Chapter 2

Olive-Pressed Solid Residues as a Medium for Growing Mushrooms and Increasing Soil Fertility

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Additional information is available at the end of the chapter

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Abstract

Organic fertilizer is the core of organic farming, which represents the most important way to provide crops and agricultural products that are safe and free of any chemical components and pesticides. From this point of view, the purpose of this study is to provide a source of organic fertilizers which was formerly an environmental problem. The northwestern region of Saudi Arabia is flourishing with olive production, leaving huge amounts of residues called olive press cake (OPC). These wastes are a major environmental pollution despite their good content of carbohydrates, protein, oil and cellulose alongside phenols and lignin. We tested the cultivation of *Gliocladium roseum, Pythium oligandrum* and *Trichoderma harzianum* and the mushroom *Pleurotus ostreatus* on OPC in order to reduce the high percentage of phenols that impede the germination of some plant seeds. *Gliocladium roseum, Pythium oligandrum* and *Pleurotus ostreatus* were able to reduce the percentage of phenols to more than 40% and thus support germination of seeds of *Eruca sativa*. This study gave than one benefit: firstly, reducing phenols that impede the germination of seeds. Secondly, *Gliocladium roseum* and *Pythium oligandrum* work against some plant diseases and also produce plant-like hormones that increase growth of plants.

Keywords: biofertilizer, *Eruca sativa*, *Gliocladium roseum*, *Pythium oligandrum*, *Trichoderma harzianum* and *Pleurotus ostreatus*, northwestern region of Saudi Arabia, olive press cake

1. Introduction

Olive trees are widespread in the Mediterranean countries, where the climate is in line with the pattern and physiology of the growth of these trees. There is almost no Mediterranean

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Figure 1. Mediterranean countries distributed in Africa, Asia, and Europe.

country without thousands of hectares of olive trees, where the majority of people thrive on their products, fruits, and oil [1, 2]. Olive is one of the most important horticultural crops, both for direct consumption of fruit and for oil extraction which has nutritional value and a high historical reputation. The scientific name of the olive plant is *Olea europaea* L. and follows the family of Oleaceae. Olive trees grow wild in many parts of the world, especially in southern France, Syria, Palestine, Jordan, Morocco, Algeria, and India and also grow wildly in the southwest of Saudi Arabia. Olive trees are durable and can live for centuries. They are strong, energetic, and resistant to various conditions, including water shortage. The tallest tree reaches 15 m and the average height is 5–8 m. Leaves are spear shape, covered with a thick cutin and some disc hairs. Trees bear two types of flowers: bisexual and male flowers, according to the variety. Pollination is done by wind, and the fruit is drupe, rounded, or oval, depending on the variety and turns to black color when matured. Olive trees are subtropical. During winter, most varieties need low temperatures until flowering buds are formed. Trees thrive in the spring. If temperatures are high in winter, flowering is greatly reduced. Olive trees can withstand summer temperatures up to 48°C. Small olive trees need to be irrigated on a regular basis during the first 3 years of planting. After that, they can tolerate very little irrigation. Olive trees are cultivated in many areas in Spain, Tunisia, and Libya, relying on rain only without the need for artificial irrigation when rainfall is up to 300 mm per year. Small olive trees also need little fertilization but respond to good fertilization later, where it is necessary to add organic and chemical fertilizers, especially nitrogen, potash, and phosphates (www.fao.org).

Most of the world's olive production is concentrated in Mediterranean countries, as well as some countries outside the Mediterranean basin such as Peru, Australia, Chile, Iran, Albania, Argentina, USA, and Saudi Arabia (**Figure 1**). Since 2010, there have been significant variations in production from year to year until 2018 (www.fao.org). This may be due to:

- Development of new varieties characterized by their high production;
- Unusual temperature change
- Recent climate changes on earth
- Political problems and wars in some countries

It should also be noted that the level of global consumption of olive products, especially olive oil, has increased steadily in parallel with increasing awareness of the strong role of olive products in human health as well as increasing world population. For this reason, global demand for olive products in general and olive oil in particular has increased. All modern methods have been used to increase production and increase the efficiency of olive squeeze operations (**Figure 2**).

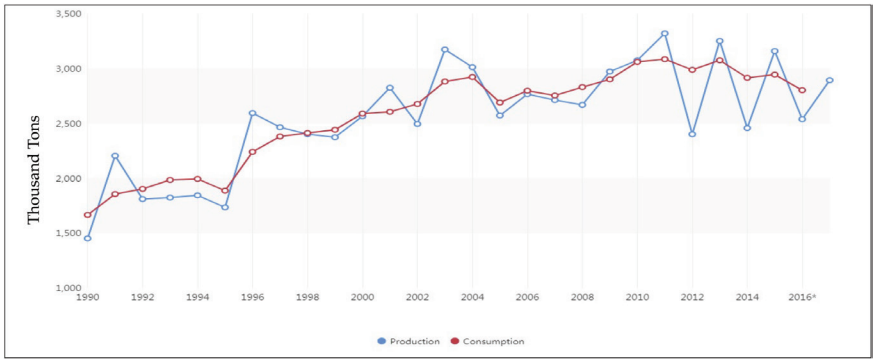


Figure 2. World olive oil production and consumption, 2016/2017, in years (www.fao.org).

