Acta Ecologica Sinica 39 (2019) 36–41

Contents lists available at ScienceDirect

Acta Ecologica Sinica

journal homepage: www.elsevier.com/locate/chnaes

Influence of *Ficus carica* and *Olea europaea* leaves extracts on the mycelial growth of mushrooms *in vitro*

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ARTICLE INFO

Article history:
Received 15 May 2018
Received in revised form 14 June 2018
Accepted 15 June 2018
Available online 21 June 2018

Keywords:
Autoclave
Heat
Herbs
Millipore filter
Mycelial growth rate
Pressure

ABSTRACT

The use of 20% plant leaves extracts included fig (*Ficus carica*) and olive (*Olea europaea*) and their mixture 1:1 as an amendment in the solid agar medium (PDA) is beneficial to promote the growth of four mycelial mushrooms. These are *Pleurotus ostreatus* (Grey oyster mushroom), *Pleurotus cornucopiae* (Yellow oyster mushroom), *Coriolus versicolor* (Turkey Tail mushroom), and *Ganoderma lucidum* (Reishi mushroom). *C. versicolor* showed better growth reached 67 mm significantly ($p < 0.05$) on OC medium after five days. While, *P. cornucopiae* recorded the lowest growth on FC medium reached 35.3 mm. Induction percentage of mycelial growth is changing according to the type of medium and species of fungus. In general, FOH medium exhibited the best percentage of induction was 14.89%, followed 12.48% and 9.43% by OH and OC media, while the lower percentages were 5.02% and 5.12% on FH and FC media, respectively. FC medium did not induce growth of *P. cornucopiae* and *C. versicolor*. The sterilization by Autoclave and Millipore filter showed different induction percentages. Finally, the extracts of fig and olive were useful to add in the culture media to improve the growth of mycelial mushroom *in vitro*.

1. Introduction

The macrofungi are saprophytic or parasitic organisms have been using organic materials as a carbon and nitrogen sources and as supplements for their growing and cultivating in nature or industrial applications in the mushroom farm [1]. Edible and medicinal mushrooms are essential as food [2] and medication against many human diseases such as bacteria, yeast, fungi [3], parasites [4], and tumors [5] spaciously.

The mushroom is developed on many natural media from agricultural residues as a support medium for the availability of the nutrient in Petri dishes [6]. Hence the effect of plant extracts on the growth of fungi, in particular, is vital to check their suitability for mycelial mushroom growth [7].

Ficus carica is a deciduous tree belongs to Moraceae which grows in tropical and subtropical zones, and its common name is fig tree [8]. Leaves of fig have many nutritional and pharmaceutical compounds like aromatic constituents [9], coumarin, ketones, polyphenols, flavonoid [10]. Thus, *Ficus carica* leaves have anti-inflammatory and antioxidative effects toward Hyperglycemia in rats [11], anticancer, antispasmodic, antipyretic, hepatoprotective [12] and antioxidant activity [10].

From another hand, *Olea europaea* L. (Olive) is one of the most important plants in the Mediterranean countries [13] and belongs to the family Oleaceae. Leaf of olive contains Oleuropein, Apigenin-7-*O*-glucoside, Verbascoside and Luteolin-7-glucoside. Thus, it has antibacterial effects against some gram positive and gram negative bacteria and antifungal effect against *Candida albicans* [14]. Olive leaves are useful for the nutrition of creatures. These leaves mixed with Fenugreek seeds improved the physiological performance and blood indices and increased hemoglobin in laying hen breeders [15]. Also, the solid olive waste was used as a supplement for the cultivation of *Pleurotus ostreatus* [16].

Wastes of olive fruit were essential for growing saprophytic mushroom such as *Pleurotus ostreatus* [16] and *Ficus vasta* leaves were used for cultivation *Pleurotus ostreatus* after mixing with other organic and cellulosic materials [17]. Recently, the cultivation and production of oyster mushrooms on fig leaves have been successful due to the abundance of its presence. The testing of fig leaves and olive leaves were done in alone or their mixtures (1:1) at percentage 20% of the composition of the culture medium as supplements for the development of other mushroom species *in vitro*.

