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Effect of Phosphorus Form and Culturing Mode on Mycelial Growth of *Pleurotus Pulmonarius* and *Pleurotus Floridanus* on Rice Straw

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Abstract

Oyster mushroom (*Pleurotus* spp.) cultivation gained a growing concern due to their limited growth requirements and high nutritional value and bioactive compounds. Present work investigates the effect of rice straw supplementation with inorganic P (KH_2PO_4) and organic P (wheat bran) with presence or absence of agar layer above straw on mycelial growth of two oyster mushrooms: *P. pulmonarius* and *P. floridanus.* Mycelial growth was better with bran than with KH_2PO_4, particularly without agar. Such a differential effect of P form and agar layer was more evident on *P. pulmonarius* than on *P. floridanus*. Mycelial growth mostly exhibited a saturable dose-dependent pattern and increased up to a certain optimal P supply of 2–5 g P/g straw, post which it approached a plateau; only in the no agar-bran culture of *P. floridanus* a progressive P-dependent increase was found. The time-course of fungal growth exhibited contrasting patterns in the two fungi; being sigmoidal in *P. pulmonarius* but almost linear in *P. floridanus*. In *P. pulmonarius*, the lag growth period (T_{10}) was longer with KH_2PO_4 than with bran, with different dose-dependent patterns in the two P sources and marginal effect of the agar layer. Relative growth rate (RGR) of *P. pulmonarius* was higher than that of *P. floridanus*, with different dose-dependent patterns according to fungal species and presence of agar. In *P. pulmonarius*, both T_{10} and RGR attained comparable values of about one day and 0.4 day^{-1}, respectively at 5 mg P/g straw for the different P form × culturing mode combinations.

Keywords: Fungal growth rate, oyster mushroom, Pleurotus spp., organic phosphorus, wheat bran.

Introduction

Oyster mushrooms are cosmopolitan fungi, belonging to the genus *Pleurotus* (family Pleurotaceae, order Agaricales, class Agaricomycetes) that grow on dead organic matter in the tropical and temperate regions. The genus *Pleurotus* includes 200 species that have decurrent blades of white or hyaline color sculptured with cylindrical or oval oyster shell-like shapes (El-Ramady et al., 2022). By virtue of their unique ability to degrade lignocellulosic wastes; oyster mushrooms are also known as white rot fungi (Torres-Martínez et al., 2022). *Pleurotus* spp. have many advantages over other mushrooms since they are easier to cultivate on several substrates such as straw, cotton waste, walnut shell and wood. In

addition, they are robust against the attack of diseases and pests and have high harvest index and high content of nutraceuticals (Bellettini et al., 2019; Raman et al., 2020).

Oyster mushrooms have distinct texture, aroma and taste, in addition to their health benefits that are attributable to the presence of bioactive compounds including polysaccharides (β-glucans), proteins, enzymes, peptides, lectins, terpenoids, polyketides and phenolics (El-Gharabawy, 2012; orres-Martínez et al., 2022). Furthermore, mushrooms, in general, are the only vegetarian source of vitamin B_{12} and can provide considerable supply of vitamin D, which content can be considerably increased upon exposure of harvested mushrooms to UV light or sunlight (El- Fallal et al., 2013). *Pleurotus* spp. have a range of therapeutic potentialities including antimicrobial, antiviral, antitumor, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating and antihyperglycemic activities (Elhusseiny et al., 2021; El-Fallal et al., 2022).

Cultivation of mushrooms represents a tempting option in modern agriculture. Mushroom cultivation is a fermentation industry that involves the bioconversion of cellulose wastes into edible biomass and value-added substances. Since mushrooms can be grown on straw and other agricultural wastes, their cultivation can aid in conservation of environmental resources and cutting down of pollutants arising from accumulation of agricultural wastes. Unlike plants, mushroom cultivation is an indoor activity, with limited land requirements, possibility of vertical cultivation and wide choice of seasonal cultivation. Furthermore, mushroom cultivation has a relatively low water and carbon footprints compared to other foods (Ferdousi et al., 2019). Currently, six mushroom species, namely shiitake (*Lentinula* spp.), oyster (*Pleurotus* spp.), wood ear (*Auricularia* spp.), button (*Agaricus* spp.), winter mushroom (*Flammulina* spp.) and paddy straw mushroom (*Volvariella* spp.) account for 90% of the total world mushroom production (Thakur, 2020). *Pleurotus pulmonarius* and *P. floridanus* are fleshy edible oyster mushrooms of high nutritional value.