Ranking of countries in terms of olive production, as shown in **Table 1**:

Country	**2017/2018 (average)**
Greece	260–280 (270)
Italy	300–318 (309)
Spain	1.100–1.250 (1.175)
Portugal	90–100 (95)
Morocco	100–110 (105)
Tunisia	250–270 (260)
Turkey	230–250 (240)
Syria*	100–150 (125)
Total	**2.430–2.730 (2.580)**

Production (1.000 tm). *Affected by war.

Table 1. Latest production in the eight leading olive oil producers that make up to 90% of the world's olive oil production (www.fao.org).

2. A research on the presence conversion of the solid waste of pressing olives to soil fertilizers using some useful fungi [part of this work has been reported elsewhere]:

It is important to outline the stages of oil production from olive. Olive fruits must be purified from all impurities, either by manual method or by special sieves, and then washed with hot water to eliminate the effect of some substances on taste and quality of olive oil [1, 2]. Processing olive fruits for the mechanical stages in the production line are as follows:

- After washing the fruits well, they are ground by different crushing processes. This is the first process designed to compress the fruits and separate largest amount of liquids in them.
- This process is carried out accurately and at suitable temperatures, until the oil is assembled together to facilitate separation from other components, especially water, where the temperature directed plays an important role in affecting the viscosity of the oil. Temperature is about 30°C. The aim is not to affect the viscosity of the water but to prevent the mixing of water with oil and influence its density, as well as to protect the oil material from being affected by temperature change of its physical properties such as changing its color to red, or its acidity.
- Separation of the components:

  The previous phase contributes to some degree in the separation of oil molecules from milled materials, but they are without the end filter work because some oil particles are stuck in the mixture. They need a more precise separation process such as process of separation of liquids from solids, separation of oil from liquid materials, and the process of refining it more than once depending on the value of its standard density. This process is affected by a number of factors:

  1. Density: It plays an important role in separating oil from other liquids. This depends on the speed at which the material is removed due to the force, resulting from the rotational movement of the center of motion.
  2. Size: The small size of the oil molecules increases the difficulty of collecting and removing them from the mix.
  3. Viscosity: Differences in the degree of viscosity between components of the mixture contribute to the speed and ease of separating oil from rest of the materials, as well as temperature that was previously mentioned.
- After the oil is separated by centrifugation, solid and liquid residues are discarded, and the oil is finally obtained.

What concerns us here is the solid remains that are the residues of grinding seeds and cellulosic cell walls and organelles of olive fruit cells, as well. This mixture is called olive press cake (OPC) (**Figure 3**).

The huge quantities of waste produced from olive mills have the following properties:

- These residues contain cellulose, protein, carbohydrate, and oil, and they represent a good medium for use as soil fertilizers.

- High content of phenols may cause inhibitors of plant seed germination.
- High content of nutrients in these residues may be an appropriate environment for hordes of insects, spiders, bacteria, and fungi, and some of them may be harmful.

Therefore, we have conducted studies on the abovementioned topics, focusing on the use of these residues as organic fertilizers that can be used to improve mechanical, natural, chemical, and biological soil properties.

The importance of organic agriculture in many areas is of interest to farmers, consumers, society, and the environment. Farmers benefit from the adoption of organic means to increase the production and quality of their crops, due to improved soil fertility and productivity over a long term. Organic agriculture also prohibits the use of insecticides, fungicides, herbicides, nematocides, and other chemicals, reducing dependence on off-farm inputs, thereby reducing production costs and improving health and vitality of animals and plants, while preserving biological and environmental diversity. For the consumer, it increases their confidence in high-quality organic agricultural products, ensuring that they are free of pesticide residues, chemical fertilizers, and genetically modified organisms. All this makes the community healthy, reduces the risk of soil and water contamination with chemical residues, and promotes the sustainability of natural resources and the ecosystem. For soil fertility, there is no accepted concept that includes or is known specifically and clearly. Some soil scientists have pointed out that soil fertility means "the state of nutrients in the soil, in terms of quantity, availability, equilibrium,



Figure 3. A, B: a large pile of solid olive residues (olive press cake, OPC) from olive presses in Sakaka, Jouf, Saudi Arabia. C: amount of OPC in a pail in preparation for some laboratory experiments.