2. Materials and methods

2.1. Mushroom species

Four mushroom species were obtained from UK (MushroonBox) namely *Pleurotus ostreatus* (Grey oyster mushroom), *Pleurotus cornucopiae* (Yellow oyster mushroom), *Coriolus versicolor* (Turkey Tail mushroom), and *Ganoderma lucidum* (Reishi mushroom). They

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https://doi.org/10.1016/j.chnaes.2018.06.003
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are subcultured on PDA and stored at 4 °C until achievement of this investigation.

2.2. Collecting the plant samples

Fresh leaves of *Ficus carica* (Fig) and *Olea europaea* (Olive) were collected from gardens of Hit district west of Iraq on September 2017 as in Fig. 1. These leaves were used as amendments to induce mycelial growth of macrofungi *in vitro*.

2.3. Aqueous extraction of the plant leaves

Fresh fig and olive leaves were cleaned from soils and debris, weighted, washed by DW twice and then chopped into small pieces. About 10 g fresh green leaves were taken from each plant and extracted by boiling into 100 ml DW for 15 min on the magnetic stirrer hotplate. The aqueous extracts were cooled and filtrated using 4-fold gauze then centrifuged at 3000 cycle/min for 10 min, and the filtrates were kept at 2–4 °C until use in the next step (Fig. 1).

2.4. Sterilization of aqueous extracts

The two extracts were divided to three parts (100% fig, 100% olive, 50% fig + 50% olive) and sterilized using Millipore filters 0.22 μ (as cold sterilization) named FC, OC, and FOC respectively. From another hand, using autoclave at temperature 121 °C and pressure 15 psi for 15 min as hot sterilization for the watery extracts above named FH, OH, and FOH respectively as described in Table 1.

Table 1
The culture media of the plant's extracts.

The medium of extracts	Sterilization mode					
	Millipore filters 0.22 μ			Autoclave 121 °C, 15 psi for 15 min		
The abbreviators	FC	OC	FOC	FH	OH	FOH
Extract of Fig	20%	–	10%	20%	–	10%
Extract of Olive	–	20%	10%	–	20%	10%

2.5. Culture media preparation

Six treatments were applied in this test as in Table 1, and PDA medium was used as a control. PDA consists of 10 g dextrose, 7.5 g agar, and extract of 100 g fresh potato (100%) completed to 500 ml of distilled water (DW). While the plant leaves extracts media consists of 10 g dextrose, 7.5 g agar, and extract of 80 g fresh potato (80%) completed to 500 ml by 100 ml (20%) of six plant extracts individually. Six flasks of 400 ml PDA were prepared. 100 ml of extracts (fig leaves, olive leaves,