A variety of lignocellulosic wastes– such as straw, cotton waste, wood shavings, banana pseudo-stem, waste paper, sawdust and corn–cobs can be used as substrates for oyster mushroom cultivation after homogenization and adjusting of water content and pH. Supplementation of these waste substrates with a variety of additives can increase nutrient content and mushroom yield (Josephine, 2015). The present work aims to investigate the effect of supplementation of rice straw with phosphorus either in the form of inorganic P (KH_2PO_4) or organic P (phytin, in the form of wheat bran) with the presence of or without an agar layer above the straw surface on the mycelial growth of the two oyster mushrooms *P. pulmonarius* and *P. floridanus.* The questions are: 1) which form of P is more favorite for fungal growth, and does this preference vary among the two mushroom species? 2) does the presence of an agar layer above the straw affect the availability of both forms of P equally in the two mushroom species?

Materials and methods

Materials

Pleurotus pulmonarius was obtained from the Consultative Comet Company of Mushroom cultivation-Egypt (CCCM). *Pleurotus floridanus* was obtained from the American Type Culture Collection, USA (ATCC). Rice straw was obtained from a recently harvested paddy rice crop. Rice straw was air-dried to a constant weight, chopped into small pieces, oven-dried at 80°C for 48 h and ground into a fine powder. The straw powder was then sieved through a 1-mm sieve to get a homogeneous substrate. Wheat bran was obtained from the local market, oven-dried to a constant weight at 80°C. Water holding capacity of rice straw and wheat bran was determined according to Jin et al. (2020) and was found to be 500%. The phosphorus content of wheat bran was estimated as 1% DW (Onipe et al., 2015) and that of rice straw as 0.03% (Feng et al., 2020).

Experimental design

For each mushroom species and mode of culturing (with or without an agar layer above straw), an aliquot of the sieved rice straw was divided into two equal portions (one for KH_2PO_4 and the other for wheat bran), and each

portion was subdivided into 5 equal subgroups to receive five doses of P either as inorganic P (KH_2PO_4) or as organic P (wheat bran). Phosphorus was added at five levels (0, 1, 2, 5, 10 mg P/g straw DW) as KH_2PO_4 to the first group and as wheat bran to the second group. In the KH_2PO_4 group, graded volumes (0, 1, 2, 5, 10 mL) of KH_2PO_4 stock (4.4% KH_2PO_4, equivalent to 1% P) were added to 10 g sieved rice straw for 0, 1, 2, 5, 10 mg P/g straw DW, respectively and water was added to bring moisture content of the straw to 50% of its water holding capacity. In the bran group, graded weights (0, 1, 2, 5, 10 g) of wheat bran (1% P) were added to 10 g of rice straw for 0, 1, 2, 5, 10 mg P/g straw DW, respectively and water was added to bring moisture content of the straw-bran mixture to 50% of its water holding capacity. The contents (the volumes of KH_2PO_4 stock or the weights of bran and rice straw) were mixed thoroughly before adjusting water content of substrate. The homogenized moistened substrates of each level and form of P were dispensed into three 12-cm Petri dishes, up to two-third of the dish capacity, for three replications of each treatment. The substrates were autoclaved at 121 ˚C under 1.5 bar for 20 minutes. After cooling to room temperature, the Petri dishes of each form and dose of P were divided into two equal groups, the first group was inoculated with a 1-cm disc of 7-day fungal culture directly above the surface of straw; while to the second group a thin 2-mm layer of molten 1.5% (w:v) agar was poured over the straw and left to solidify, then the fungal discs were positioned at the center of the agar layer. Experimental layout and details of experimental procedure are presented in Tables 1 and 2. Growth of fungi was monitored by measuring the diameter of the growth zone at frequent time intervals (0, 2, 4, 5, 6 days from inoculation).

The experiment was factorial with four levels and three replications in a completely randomized design with a total of 120 experimental units each was a Petri dish. The main factors were: 1) mode of culturing with two levels: either with the presence or absence of an agar layer above straw, 2) form of P with two levels: either KH_2PO_4 or bran, 3) dose of P with five levels: 0, 1, 2, 5, 10 mg P/g straw, and 4) incubation period with five levels: 0, 2, 4, 5, 6 days. The data were subjected to four-way ANOVA to test the significance of the main factors and their interactions. Mean separation was performed according to the Duncan's multiple range test at $P<0.05$.