and other nutrients." According to this definition, it has a well-balanced source of nutrients in a soft form to meet its needs during various stages of its growth. Soil may contain necessary essential nutrients in a readily available form. However, their production capacity is low, or unproductive, due to the negative impact of physical, chemical, and biological soil properties. In other words, soil fertility, whether physical or chemical, refers to "the ability of the soil to supply the plant with nutrients." In these two ideas, soil fertility is only an estimate, since the biological effects and their relation to certain aquatic or hydrothermal factors are not considered important, making this interpretation non-exhaustive, although it is used by most soil fertility researchers. Soil fertility is also indicated by its ability to meet the needs of the entire crop of nutrients and water. Soil fertility is sometimes defined as an expression of the state of the nutrient soil, that is, the amount of nutrients it contains in a prepared, adequate, and balanced form for optimal production of a particular crop. In general, soil fertility is a cumulative estimate that can deteriorate as a result of continuous agricultural exploitation and can be developed, maintained, and sustained through good fertilization programs and appropriate soil management.

2.1. Biofertilizers

Modern scientific progress has allowed many processes to take place in nature, prompting scientists to develop new technologies and introduce them into agriculture to protect the environment and increase crop productivity. Using of microorganisms in agriculture was proven to take advantage in processing nutrients needed by the plant in its growth and productivity, and in increasing its biological ability to control pathogens. Biofertilizers can be used to improve soil properties when applying organic farming systems as a natural catalyst for plant growth and productivity. Many studies have shown that some added microorganisms produce antibiotics to protect themselves, killing many pathogenic fungi. At the same time, these microorganisms secrete stimulant-like substances such as auxins to increase seed germination rate as well as increasing root and vegetative growth of the plant. In addition, these stimulants increase the surface area of the root hairs, which contributes to increase the ability of the plant to absorb water, salts, and nutrients. For the previous mentioned reasons, these microorganisms contribute to improving physical and chemical properties of agricultural soils and thus their fertility and productivity. Therefore, some countries have been interested in settling organic agriculture in many parts of the country. This is done by converting organic waste and agricultural products to organic fertilizers, especially in countries characterized by drought due to lack of rainfall, scarcity of vegetation, and high temperatures. In desert countries, lack of intensive cultivation methods resulted in a decrease in biofertilizers and low organic matter, resulting in reduced soil fertility, http://www.fao.org/organicag/oa-faq/oa-faq1/ar.

From this point of view, one of the main objectives of this study is the use of olive press cake (OPC) from many olive mills spread in Jouf region in the northern part of Saudi Arabia, as biofertilizers in organic agriculture. The number of fruitful olive trees in such area was estimated to be more than 15,000,000 trees, produced more than 12,000,000 l of olive oil.

The agricultural land of the city of Sakaka and its suburbs, belonging to the Jouf region, of the northern part of Saudi Arabia is characterized by the lack of suitable physical, chemical, and biological properties. Therefore, we have considered using enormous amount of residual OPC in raising efficiency of agricultural soil through a number of successive researches in this field.

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As a result of the huge quantities of the remnants of the process of refining olives, large quantities of waste are formed with other pollutants from the wastewater of these processes [2, 3]. These pollutants are of big environmental problem because of their high organic load [4]. The addition of OPC to agricultural soil increases organic matter and inorganic elements essential for plant growth [5]. By contrast, the application of OPC to the soil causes phytotoxic properties due to the high content of phenolic compounds [4, 6, 7]. Generally, the mushroom fungus (*Pleurotus*) can grow well on organic residues containing lignin and lignocellulose, since these fungi are able to analyze these substances and produce simpler, more nutritious residues, and more benefits to plants. Previous studies indicate that the first stage of mycelial growth of the mushroom and some terrestrial fungi is to be biomass, followed by a decrease in the concentration of harmful phenolics, which turns waste into organic residues enriched and useful for agricultural soil [8, 9].

Analysis of components of OPC is found to contain ash, lipids, minerals, polyphenols, polysaccharides, proteins, sugars, and tannins [10]. The concentration of phenolic compounds reaches up to 10 g/L [11], which causes high plant toxicity and antibacterial properties.

We have benefited from these data that we designed researches based on the use of certain fungi in the withdrawal of high phenols of OPC and then converted it into organic fertilizers added to agricultural soil. Useful fungus of *Gliocladium roseum*, *Pythium oligandrum* and *Trichoderma harzianum,* and the mushroom of *Pleurotus ostreatus* were used in this respect. It is well known and noted through many previous studies that *G. roseum*, *P. oligandrum,* and *T. harzianum* have a long history of biological control of many fungal plant diseases [10]. The mushroom of *P. ostreatus* mushroom is also known for its high nutritional value and a good source of protein for many people. Therefore, the use of *G. roseum*, *P. oligandrum,* and *T. harzianum* has more than one benefit. The first is the withdrawal of the high concentration of phenolic materials from OPC to be suitable for agriculture. The second that these fungi are important in the biological control constitutes a wonderful medium to exist within these organic fertilizers. It is worth mentioning that *G. roseum*, *P. oligandrum,* and *T. harzianum* have the ability to secrete substances similar to plant hormones (auxins) that cause increased vegetative growth and productivity of plant crops [12].