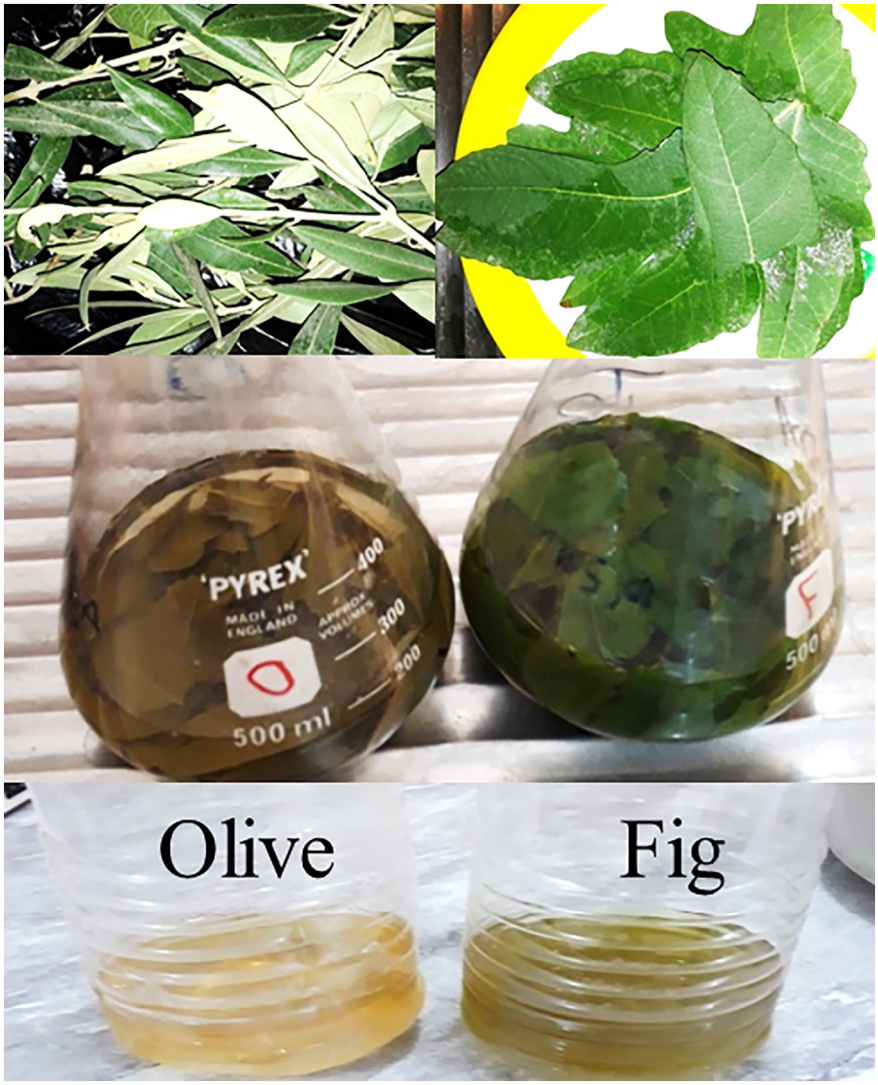

Fig. 1. The watery extracts of the plant leaves.

Table 2
Mycelial growth rate of mushrooms after four days (mm).

Mushroom species	FOC	FOH	OC	OH	FC	FH	PDA	Mean of mushrooms
Pleurotus ostreatus	43.0 ± 0.0H	46.6 ± 0.3G	47.0 ± 1.5G	49.0 ± 1.0F	43.0 ± 0.5H	41.3 ± 0.3IKJ	35.3 ± 0.0N	43.61B
Pleurotus cornucopiae	37.3 ± 0.3M	48.0 ± 0.5GF	36.6 ± 0.3NM	42.6 ± 0.3HI	35.3 ± 0.0N	36.6 ± 0.3NM	35.3 ± 0.0N	38.84D
Coriolus versicolor	66.0 ± 0.0AB	65.6 ± 0.3ABC	67.0 ± 0.5A	65.0 ± 0.0BC	63.0 ± 0.0D	61.0 ± 0.0E	64.6 ± 0.0C	64.60A
Ganoderma lucidum	43.3 ± 0.3H	42.6 ± 0.3HI	42 ± 0.0IHJ	41.3 ± 0.3IKJ	40.6 ± 0.3LKJ	40.3 ± 0.3LK	39.6 ± 0.0L	41.41C
Mean of Medium	47.41D	50.75A	48.16C	49.50B	45.49E	44.83F	43.70G	

Legend: LSD ($p < 0.05$), mean ± standard error.

and their mixture) were individually added to three flasks before sterilization by autoclave and named FH, OH, and FOH respectively. While the rest three flasks left to cool to 45 °C, then added 100 ml of extracts sterilized by Millipore filters 0.22 μ and mixed well namely FC, OC, and FOC, respectively. All extracts were adjusted to pH 6.5. About 20–25 ml of each medium was poured into 85 mm Petri dish and left in the room temperature for solidity, and it will ready for use.

2.6. Inoculation and incubation of media

Seven days of mycelial growth on disk (6 mm) of four mushroom species were individually placed in the center of the plates with triplicates. Fresh PDA was applied as a control for all mushroom species. All plates were kept in the incubation at 25 ± 0.8 °C. By a ruler, the diameter of the fungal colony was daily determined. The diameter of fungal colonies (mm), period of overgrowth (day), cumulative growth rate (mm/day), mycelial growth rate after four days (mm/4th day), line chart of daily growth rate (mm), and enhancement/induction of the growth (%) were calculated after 5 days as in the equation below:

$$\text{Induction of Growth}\% = \frac{(\text{Diameter of the control [PDA]} - \text{Diameter of the treatment})}{\text{The diameter of the treatment}} \times 100$$

2.7. Statistical analysis

The experimental design was applied with triplications. Two factors effect included strain type and medium type and the interactions between were significant ($p < 0.05$). The statistical analysis applied by two-way analysis of variance using Completely Randomized Design (CRD) by SAS software.