Table 1. Layout of the experiment

Agar layer	Form of P	*Pleurotus pulmonarius* mg P/g straw					*Pleurotus flridanus* mg P/g straw				
		0	1	2	5	10	0	1	2	5	10
No agar	KH_2PO_4	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
	Bran	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
Plus agar	KH_2PO_4	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
	Bran	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx

Table 2. Details of treatment application

	mg P/10 g straw				
	0	10	20	50	100
KH_2PO_4: 4.4% stock, equivalent to 1% P					
mL stock KH_2PO_4/10 g straw	0	1	2	5	10
mL water/10 g straw	25	24	23	20	15
Bran: 1% P					
g bran/10 g straw	0	1	2	5	10
mL water/10 g straw and bran	25	27.5	30	37.5	50

Definitions and calculations

T_{10} is the lag period of fungal growth in case of sigmoidal growth curves. It is the duration from incubation to the start of the exponential phase of growth and estimated as the time corresponding to 10% of the final fungal growth.

Relative growth rate (RGR) was calculated according to the following formula:

$$RGR = \frac{ln\ D_2 - ln\ D_1}{t_2 - t_1} \quad day^{-1}$$

where D_2 and D_1 are the diameters of fungal culture (cm) at times t_2 and t_1, respectively.

Results

The four-way ANOVA revealed highly significant effect ($P<0.001$) of the main factors (mode of culturing, form and dose of phosphorus and incubation time) and their interactions on growth of the two mushroom

fungi *P. pulmonarius* and *P. floridanus*. However, considering the magnitude of the F ratio, the effects of the factors, except for mode of culturing (the presence of the agar layer), were more evident on *P. pulmonarius* than on *P. floridanus* (**Table 3**).

Table 3 Four-way NOVA showing the effect of the main factors (**mode of** culturing, form and dose of phosphorus and incubation time) and their interactions on growth of *Pleurotus pulmonarius* and *Pleurotus floridanus* mushroom fungi.

Fungus and source of variation	df	MS	F	P
Pleurotus pulmonarius				
Culturing mode (Mode)	1	11.17	312.4	0.000
P form (Form)	1	57.48	1607	0.000
P dose (Dose)	4	4.218	118.0	0.000
Time	4	208.8	5838	0.000
Mode × Form	1	5.576	155.9	0.000
Mode × Dose	4	0.463	12.93	0.000
Mode × Time	4	3.754	105.0	0.000
Form × Dose	4	0.544	15.21	0.000
Form × Time	4	5.829	163.0	0.000
Dose × Time	16	0.434	12.13	0.000
Mode × Form × Dose	4	0.135	3.761	0.006
Mode × Form × Time	4	1.772	49.55	0.000
Mode × Dose × Time	16	0.098	2.752	0.001
Form × Dose × Time	16	0.077	2.142	0.008
Mode × Form × Dose × Time	16	0.087	2.436	0.002
Error	200	0.036		
Pleurotus floridanus				
Mode of culturing (Mode)	1	140.2	2084	0.000
P form (Form)	1	1.800	26.77	0.000
P dose (Dose)	4	3.550	52.78	0.000
Time	4	313.2	4657	0.000
Mode × Form	1	9.448	140.5	0.000
Mode × Dose	4	0.115	1.717	0.148
Mode × Time	4	17.32	257.5	0.000
Form × Dose	4	0.615	9.143	0.000
Form × Time	4	0.588	8.748	0.000
Dose × Time	16	0.384	5.711	0.000
Mode × Form × Dose	4	1.198	17.80	0.000
Mode × Form × Time	4	0.765	11.37	0.000
Mode × Dose × Time	16	0.093	1.384	0.152
Form × Dose × Time	16	0.192	2.857	0.000
Mode × Form × Dose × Time	16	0.154	2.286	0.004
Error	200	0.067		

df = degrees of freedom; MS = Mean square; F = F-statistic, the ratio of mean square of the factor and mean square of error; P = the significance level associating the F ratio

Growth of *P. pulmonarius* was significantly higher with bran as a source of P than with KH_2PO_4. The advantage of bran over KH_2PO_4 was more evident in absence of the agar layer than with its presence and amounted to 84% and 30%, respectively as an average for the whole growth period and to 73% and 17%, respectively for the final growth period (the 6th day of growth). The differential effect of form of P on fungal growth was ~~generally~~ mild in *P. floridanus*, with an average 24% higher growth with bran over KH_2PO_4 in absence of agar versus non-significant (P>0.05) improvement with KH_2PO_4 over bran in the presence of agar layer (**Table 4**). In turn, the presence of the agar layer above straw significantly improved fungal growth relative to absence of agar. The advantage of the agar layer was more evident for *P. floridanus* than for *P. pulmonarius* and with KH_2PO_4 as a source of P than with bran. The effect of the agar layer relative to absence of agar on growth of *P. pulmonarius* amounted 48% and 5% increases with KH_2PO_4 and bran, respectively as an average for the whole growth period and to 30% increase versus 12% decrease, respectively at the final growth period. The advantage of the agar layer above no agar on growth of *P. floridanus* amounted to 80% and 40% with KH_2PO_4 and bran, respectively as averages for the whole period and the final period of growth (**Table 4**).