The overall aim of this study was to use *P. ostreatus* mushrooms as well as *G. roseum*, *P. oligandrum,* and *T. harzianum* to grow on OPC to benefit from the productivity of mushrooms and make it suitable as a biofertilizer.

2.2. Materials and methods

G. roseum (JU 121, Jouf University, Saudi Arabia), *P. oligandrum* (JU 221, Jouf University, Saudi Arabia), and *T. Harzianum* (JU 321, Jouf University, Saudi Arabia) were isolated from 25 agriculture fields distributed in Khoaa village, Sakaka (29° 48′ 6′ N, 40° 26′ 27″), Jouf Governorate, located in the northern part of Saudi Arabia [1]. *P. ostreatus* (MUAGRI 1102, Egypt) fungus was kindly obtained from the Ministry of Agriculture, Egypt, as a ready spawn grown on grains of sorghum; afterward the spawn prepared by subculturing the fungus on the medium of Malt Extract Agar) was used (**Figure 4**).

OPC was obtained from an olive mill located in Sakaka city, Jouf, Saudi Arabia, and used spontaneously after sterilization by autoclaving.



Figure 4. Spawn of mushroom of *P. ostreatus* grown on grains of sorghum.

2.3. Mushroom (*P. ostreatus*) cultivation

Experiments were performed in a glass house, and two treatments were used (control + five replicates). Subsequently, results were statistically arranged and all treatments were compared using Duncan Multiple Range test. Ninety-five percent vermiculite and five percent gypsum were the only components of the control. Treatments were prepared as 95% olive press cake and 5% gypsum (dry weight).

2.4. Substrate medium sterilization for cultivation of *P. ostreatus*

Gypsum was added to each treatment and mixed thoroughly and then placed in a cloth bag. Autoclaving was done for two successive days at 121°C for 1 h and left 3 days before use. The glasshouse was disinfected using sodium hypochlorite. The medium was re-placed in big plastic bags in order to allow the manipulation of mixing the spawn with the substrate by thoroughly shaking. Subsequently, medium was inoculated with 5% (dry weight) spawn of *P. ostreatus*. The bags were sealed tightly with a strong thread and punctured with a sterile metal screwdriver.

2.5. Adjustment of culture circumstances

Substrates were incubated at 20–25°C, under 80–95% (R.H.) humidity in the dark during starting days until the emergence of white mycelial growth. The colonized substrates were subjected to a cold shock at 5°C for 48 h to stimulate the emergence of first flush. It is worth mentioning that ventilation was very important during the fruiting period; therefore, the upper side of the bags was opened. Precautions must be taken for the temperature to be around 25°C and the relative humidity was between 80 and 90% by watering the bags twice daily and placing vast water containers on the floor.

2.6. Harvesting mushroom crop

Basidiocarps (fruiting bodies) of *P. ostreatus* had been collected when pilei were matured and just before started to curl up. Residues attached on stipes of mushrooms were gently disposed of by wiping them with a tissue paper before weighing. After harvesting mushroom, the average weight of singular basidiomata calculated as the quotient of the total weight of fresh bodies collected by their total number, the average production for each parameter and diameter of the pilei, and the average diameter were measured.

2.7. Culturing *G. roseum*, *P. oligandrum,* and *T. harzianum* on OPC

Olive press cake (OPC) was collected from an olive mill (Aljouba, Sakaka city, Jouf, Saudi Arabia). Fungi were developed and preserved in potato dextrose agar (PDA) (part of this work has been reported elsewhere [1]). Potato dextrose agar discs containing fungal growth were used for OPC inoculation and subculturing, as well. Incubation procedure was performed in 1-L Erlenmeyer flasks, each containing 200 g of OPC and 150 ml distilled water at 28°C for a time course of 1–4 weeks (**Figure 5**).

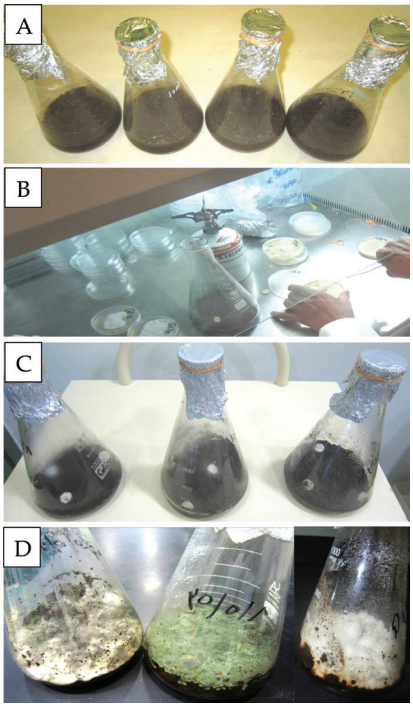


Figure 5. A. OPC and water in flasks after sterilization in the autoclave. B. Flasks during inoculation by fungi. C. Flasks after inoculation. D. Fungal growth after incubation for 2 weeks at 28°C in the dark.