3. Results and discussion

This study is concerned with the study of the effect of heat and pressure on the plant extracts described as in Table 1 in the composition of the culture medium for stimulation the mycelial growth of four types of edible and medicinal mushrooms. Through Table 2, *Coriolus versicolor* mushroom has recorded the best growth reached 67.0 mm significantly ($p < 0.05$) since the fourth day on OC medium. However, FOC and FOH media encouraged the growth of this mushroom in plates reached 66.0 mm and 65.6 mm respectively. While, *Pleurotus cornucopiae* has shown the lowest growth on FC medium (35.3 mm) compared with the control (PDA) which reached 35.3 mm, and followed by 36.6 mm on OC and FH media. In general, FOH medium has given the highest rate of significant ($p < 0.05$) growth, reached 50.75 mm followed by OH medium (49.50 mm), while FH medium has recorded the growth rate 44.83 mm compared to 43.70 mm by the control. Generally, depending on Table 2 for the variation in the growth of the fungus species. *C. versicolor* mushroom has shown the best growth was 64.60 mm significantly ($p < 0.05$) than other species under the study were including *Pleurotus ostreatus*, *Ganoderma lucidum*, and *P. cornucopiae* which have recorded 43.61, 41.41, and 38.84 mm, respectively.

Average of mycelial growth was variable as watched in plates of plants extracts compared with PDA (control). Fig. 2 exhibits the growth of *C. versicolor* on the fourth day which was similar approximately to the control. While the growth of *P. ostreatus* has shown apparent differences on the seventh day, see Fig. 3. As [16] mentioned OC and OH media (olive leaves extracts) had shown better growth than FC and FH media (fig leaves extracts). However, fig leaves extract exhibited induction of the growth when it mixed with olive leaves extract at ratio 1:1 as in FOH and FOC media generally. The reason of this state may go back to enhance properties of the crude extract, and that agrees with [15] who referred that olive leaves are useful for the nutrition of creatures. Thus, these leaves when mixed with Fenugreek seeds led to improving the

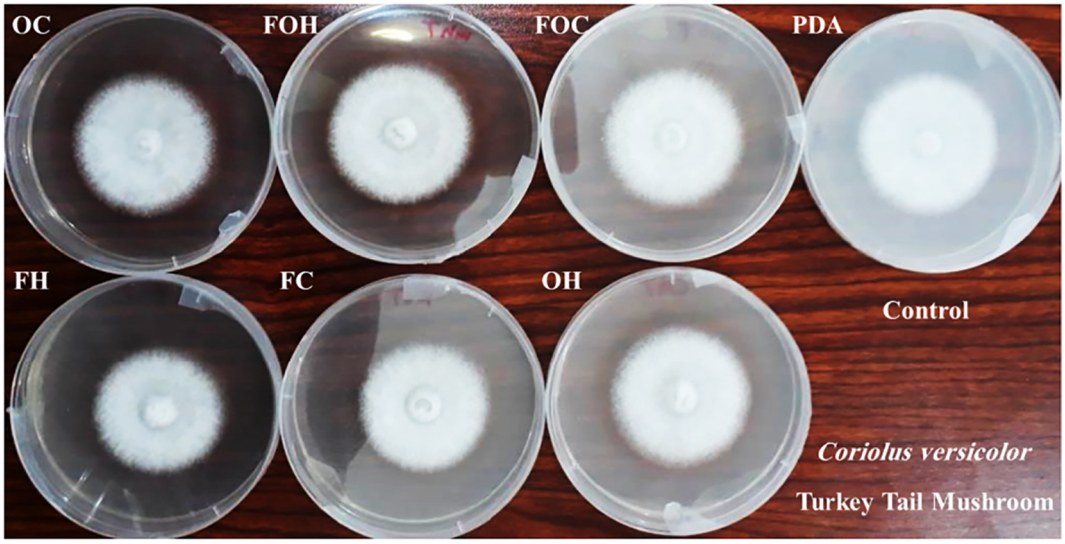

Fig. 2. Appearances of mycelial growth of *C. versicolor* on the fourth day.