Table 4. Effect of mode of culturing and form of phosphorus supply on growth of *P. pulmonarius* and *P. floridanus* mushroom fungi estimated as the diameter of growth zone (cm) either averaged across a six-day incubation period or at the final period (6th day of incubation). Each value is the mean of the five doses of P, with r replicates ± SE. Means with common letters are non-significantly different at P<0.05. For average incubation period r = 75, for the 6th day of incubation r = 15.

Culturing mode and P form	Average of incubation period		6th day of incubation	
	P. pulmonarius	***P. floridanus***	***P. pulmonarius***	***P. floridanus***
No agar				
KH_2PO_4	1.37 ± 0.15^{a}	2.06 ± 0.16^{a}	3.46 ± 0.15^{a}	3.87 ± 0.08^{a}
Bran	2.52 ± 0.24^{c}	2.57 ± 0.21^{b}	5.96 ± 0.18^{d}	4.81 ± 0.26^{b}
Agar				
KH_2PO_4	2.03 ± 0.19^{b}	3.79 ± 0.30^{d}	4.48 ± 0.09^{b}	6.89 ± 0.08^{cd}
Bran	2.63 ± 0.23^{d}	3.59 ± 0.30^{c}	5.25 ± 0.13^{c}	6.78 ± 0.12^{c}

Apart from *P. floridanus* grown with bran in absence of the agar layer, the dose-response relationship of fungal growth versus P supply followed a saturable pattern, where growth increased up to a certain optimal P supply, post which it approached a plateau or exhibited slight decline. The optimum P supply and the magnitude of growth promotion in response to pre-optimal P supply varied according to fungal species, form of P and mode of culturing. In *P. pulmonarius*, cultured with

no agar layer, the fungus attained maximal growth at 2 mg P/g straw with 44% increase above control as an average for KH_2PO_4 and bran, followed by 23% average reduction with further increase in P supply up to 10 mg P/g straw. But, in *P. pulmonarius*, cultured with an agar layer, the fungus attained maximal growth at higher P supply (5 mg P/g straw) with limited enhancement (24% increase above control as an average for KH_2PO_4 and bran) with a tendency towards a plateau at higher P supply (**Fig. 1A**). In *P. floridanus*, except for the no agar-bran culture, fungal growth exhibited a saturable pattern with the increase in P supply, approaching optima of 5 mg P/g straw with limited stimulation that amounted to 20%, 13.5% and 9% above the control for KH_2PO_4-no agar, KH_2PO_4-plus agar and bran-plus agar, respectively. In the no agar-bran culture, *P. floridanus* growth exhibited a progressive increase of 64% with the increase of P supply from 0 to 10 mg P/g straw (**Fig. 1B**).

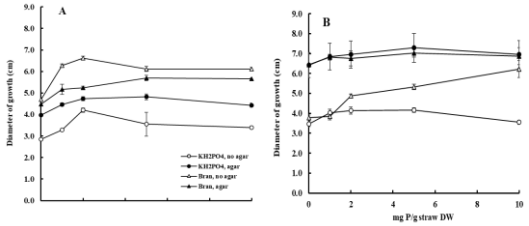

Fig. 1 Effect of mode of culturing and dose and form of phosphorus supply on growth of the two oyster mushroom fungi *P. pulmonarius* (A) and *P. floridanus* (B) estimated as the diameter of growth zone on the 6th day of incubation. Each value is the mean of three replicates ± SE.

The time-course pattern of fungal growth varied considerably in the two mushroom fungi; it exhibited a clear sigmoidal

KH_2PO_4 or wheat bran with no agar layer (A and B, respectively) or with an agar layer over the straw (C and D, respectively). pattern in *P. pulmonarius* versus an almost linear pattern in *P. floridanus* (**Figs. 2 and 3**). For *P. pulmonarius*, with the sigmoidal pattern of growth, the length of the lag period (T_{10}) varied depending on the form and supply of P, with marginal interference from mode of culturing. The T_{10} was consistently longer with KH_2PO_4 than with bran, independently of the presence of the agar layer, which exerted a marginal effect (**Fig. 4A**). Furthermore, the P supply-T_{10} relationship exhibited a periodic pattern (approximated to a third-degree relationship) with different patterns in the two P forms but with marginal effect of the agar layer (**Fig. 4B**). In KH_2PO_4 culture, the T_{10} slightly declined with the increase in P supply reaching minima at about 6 mg P/g straw followed by marked increase at higher P supply. In bran culture, the value of T_{10} decreased with the increase in P supply, approaching minima at 2 mg P/g straw, followed by an increase with further increase in P supply (approaching maxima at 8 mg P/g straw) and a small decline again at 10 mg P/g straw. It is evident from **Fig. 3B** that the value of T_{10} attained comparable values of about one day at P supply of about 5 mg P/g straw for the different P form-culturing mode combinations.