2.8. Effect of growth of *G. roseum*, *P. oligandrum*, *T. harzianum,* and *P. ostreatus* on the amount of phenols in OPC

The total phenolic contents of OPC were estimated according to the method of [13], via tannic acid as a standard, and expressed as grams per kilogram of OPC. Analyses were done for each treatment before and after growth of tested four fungi within 1–4 weeks.

2.9. Testing the ability of *Eruca sativa* seeds to grow in the waste before and after the growth of fungi

The OPC before and after culturing with each of the tested four fungi was analyzed for their appropriateness for growing seeds of *E. sativa*. Quantities of every 100 g of OPC were added

to plastic pots. Fifty *E. sativa* seeds were distributed on the surface of each pot containing tested OPC. Pots were incubated in an illuminated growth cabinet at 25°C with 12 h photoperiod (91 μmol m^{-2} s^{-1}). Emergence seedlings were counted in the course of 5–20 days.

2.10. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) through Minitab statistical software (version 12) unless elsewhere mentioned.

3. Results

3.1. The effect of different amounts of OPC on growth parameters (incubation period, yield, average weight, and average diameter of pilei) of *P. ostreatus*

Table 2 shows that that period required for incubation of OPC substrate was around 13 days compared with the control treatment which needed 5 extra days. Highest mushroom production was recorded in control, but in OPC it showed significant differences between them and the yield fell by almost half. In control, the average weight was 25.26 (g/cap), whereas it decreased to 17.99 (g/cap) in OPC. There were no significant differences between control treatment and OPC in their average diameter of fungal pileus.

3.2. Culturing *G. roseum*, *P. oligandrum, T. harzianum,* and *P. ostreatus* on OPC

G. roseum, *P. oligandrum, T. harzianum,* and *P. ostreatus* showed excellent growth on OPC, which began from the first week of cultivation and more intense growth between the second and the third week of the incubation period (**Figure 5**).

3.3. Total phenols of OPC before and after the growth of *G. roseum*, *P. oligandrum, T. harzianum,* and *P. ostreatus*

Total phenols significantly decreased when *G. roseum*, *P. oligandrum,* and *P. ostreatus* grew on OPC from the first week up to the fourth week of growth. On the other hand, *T. harzianum* did not show any significant decrease in phenol content of OPC before and after growth on OPC (**Figure 6**).

Treatments	Incubation period (days)	Yield (g/0.5 kg)	Average weight (g/cap)	Average diameter (cm/cap)
Control	18a[1]	588.69a	25.26a	8.31a
OPC	13b	270.16c	17.99c	7.28a

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

Table 2. Effect of adding olive press cake on incubation period, yield, average weight, and average diameter of *P. ostreatus.*

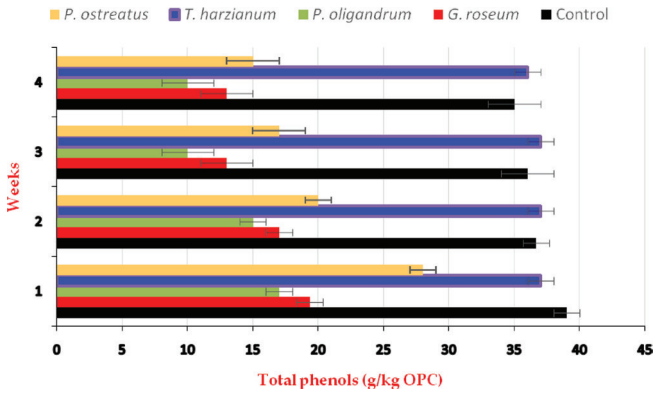


Figure 6. Phenol content (g/kg OPC) of OPC treated with each of *G. roseum*, *P. oligandrum*, *T. harzianum*, and *P. ostreatus* during different treatment times (1–4 weeks). Data are averages (±S.E.) of five replicates and significant values against control represent **highly significant at $p < 0.01$, ***very significant at $p < 0.001$.