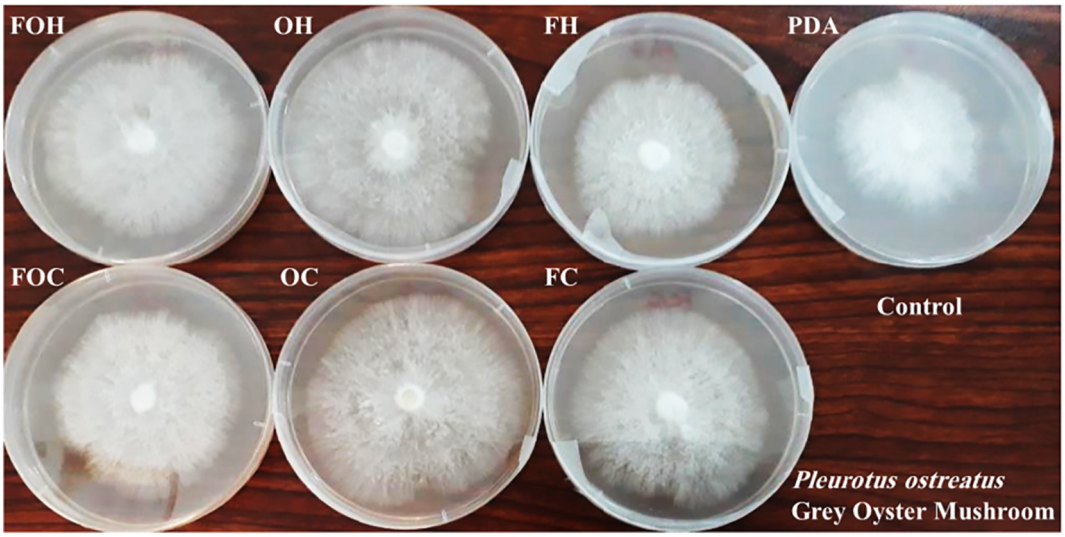

Fig. 3. Appearances of mycelial growth of *P. ostreatus* on the seventh day.

physiological performance and blood indices and increased hemoglobin in laying hen breeders. Also, *in vitro*, the suitability of using fig leaves agrees with [17] who referred that oyster mushrooms significantly can be converted solid wastes of fig leaves mixed with other organic substances into vitamin and some essential mineral rich food and with low fat and calorie. Leaves of fig and olive have a nutritive value such as elements macro and microelements, proteins, carbohydrates and the organic matter [18–21].

Overgrowth period of mushroom in plates was changeable for the completion of growth as in Fig. 4. Turkey tail, *C. versicolor* had the least time to overgrow in all treatments reached seven days thus this fungus was the best significantly ($p < 0.05$). After that, *P. ostreatus* recorded eight days on OC, and OH media, followed by FOC medium and then *P. ostreatus* and *G. lucidum* on FC medium reached nine days compared with the control for these two mushrooms (10 days). Also, *G. lucidum* recorded ten days on all treatments except FC medium (9 days). Finally, *P. cornucopiae* recorded the highest time from 10 to 11 days compared with 13 days by the control (PDA).

The differences of growth of mushroom belong to inheritance characteristics of each fungus species which increases the growth of macrofungi in various culture media [22]. The differentiation of growth in PDA illustrates the natural ability of the growth of the studied species in this study as seen in Fig. 5. *C. versicolor* was faster mushroom grown in PDA than other species through 7 days. Moreover, *P. ostreatus* and *G. lucidum* recorded ten days for overgrowth in the control plates. While, *P. cornucopiae* recorded more extended time for completion of the full growth in the same dishes reached 13 days because of its slow growth. The differences in the mycelial growth in PDA (Fig. 5) are confirmative for differences of overgrowth of the mycelial mushrooms in the culture media of plant leaves extracts (FC, OC, FOC, FH, OH, and FOH media) in Fig. 4.