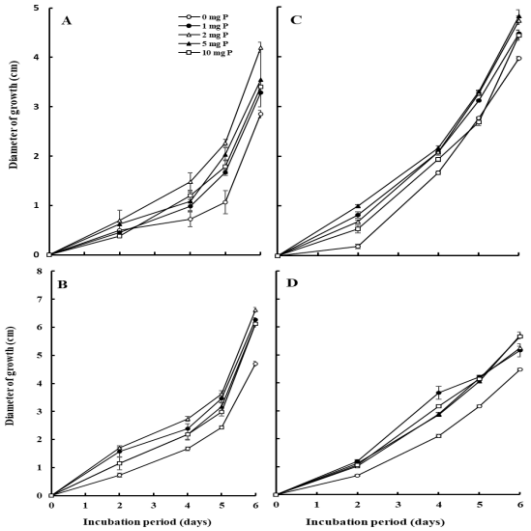

Fig. 2 Time course of growth of *P. pulmonarius* in response to the mode of culturing and form and dose of phosphorus. Each value is the mean of three replicates ± SE. Phosphorus was applied to sterilized rice straw at 0, 1, 2, 5 and 10 mg/g DW either as

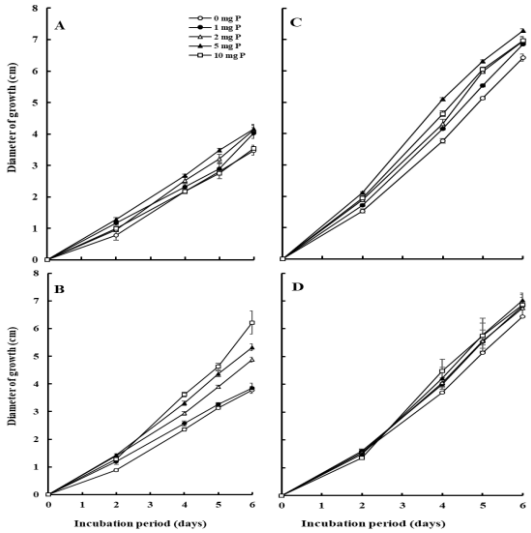

Fig. 3 Time course of growth of *P. floridanus* in response to the mode of culturing and form and dose of phosphorus. Each value is the mean of three replicates ± SE. Phosphorus was applied to sterilized rice straw at 0, 1, 2, 5 and 10 mg/g DW either as KH_2PO_4 or wheat bran with no agar layer (A and B, respectively) or in the presence of an agar layer over straw (C and D, respectively).

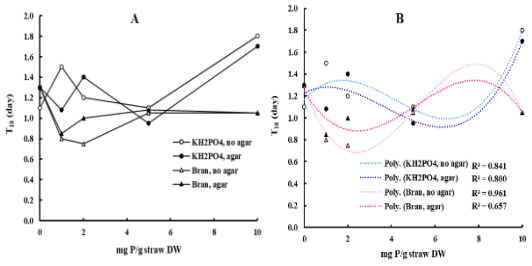

Fig. 4 Effect of P supply on the lag period of *P. pulmonarius* growth estimated as the time corresponding to 10% of the final fungal growth (T_{10}) in interaction with the mode of culturing and form of phosphorus (A). The best fit curves summarizing the dose-response relationship of P-T_{10} (B).

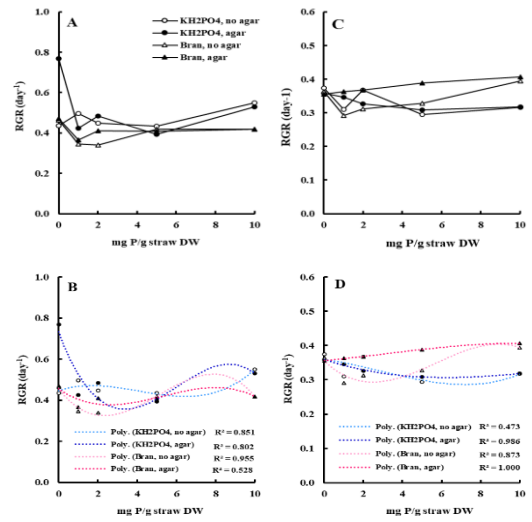

Fig. 5 Effect of P supply on relative growth rate (RGR) of *P. pulmonarius* (A) and *P. floridanus* (C) across the period 2–6 days in interaction with the mode of culturing and form of phosphorus. The best fit curves summarizing the dose-response relationship of P-RGR of *P. pulmonarius* (B) and *P. floridanus* (D).

