantly different (compared with the control in OPC) according to Duncan’s Multiple range test ($P= 0.05$).

Discussion

Our results show that the olive mill dry residues have phytotoxic properties. Using olive press cake (OPC) inhibited germination of tomato plants. Many Phenols are considered one of the main responsible of the toxicant effect of wastes on plant health (Wang et al., 2002). It is possible that the phenolic content of OPC could be the responsible of their phytotoxicity. Most of phenolic acids began to manifest their phytotoxicity at higher concentrations (Wang et al., 1967). In this study, OPC showed 40 g Kg$^{-1}$ of total phenolic compounds, addition of OPC to the soil resulting in impaired growth of a lot of plant seeds and hinder the growth of a lot of growing plants.

It is known that some fungi can grow on media containing large amounts of phenols. Those organisms can be used in operations suction of phenols and degrade this harmful chemicals to the plant (Moreno et al., 1990).

Several species of Aspergillus were efficient at decomposing phenolics in OPC. Strains of A. niger were appeared to flourish and produce dense mycelia on OPC (Hamdi and Ellouz, 1992).

One of the ways in which microorganisms use to remove toxic phenols have been attributed to their ability to metabolise phenolics (Wang et al., 2002). Previous results indicated that Coriolopsis rigida decrease phenolics of dry olive residues (Sampedro et al.,...
The same fungus of *C. rigida* increased dry weight of tomato plants (Sampedro *et al*., 2004b). Application of OPC to soil without incubation with *C. rigida* did not repressed the shoot and root dry weight of tomato inoculated with *Glomus deserticola* compared with the noninoculated controls (Ocampo, 1993). Usage of basidiomycetes was lately adopted for decreasing phenolics in OPC. Fungi belonging to white-rots were showed efficient metabolization of phenolics in OPC (Hammel, 1989). Using the edible mushroom of *Pleurotus* for growing in OPC and reducing total phenols has been carried out. Sanjust *et al.* (1991) proved the development of normal appearing basidiomata on Perlite-OPC cultured with *P. ostreatus* and *Pleuratus eryngii*. They further postulated that residual toxicity of OPC was significantly reduced.

Results in Fig. 2 consistent with the previous studies on the possibility of culturing *G. roseum* and *P. ostreatus* on OPC derived from an olive press mill, located in Sakaka city, Aljouf, Saudi Arabia. Both fungi were able to significant reduce the level of total phenols from more than 38 g / kg OPC to about 10 g total phenols/kg OPC through 1-4 weeks. *G. roseum* showed excellent growth on OPC, starting from the first week and appeared more intense growth between the second and the third week of incubation (Fig. 1). Results also showed a good growth of *P. ostreatus* on OPC despite the lack of yield intensity compared to the control.

As a result, tomato seeds germinated well on OPC that have already been cultured with *G. roseum* and *P. ostreatus*.

It is worth mentioning that *G. roseum* has a biocontrol activity against many fungal plant diseases. Therefore, using this fungus in bioremediation of OPC has two benefits: first, remove toxicity of OPC due to reduce phenols; second, growth of *G. roseum* in OPC act as inocula of this bioagent to the soil. Furthermore, cultivation of *P. ostreatus* on OPC has also two benefits: first, production of edible mushroom and the second is to use the leftover media of mushroom cultivation for the production of organic soil.

**Conclusion**

Fungi of *G. roseum* and *P. ostreatus* can be used in transforming OPC after its incubation for 2-4 weeks, therefore, germination of tomato seeds increased significantly compared with seeds on the crude (without growing fungi on it) OPC.

These findings shed light on the possibility of conversion of the solid waste of pressing olives to soil fertilizers using the bio-control fungus (*Gliocladium roseum*) and the edible mushroom (*Pleurotus ostreatus*). Further studies on using other beneficial fungi to optimize transformation of OPC to organic fertilizers, should be done.

**Acknowledgement**

This work was supported by the grant (project # 140/33) from the University Agency for Graduate Studies and Scientific Research, Aljouf University, Saudi Arabia. The financial support is gratefully acknowledged.
References