Treatments	Time of incubation (days)			
	5	10	15	20
Vermiculite	42*	42**	42	42
OPC	0	0	0	0
OPC previously incubated with *G. roseum* for 1 week	29c	33c	33c	33c
OPC previously incubated with *G. roseum* for 2 week	38c	41c	41c	41c
OPC previously incubated with *G. roseum* for 3 week	37c	40c	40c	40c
OPC previously incubated with *G. roseum* for 4 week	39c	41c	41c	41c
OPC previously incubated with *P. ostreatus* for 1 week	22c	28c	30c	31c
OPC previously incubated with *P. ostreatus* for 2 week	32c	38c	40c	41c
OPC previously incubated with *P. ostreatus* for 3 week	35c	38c	39c	40c
OPC previously incubated with *P. ostreatus* for 4 week	35c	43c	40c	42c
OPC previously incubated with *T. harzianum* for 1 week	0	0	0	0
OPC previously incubated with *T. harzianum* for 2 week	0	0	0	0
OPC previously incubated with *T. harzianum* for 3 week	0	0	0	0
OPC previously incubated with *T. harzianum* for 4 week	0	0	0	0
OPC previously incubated with *P. oligandrum* for 1 week	33c	33c	31c	31c
OPC previously incubated with *P. oligandrum* for 2 week	35c	40c	42c	42c
OPC previously incubated with *P. oligandrum* for 3 week	43c	45c	45c	45c
OPC previously incubated with *P. oligandrum* for 4 week	45c	46c	46c	46c

*Number of emerged *Eruca sativa* seeds out of 50.

**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).

Table 3. Emergency of 50 *Eruca sativa* seeds inoculated or not with *G. roseum*, *P. oligandrum*, *T. harzianum*, and *P. ostreatus* in the presence or absence of olive press cake (OPC) incubated within 20 days.

3.4. Germination of *E. sativa* seeds on OPC previously cultured with *G. roseum*, *P. oligandrum, T. harzianum,* and *P. ostreatus*

OPC previously cultured with each of *G. roseum*, *P. oligandrum, T. harzianum,* and *P. ostreatus* during 1–4 weeks increased the emergency of *E. sativa* seedling, whereas seeds never germinated in crude OPC (**Table 3**, **Figures 7** and **8**).

Ability of Eruca sativa seeds to grow on OPC before and after the growth of *P. oligandrum* after 30 and 40 days

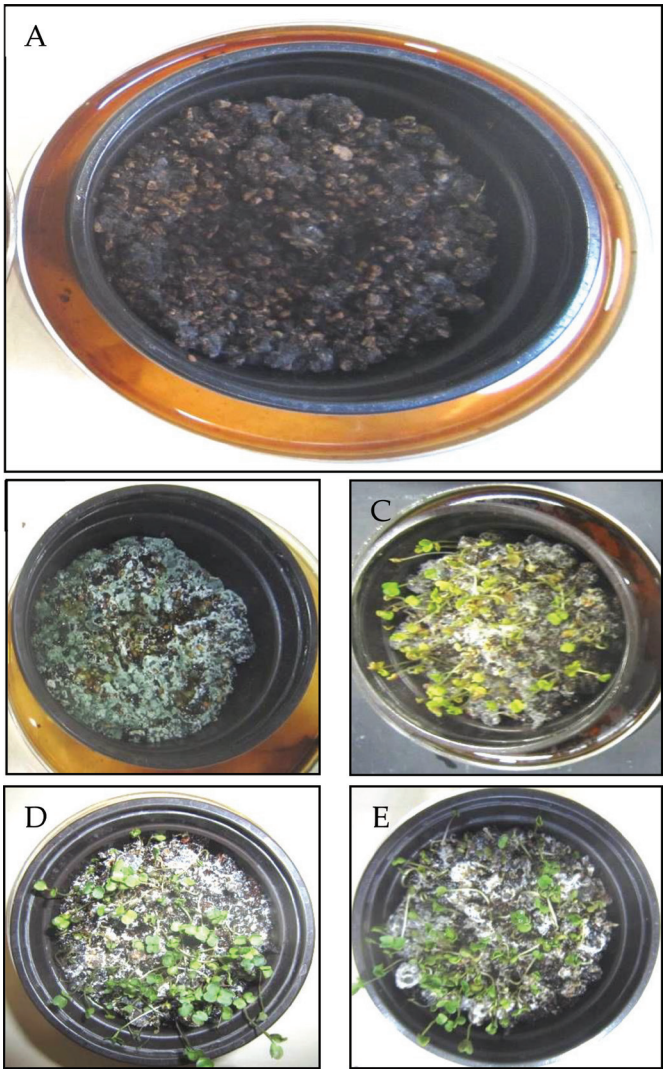


Figure 7. (A–E) Germination of *Eruca sativa* seeds on OPC after 2 weeks culturing with *G. roseum* (B), *T. harzianum* (C), *T. harzianum* (D), *P. oligandrum* (E), *P. ostreatus,* and control (A) whereas seeds were seeded on crude OPC after 7 days at 25°C under 12 h photoperiod (91 μmol m^{-2} s^{-1}).