During the exponential phase of growth (the period 2nd–6th day), RGR of *P. pulmonarius* was higher than that of *P. floridanus*, with relatively marked effect of treatments (form and supply of P and mode of culturing) in the former species (**Fig. 5A, 5C**). In *P. pulmonarius*, the RGR-P supply relationship followed a periodic third-degree pattern. In the KH_2PO_4 the RGR-P supply relationship of *P. pulmonarius* exhibited marked minima and maxima around 3 and 9 mg P/g straw DW, respectively in the presence of agar layer versus faint maxima and minima at 2 and 8 mg P/g straw DW, respectively in absence of agar. But, in bran-cultured *P. pulmonarius*, the periodic pattern was independent of the agar layer, with minima at 2 mg P/g straw DW and maxima at 8 mg P/g straw DW, which were relatively faint in the presence of agar but marked in its absence. Similar to the pattern of T_{10}, RGR of *P. pulmonarius* attained comparable values of about 0.4 day^{-1} at 5 mg P/g straw for the different P form × culturing mode combinations (**Fig. 5B**). In *P. floridanus*, the RGR-P supply relationship of the KH_2PO_4 culture was almost linear mild progressive reduction with the increase in P supply irrespective of the presence of the agar layer. In the bran culture, there was a small increase in RGR with the increase in P supply that was comparable for the two agar treatments; but while the increase was progressive in the presence of agar it occurred post a minimum at about 2 mg P/g straw DW in absence of agar.

Discussion

Phosphorus is one of the most essential elements for the growth of fungi in general and mushrooms since it is involved in the formation of the cytoskeleton, phospholipids, energy transfer molecules, intermediary metabolism, synthesis of nucleic acids and as a part of co-enzymes. Phosphorus plays a central role in the integration of signals relevant to nutrient acquisition and adaptation to stress conditions. In this regard, phosphate deprivation generally impairs fungal growth and virulence of fungal pathogens (Bhalla et al., 2022). It has been claimed that phosphorus deficiency causes greater inhibition of mushroom mycelial growth than any other mineral deficiency (Kamal et al., 2012).

Strikingly, the present work reveals that in contrast to autotrophs such as higher plants (Lambers, 2022) and algae (Dyhrman, 2016) which generally exhibit preference of inorganic P (Pi) over organic P, heterotrophs, e.g. the two investigated oyster mushrooms (*P. pulmonarius* and *P. floridanus*) seem to prefer organic P (wheat bran) over P_i (KH_2PO_4). The main storage form of P in wheat bran is phytates (Guo et al., 2015). Phytates are the Ca and Mg salts of phytic acid (myo-inositol-hexaphosphate), along with traces of micronutrients such as Zn and Fe to which phytic acid express high affinity (Wang et al., 2021). The claim that the ability to assimilate organic P is ubiquitous in heterotrophs can be supported by the fact that, in higher plants, pollen grains (in fact, the microspores) upon germination give rise to the male gametophyte (germ tube) – the analogous

of fungal hyphae in being a heterotrophic entity–also exhibit a unique ability to utilize storage phytates as a source of P by the aid of phytase enzyme (Lambers, 2022). Phytates are also found in non-photosynthetic tissues of higher plants such as the roots and tubers and can be utilized as storage P (Campbell et al., 1991).

It is quite acceptable that fungi, as decomposers, have acquired the ability to utilize organic P in addition to the inherent ability to assimilate inorganic P. Generally, fungi are intimately involved in soil mineral weathering and element cycling (Amundson et al., 2007); their mycelia can efficiently colonize both organic and mineral soils with the production of a variety of active compounds such as enzymes (Voříšková et al., 2011) and organic acids (vanSchöll et al., 2006). But excitingly the two investigated mushroom fungi seem to prefer organic P over P_i. The advantage of phytates over P_i, observed in the present work, can be traced in a more than a P-supply factor. To assimilate phytates as a source of P, the fungus has to secrete phytase enzymes to release P in the form of the most available form that is P_i. But this occurs also probably with the concomitant release of some nutrients, e.g., Ca, Mg, Mn, Zn and Fe (Cheng & Hardy, 2002; Perera et al., 2018; Nielsen et al., 2013; Moretti et al., 2014) in addition to the release of some beneficial intermediates that can stimulate fungal growth further. Phytase activity is ubiquitous in edible mushrooms, including *Pleurotus* spp. with marked genotypic variability (Collopy & Royse, 2004), and this potentiality can be manipulated in solid-state fermentation systems to utilize agricultural wastes as raw materials for production of added value products (Awad et al., 2014; Huang et al., 2018). However, it is worth to note that since wheat bran contains other constituents in addition to phytate, these constituents can contribute to the observed enhancement of mycelial growth. Therefore, assigning the advantage of wheat bran to phytate must be taken with care; and this claim, in fact, needs precise verification using pure phytate.