The cumulative mycelial growth of four edible and medicinal mushrooms was done in this investigation (Fig. 6). This measure is useful for determining the suitability of fig and olive leaves extracts and their mixture (1:1) compared with PDA medium *in vitro*. *C. versicolor* recorded the cumulative growth rate was 12 mm/day on PDA plates compared with treatments media of plant extracts which induced this property to average ranged from 12.1 mm/day to 12.3 mm/day. *P. ostreatus* recorded cumulative growth obtained 11.1, 9.8, and 9.8 mm/day on OC, FC, and FOC media. While, it recorded the least growth reached 11.0, 8.7, and 8.6 mm/day on OH, FH, and FOH media respectively in comparison with the control (PDA) 8.6 mm/day. The cumulative growth of *Pleurotus ostreatus* in OC, FC, and FOC media is more conveniently compared with OH, FH, and FOH media. Because the treating by heat (OH, FH, and FOH) leads to destroy proteins and decreasing the aggregation of proteins that may be decrease the mycelial growth when compared with sterilization of extracts using Millipore filters (OC, FC, and FOC) [23]. *G. lucidum* exhibited growth rate of 8.4 mm/day on OC and OH media, whereas that increased on FC medium (9.4 mm/day) and then decreased to 8.3 mm/day on FH, FOH, and FOC media compared with

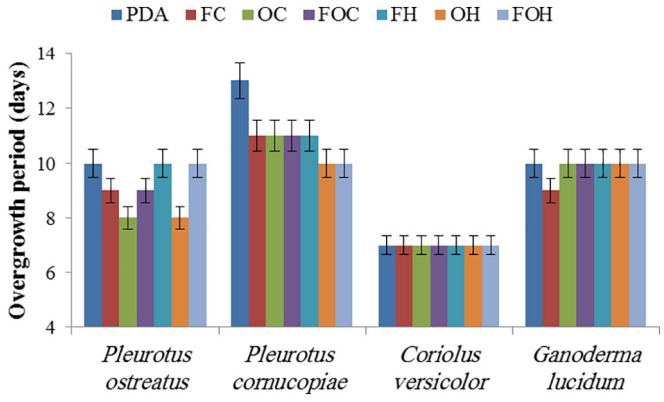

Fig. 4. The period of overgrowth of mushrooms in plates (days) ($p < 0.05$).

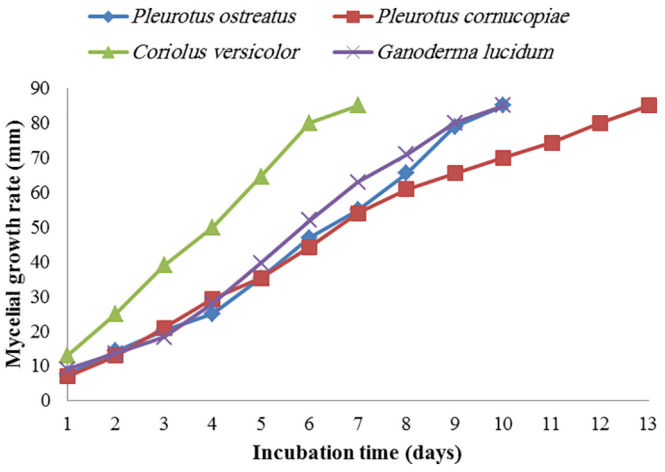

Fig. 5. Comparison of the studied four mushrooms mycelial growth in PDA medium.

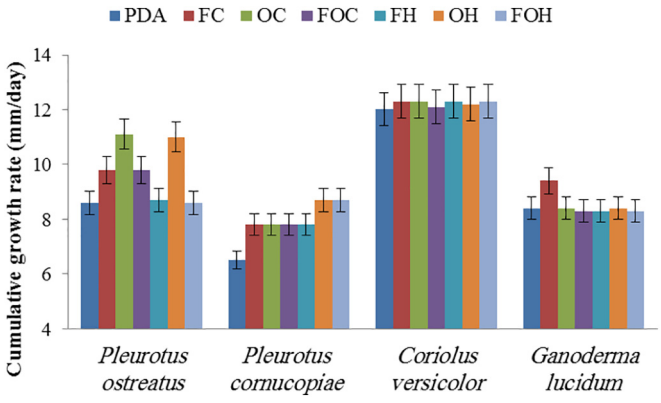

Fig. 6. Cumulative growth rates of mushrooms (mm/days) ($p < 0.05$).