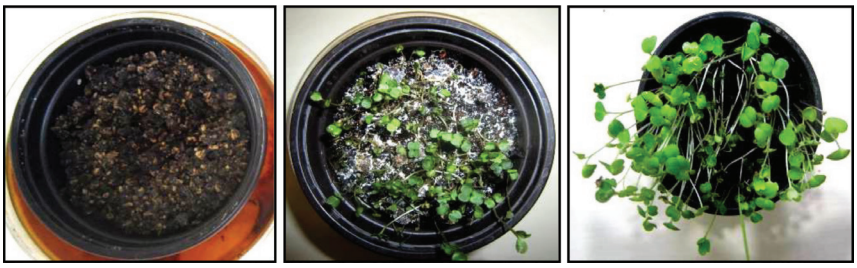


Figure 8. (A–E) Germination of *Eruca sativa* seeds on OPC after 30 and 40 days culturing with *P. oligandrum*. Control represents seeds on crude OPC after 7 days at 25°C under 12 h photoperiod (91 μmol m^{-2} s^{-1}).

4. Discussion

Experimental data show that the phytotoxic properties of OPC were responsible for inhibiting the growth of plant seeds used in this study. The olive press cake used in our study inhibited *E. sativa* seed germination. Many high concentrations of phenolic compounds were considered one of the main reasons of the toxicant effect of OPC on plant seed germination and subsequent growth [14]. For this reason, a high phenolic content of OPC could be responsible for phytotoxicity. Most of phenolic acids began to exhibit their phytotoxicity at high concentrations [15]. In this research, OPC were 40 g kg^{-1} of total phenolic compounds. The application of OPC to agricultural soil causes the inhibition of plant seeds and retards the growth of many growing plants.

It is worth mentioning that many fungi can grow and flourish in food environments containing high concentrations of phenols. From the previous point, this phenomenon can be used to withdraw or even reduce the high percentage of phenols in any medium [16].

From the study, many *Aspergillus* spp. are capable of decomposing phenolics in OPC. Subsequently, many isolates of *A. niger* were observed to flourish and produce dense growth on OPC [17]. One of the methods used by some fungi to remove the toxic effect of high concentrations of phenols had been attributed to their capacity to metabolize phenols [14]. Earlier results showed that *Coriolopsis rigida* decreases phenolics of OPC [4]. In addition, the same fungus increased the dry weight of tomato fruits [18].

Results of this study showed that some of the tested fungi, which were *G. roseum*, *P. oligandrum,* and *P. ostreatus*, had the ability to grow on OPC and withdraw a large amount of phenols up to 75% of the main concentration. By contrast, *T. harzianum* was able to grow on OPC while it could not affect the level of phenols and therefore remained the amount of phenols as they were throughout the incubation period. This may be explained by the ability of *G. roseum*, *P. oligandrum,* and *P. ostreatus* to metabolize the phenols while *T. harzianum* cannot. It is therefore very important to test the ability of fungi (even in the level of isolates) for analyzing phenols after testing their ability to grow on OPC to be used in the clearance OPC from the high concentration of phenols.

Fortunately, a high level of nutrients in OPC strengthened and helped to grow tested fungi intensively without any dietary additives. Therefore, we have used environmentally friendly

fungi and have benefits in the biological control and production of plant-like auxins for plant growth in addition to its ability to reduce the high concentration of phenols. So we hit two birds with one stone, which is that we have made OPC suitable to add to the agricultural soil to improve their properties and at the same time add fungi that resist plant diseases and increase the vegetative growth and productivity of plants.

Another useful dimension is the extent to which OPCs are used as a medium for growth of an edible species of mushrooms (*P. ostreatus*). After mushroom cultivation course, OPC can then be used as high-value organic fertilizers. Mushrooms were recently used for decreasing phenolics in OPC. Other basidiomycota belonging to white rots were proved to be efficient metabolizers of phenolics in OPC [19]. *Pleurotus* was able to grow on OPC and reduced total phenols. It has been evidenced that the development of normal basidiomata on OPC cultured with *P. ostreatus* and *P. eryngii* [20]. They further postulated that the residual toxicity of OPC was significantly reduced. Our experiments showed that by growing *P. ostreatus* on OPC, the percentage of phenols decreased by about 40% of the ratio in the raw OPC after 4 weeks of mushroom cultivation.

It is worth mentioning that each of *G. roseum*, *P. oligandrum,* and *P. ostreatus,* which gave positive results toward the reduction of high phenols and make the residues suitable for seed germination and then used in organic agriculture as fertilizer for agricultural soil, was used separately from each other.

It is therefore very appropriate to test the integration of *G. roseum* and *P. oligandrum* in their work as depressants of the high concentration of phenols and their ability as biological controls. There have been no experiments on this integration in this study here, and therefore we recommend further studies in this regard.