On the other side, phytic acid can act as a cation trap that eliminates excessive cellular concentrations of K^+ and Mg^{2+}. The high affinity of phytic acid for Zn^{2+}, Fe^{2+} and other heavy metals may be important for heavy-metal binding and, thereby, detoxification. This means that phytate plays a prominent role in mineral homeostasis through buffering of the mineral content of the substrate within favorable limits. Another aspect of the beneficial role of phytate for fungal growth can be derived from the fact that upon degradation of phytate, various inositol phosphates intermediates are produced, some of them constitute a significant proportion of the phospholipid fraction of membranes. In addition, inositol-1,4,5(tri) phosphate–an intermediate of phytate degradation–can act as a secondary messenger regulating Ca^{2+} channels in cell membranes (Isayenkov et al., 2010) which modulates the activity and cellular location of Ca^{2+}-binding proteins and affect gene expression of the organism, its physiology and growth (Kim et al., 2018).

The present work suggests that the advantage of bran over KH_2PO_4 was particularly evident in absence of the agar layer above the rice straw substrate. In addition, the agar layer, by virtue of its differential beneficial role in favor of KH_2PO_4, can narrow the gap between bran and KH_2PO_4. The advantage of the agar layer for KH_2PO_4 culture can be attributed to moderation of P flow from KH_2PO_4 to the fungal body which ensures uniform and steady supply of P to the mycelium. In the bran culture, it is also probable that the agar layer can moderate the contents of micronutrients that arise from phytate hydrolysis, some of which might occur at too high levels to hinder fungal growth. The agar layer is expected also to slow down the flow of phytase enzyme from fungal mycelium to the substrate and the back flow of phytate hydrolysates upward to the fungal mycelium. These two contradictory roles of the agar layer with the bran culture can account for the limited beneficial effect of the agar layer with bran as a source of P relative to the marked beneficial effect with KH_2PO_4.

The present findings point also to the genotypic differences among the two oyster mushrooms in response to the source of P and presence of the agar layer, since the preference of bran over KH_2PO_4 and also the advantage of the agar layer followed different patterns in the two fungi. Whereas the advantage of bran over KH_2PO_4 for *P. pulmonarius* was markedly evident in absence of the agar layer and still existent but less evident in its presence; the advantage of bran over KH_2PO_4 for *P. floridanus*, was evident only in absence of agar. Likewise, whereas the advantage of the agar

layer was consistently evident in *P. floridanus* in the two P forms, but with particular emphasis in KH_2PO_4, the advantage of the agar layer was evident only with KH_2PO_4 in *P. pulmonarius*. In addition, presence of the agar layer moderated the dose-response pattern of mycelial growth, either the pre-optimum increase or the post-optimum decrease and narrowed the gap between bran and KH_2PO_4. In general, the growth response to P supply was more evident in *P. pulmonarius* than in *P. floridanus*.

Another aspect of the genotypic differences among the two oyster mushrooms in response to source of P and presence of the agar layer emerged from considering the time-course pattern of fungal growth under the different combinations of P form, P dose and mode of culturing. While growth of *P. pulmonarius* exhibited the typical sigmoidal time-course pattern with distinct lag and log phases, the time-course pattern of *P. floridanus* growth was approximately linear, which allowed calculation of the lag period in *P. pulmonarius*. In accordance with its preference of organic P, the lag period of *P. pulmonarius* growth was shorter with bran than with KH_2PO_4, particularly at high P supply, with contrasting dose-response patterns in the two P forms, where the minima of bran coincided with maxima of KH_2PO_4. Presence of the agar layer seems to smoothen the dose-dependent fluctuation in lag period only with bran. The effect of treatments (P form, P supply and presence of the agar layer) on mycelial RGR was more evident in *P. pulmonarius* than in *P. floridanus*. The RGR was higher in *P. pulmonarius* than in *P. floridanus*, with a dose-dependent periodic pattern which was more evident in the former species. Also, a smoothening effect of the agar layer on the periodic dose-dependent fluctuation in RGR– which was synchronized in the two fungi– was observed in the bran culture versus a sharpening and phase-reversal in KH_2PO_4 culture.