8.4 on PDA. Finally, *P. cornucopiae* showed growth rate of 8.7 mm/day on OH, and FOH media then decreased to 7.8 mm/day on the rest media compared with PDA medium which showed 6.5 mm/day. That is due to the pressure and heat which may be destroyed some antifungal compounds of the plant extracts in the prepared culture media [24].

In general, daily mycelial growth rates of the studied mushrooms were presented in Fig. 7 to illustrate the differences in the growth of fungal species and affect the plant extracts undoubtedly. Olive leaves contain many potentially bioactive compounds that may promote the growth of mycelial mushrooms [25]. Polyphenols of olive are essential for growing some mushrooms as mentioned by [26]. High-pressure thermal exposure may bring enzyme inactivation, resulting from conformational changes in the protein structure; also, enzymatic reactions may be enhanced or retarded by the pressure [24]. Thus, the sterilized plant extracts using Millipore filter appeared to be more convenient for growing mushroom than the extracts prepared by the autoclave because the pressure and heat have destroyed many hormones and enzymes that help to grow mushrooms [24, 27].

Induction percentage of mycelial growth is changing according to the type of medium and species of fungi as shown in Table 3. In general, FOH medium exhibited the best percentage of induction was 14.89%, followed 12.48% and 9.43% by OH and OC media, while the lower percentages were 5.02% and 5.12% on FH and FC media respectively. Generally, type of mushroom affected by media type; *P. ostreatus* significantly ($p < 0.05$) recorded the best induction of 21.22%, followed 9.42% by *P. cornucopiae*. The lower induction percentages were 1.32% and 5.01% for *G. lucidum* and *C. versicolor*, respectively. *P. ostreatus* recorded better induction on OH medium (27.8%) than *P. cornucopiae* on FOH medium (26.4%). FC medium did not induce growth of *P. cornucopiae* and *C. versicolor* that maybe belongs to the role of the fig leaves extract sterilized using Millipore filter which contains antifungal compounds that leads to inhibit fungi [28, 29]. From another hand, fig leaves extract exhibited the success in the promotion of growth and production of *P. ostreatus* [17].

The different growth in the various media is due to several reasons. These are including the sensitivity of the mushroom to the type of biological substances found in plants extracts, enzymes, useful nutrients and polyphenols, and the type of sterilization filters or exposure the extract to height heat and pressure within the autoclave [30]. Heat and pressure play a significant role in altering the structure of proteins and fraction or damaging antibiotic substances [31]. If the concentration of the extract is low (about 20% which was used in this experiment), it will lead to never inhibition effect as mentioned by [17]. If we take high concentration, it will show a disincentive effect on the growth of mushrooms [24, 30]. The results were applied *in vitro* may different compared as in farm when the plant organic matters were applied on the compost composed from various substrates that improve properties of organic matters [17].

Also, autoclaving caused less damage to the antimicrobial agents of the tested plant extracts compared with syringe filtration [32]. Leaves of fig and olive have nutritive value. Leaves extract of fig is useful for plants and fungi due to its nutritional value such as elements of Na, K, Ca, Mg, Fe, P [18], Mn, Cu, Zn, B [19], and contains 10% proteins, 12.2%