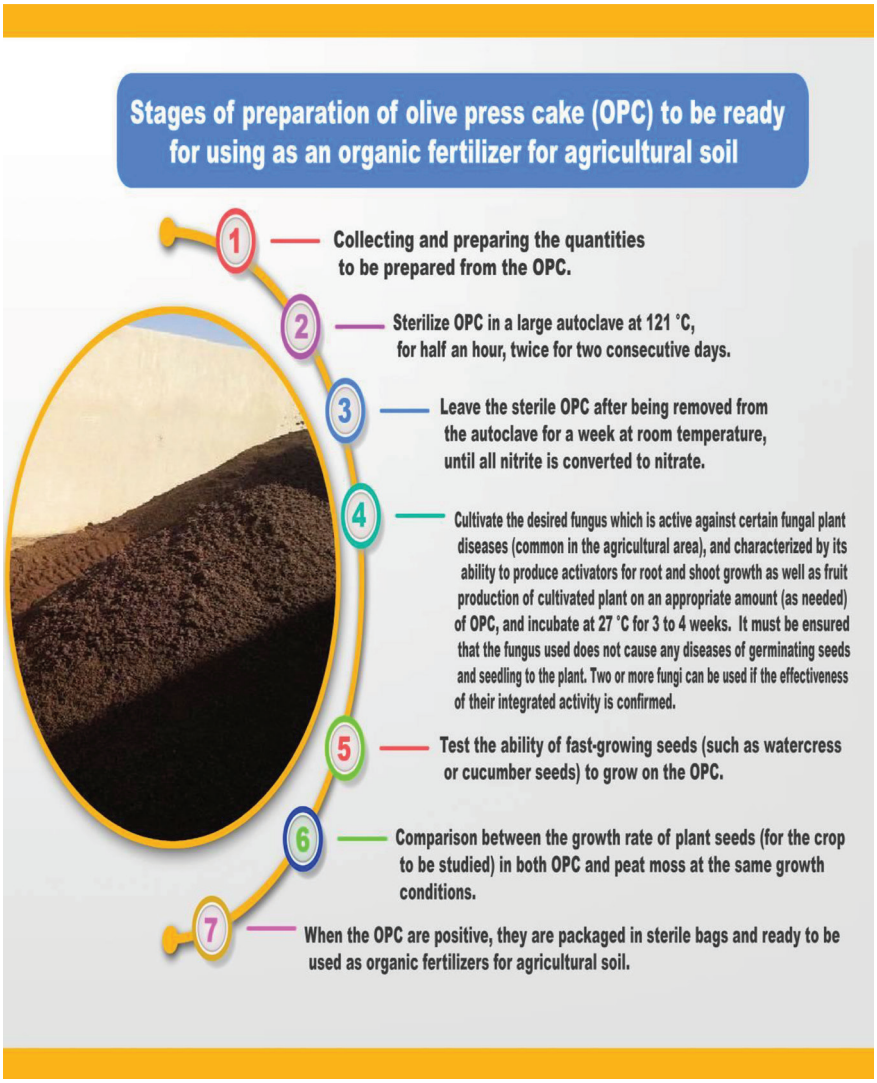
This study is a nucleus of similar studies using other useful fungi that have antifungal properties and can eliminate the OPC of the high concentrations of phenols.

5. Conclusion

It is known that there are many sources of organic fertilizers that man has dealt with throughout the ages. The basic contents of organic fertilizers contain plant and animal residues moistened and left for a certain period of time until microbial degradation occurs and eventually produce organic fertilizers containing organic sources in a simple form that the plant can benefit from. What is new here is that we used OPC as a vital source of organic fertilizer. Olive press cake contains cellulose, protein, carbohydrate, oil, and phenol. This shows the good content of the necessary compounds to ensure seed germination, plant growth, and prosperity. The problem is the high content of phenols that have hindered the germination of plant seeds in some crop plants. In this context, research studies have been conducted to benefit from the high nutritional content of OPC and to withdraw the high concentration of phenols in order to prepare these wastes as a good source of organic fertilizers .It has been found that using some of the saprophytic fungi can reduce the level of phenols in OPC. The idea was to use saprophytic fungi with the ability

to control some plant diseases, in addition to their ability to increase plant growth by producing plant-like hormones (auxins) that are responsible for increasing vegetative growth and fruit production. Therefore, we have used *G. roseum*, *P. oligandrum*, *T. harzianum*, and *P. ostreatus* known to have the ability to control fungal plant diseases and produce plant-like hormones. Our studies have shown that *G. roseum*, *P. oligandrum*, and *P. ostreatus* can play a positive role in reducing the rate of phenols and the foundation of OPC to be a good source of organic fertilizers.

Steps of preparing OPC to be a suitable medium for organic fertilizers can be illustrated in the following infographic illustration:



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