The data also reveal that at moderate P supply (5 mg/g straw) the length of the lag period and the value of RGR seem robust to variation in P form and presence of the agar layer, with almost the same value (1 day for the lag period and 0.4 day^{-1} for RGR) at the four P form × agar combinations. However, the sigmoidal growth curve of *P. pulmonarius* and the linear curve of *P. floridanus* points for instantaneous acclimation to culturing conditions in *P. floridanus* with a relatively low rate of growth versus a sluggish acclimation in *P. pulmonarius*. In agreement with the present findings, addition of P as KH_2PO_4 to *Agaricus bisporus*, *Pleurotus floridanus*, *Volvariella volvacea* mushrooms grown on liquid Czapek's Dox medium, enhanced mycelial growth and protein production with marked genotypic variability in optimal P level (Kamal et al., 2012).

Conclusions

In contrast to preference of inorganic P (P_i) by autotrophs (higher plants and algae), the two oyster mushrooms *P. pulmonarius* and *P. floridanus*–as heterotrophs– seem to prefer organic P (phytates of wheat bran). The advantage of wheat bran over P_i can be attributed partly to the release of minerals (Ca, Mg, Mn, Zn and Fe) and beneficial intermediates of phytate hydrolysis. Also, phytate, as a cation exchanger, can buffer the mineral content of the substrate within favorable limits. Presence of an agar layer above rice straw substrate can narrow the gap between bran and KH_2PO_4, probably via moderation of P flow from KH_2PO_4 to the mycelium and of the micronutrients that arise from phytate hydrolysis. The effect of P supply on mycelial growth was more evident in *P. pulmonarius* than in *P. floridanus*. While the time-course of *P. pulmonarius* growth exhibited a typical sigmoidal pattern with distinct lag and log phases, it was almost linear in *P. floridanus*; this means instantaneous acclimation to culturing conditions in *P. floridanus* versus a sluggish acclimation in *P. pulmonarius*.

Author contributions

Amira A. El-Fallal: Conceptualization, Reviewing and editing; **Taha M. El-Katony:** Methodology, Data visualization, Statistical analysis, Reviewing and editing; **Heba E. Dahap:** Conducting experiments and taking measurements, draft preparation; **Hoda M. El-Gharabawy:** Data visualization, Writing manuscript, Reviewing and Editing, Manuscript submission.

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الملخص العربي

عنوان البحث: تأثير شكل الفوسفور وطريقة الاستزراع على نمو الغزل الفطري لنوعي الفطر المحاري*Pleurotus pulmonarius* و *Pleurotus floridanus*

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اكتسبت زراعة الفطريات المحارية اهتماما متزايدا بسبب متطلبات نموها المحدودة وقيمتها الغذائية العالية وغناها بالمركبات النشطة بيولوجيا. يدرس البحث الحالي تأثير إضافة الفسفور فى صورته غير العضوية (KH_2PO_4) والعضوية (نخالة القمح) إلي قش الأرز فى وجود أو غياب طبقة أجار فوق القش على النمو الفطري لنوعين من فطر المحار *P. pulmonarius* و *P.*

floridanus. كان نمو الفطريات أفضل مع النخالة منه معKH2PO4 ، خاصة بدون أجار. كان هذا التأثير التفاضلي لشكل الفسفور وطبقة الأجار أكثر وضوحا على P. pulmonarius منه على P. floridanus. أظهرت علاقة نمو الغزل الفطري بتركيز الفوسفور في الغالب نمطا تشبعيا حيث كان التركيز الأمثل الفسفور في الوسط ٢–٥ ملجم فسفور/ جم من القش مع الميل الى الثبات عند التركيزات الأعلى . فقط في حاله مزرعه P. floridanus المتحتوية علي النخالة وبدون اجار ، اظهر نمو الفطر زيادة مطردة بزيادة تركيز الفسفور. كذلك كان المسار الزمني للنمو الفطري مختلفا في الفطرين إذا أبدى النمط السجمويدى فى فطر P. pulmonarius مقابل النمط الخطي في فطر P. floridanus. في فطر P. pulmonarius ، كانت فترة تلكؤ النمو (T10) أطول مع KH2PO4 مقارنة بالنخالة ، مع أنماط مختلفة معتمدة على جرعة الفوسفور حسب صورة الفسفور وتأثير هامشي لطبقة الأجار. كان معدل النمو النسبي (RGR) لفطر P. pulmonarius أعلى من فطر P. floridanus مع أنماط مختلفة معتمدة على الجرعة وفقا لنوع الفطر ووجود الأجار. في فطر P. pulmonarius، حقق كل من T10 و RGR قيما متقاربة تبلغ حوالي يوم واحد و٠,٤ يوم -١ ، على التوالي عند ٥ مجم فسفور/ جم قش بصرف النظر عن صورة الفسفور وطريقة الاستزراع.