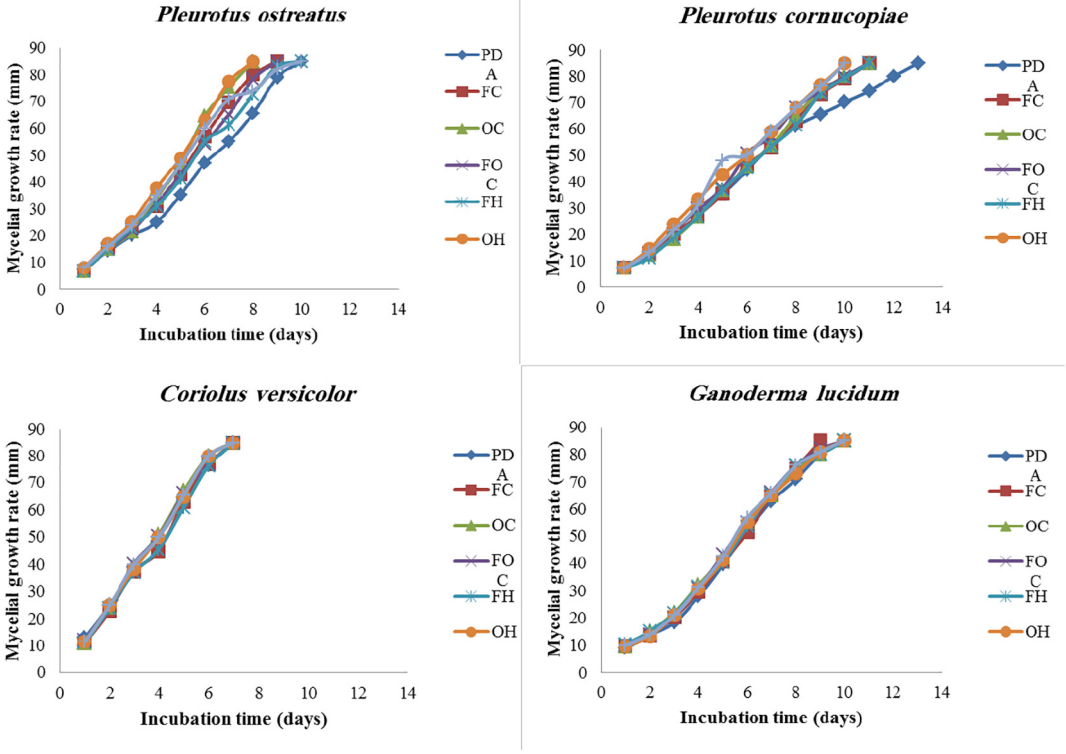

Fig. 7. Line charts of mycelial growth rates of mushrooms.

Table 3
Induction percentage of mycelial growth after five days.

Mushroom species	FOC	FOH	OC	OH	FC	FH	Mean of mushrooms
Pleurotus ostreatus	17.9 ± 0.0C	24.3 ± 0.5B	24.7 ± 2.3B	27.8 ± 1.5A	17.8 ± 1.1C	14.50.6D	21.22A
Pleurotus cornucopiae	5.4 ± 0.8FG	26.4 ± 0.8AB	3.7 ± 0.8HG	17.2 ± 0.6C	0.0 ± 0.0I	3.7 ± 0.8HG	9.42B
Coriolus versicolor	2.1 ± 0.0HI	1.6 ± 0.5HI	3.5 ± 0.8HG	0.6 ± 0.0I	0.0 ± 0.0I	0.0 ± 0.0I	1.32D
Ganoderma lucidum	8.6 ± 0.6E	7.1 ± 0.7FE	5.7 ± 0.0FG	4.1 ± 0.7HG	2.6 ± 0.8HI	1.8 ± 0.8HI	5.01C
Mean of media	8.51C	14.89A	9.43C	12.48B	5.12D	5.02D	

Legend: LSD ($p < 0.05$), mean ± standard error.

oils, 37.3 crude fiber, and 30.9% carbohydrates [18]. While leaves of olive contain organic matter, crude protein [20], and elements of Na, Mg, Al, Si, P, S, K, Ca, Mn, Fe, and Cl [21]. Thus, the induction of mycelial growth of mushroom was successful at percentage 20% of the composition of culture media (PDA), and this agrees with few studies. Wastes of the olive plant is important for growing edible mushrooms like *P. ostreatus* because it is a saprophytic fungus [16] and *Ficus vasta* leaves were used for cultivation *P. ostreatus* after mixing with other organic and cellulosic materials [17].

C. versicolor negatively influenced by using fig and olive leaves extracts compared with other mushroom species (Table 3) that may be returned to unsuitability these extract for its growth. The cold extract of fig leaves did not induce the mycelial growth of *C. versicolor* and *P. cornucopiae* because of its antifungal properties [33] which may inhibit the growth of some mushrooms compared with the cold extract of fig leaves.

Acknowledgments

The authors thank the staff of the Department of Biophysics in College of Applied Sciences, for achieving this project. This work was achieved under project No. 2/11 on 18/10/2017 in College of Applied Sciences, University of Anbar.

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