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Studies on Some Rquirements for Vegetative Growth of Two *Pleurotus* spp.

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EFFECT of some physiological factors on the growth of *Pleurotus columbinus* and *Pleurotus pulmonarius* have been investigated. The tested fungi had the same optimum temperature range 25-30°C. *P. columbinus* was slightly more tolerant to higher temperatures than *P. pulmonarius*. Both fungi preferred neutral medium and widely used carbohydrates. Sucrose and galactose were the best carbon sources for *P. pulmonarius* and *P. columbinzus,* respectively. Asparagine was efficiently utilised by the two fungi. Growth response was strong at a wide range of 0.04-0.48% nitrogen in the medium which is equivalent to 32:1-2.63:1 C/N ratios

Key words : *Pleurotus Columbinus, p. Pulmonarius,* Mushroom hysiology, Pleurotus Nutrition .

The need to exploit the potential of lignocellulosic agricultural wastes as mushroom cultivation substrates, led to the study of the influence of certain environmental parameters on the *in-vitro* growth of *Pleurotus* spp.

Oyster mushroom cultivation has been introduced in Egypt in the last two decades (Mohamed & El-Kattan, 1989). Although some studies on *Pleurotus* spp. have been undertaken on degradation of lignocellulosic wastes (Carmarero *et al.,* 1996; Lang *et al.*, 1996; Tan & Wahab, 1997; Lang *et al.*, 1998 and Kuz`mina *et al.,* 2001), little information is available on the influence of physiological factors on the growth of *Pleurotus* spp.

The aim of this work is to shed more light on the physiological requirements of *Pleurotus columbinus* and *Pleurotus pulmonarius* and thus to interpret the ways in which growing systems affect the mycelial growth.

Material and Methods

Pleurotus columbinus Quel.ap.Bres and *Pleurotus pulmonarius* Fr. were obtained from the consulative Comet Company of mushroom cultivation-Egypt (CCCM). They were maintained at 30°C on 1.5% malt agar (MA) slopes.

Measurement of growth over a range of temperatures and pH

Linear mycelial growth was measured on plates of potato dextrose agar medium (PDA). The plates were incubated at temperatures between 15-40°C for 4 days. Four replicates were prepared for each incubation temperature. In the study of pH the PDA medium was adjusted to the required pH values in the range of 3-9 by adding sterile lN-HCl or 1N-NaOH after autoclaving at 1b.per sq. inch (p.s.i) for 15 min . The ability of *Pleurotus* spp. to grow at different pH values was also determined by measuring the mycelial dry weight produced by growth of the two fungi in 25 ml of Dox liquid medium in 250 ml Erlenmeyer flasks over a range of 3-9 pH values. Phosphate buffer was prepared in the ratios given by Hale (1965). Treatments were replicated 4 times and incubated at 30°C for 7 days. Harvesting was carried out by filtering the mycelium through Whatman no. 3 filter paper of known dry weight. The filter paper and mycelium were then dried at 80°C for 48 hr and reweighed.

Nitrogen and carbon sources

Several nitrogen sources were tested for their ability to support the growth of both *Pleurotus* spp. Sodium nitrate in Dox medium was substituted with equimolecular different nitrogen sources. The following nitrogen sources were tested separately: asparagine, ammonium nitrate, potassium nitrate, ammonium phosphate, urea and glutamic acid in comparison with sodium nitrate.

The fungi were grown on 25ml liquid Dox medium, the basic procedures being the same as outlined above. The initial pH was adjusted to 7 initially.

Likewise, for determination of the best carbon source, the following carbon sources were each used to replace sucrose in the Dox medium: glucose, fructose, maltose, lactose, galactose, raffinose, sorbose, inositol and arabinose in comparison with sucrose. The concentrations used also correspond to the original carbon concentration in the Dox medium. Each treatment was replicated 4 times and incubated at 30°C for 7 days.

The results of the experiments were statistically analyzed using the Least Significant Difference (L.S.D) test at 5% level of probability (Snedecor & Cochran, 1969).

Results

Both *Pleurotus* spp. grew well over a range of temperatures from 25-30°C, although at the latter temperature both fungi grew significantly better than at 25°C (Fig. 1). At low temperatures *P. columbinus* grew slightly better than *P. columbinus*, while the latter species grew slightly better at higher temperatures. These differences, however, were small and often not significant. The sharp transition of both *Pleurotus* spp. mycelia growth above 32°C has been noticed. Figures 2&3 show the pH tolerance of both fungi in two different media. In Dox liquid medium, the increase in growth at , pH 4, 5&6 (Fig. 2) was less marked than on PDA, with an optimum at pH 7 for both *Pleurotus* spp. On PDA,the maximum colony diameter in 5 days was attained at pH values within the range 6-7 with no significant difference between the growth means of both *Pleurotus* spp. *P. columbinus* appeared more tolerant for low pH-values and less so for high pH-values than *P. columbinus*.

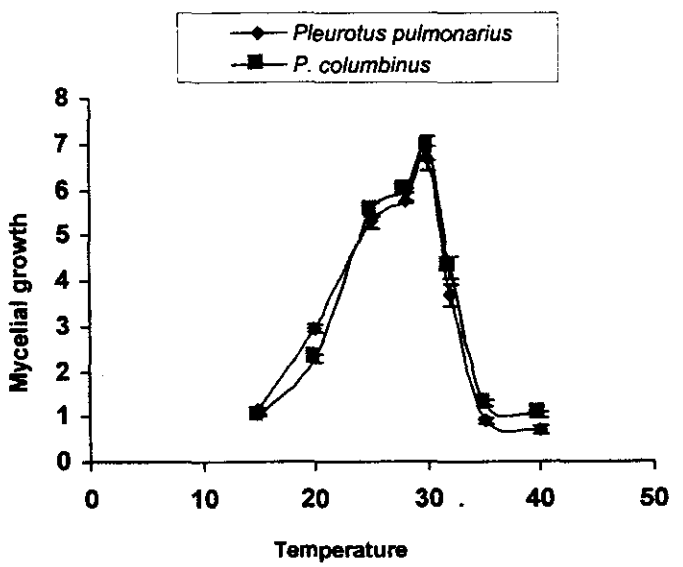

Fig .1. Effect of different temperature on mycelial growth of *Pleurotus pulmonarius* and *P . columbinus* .

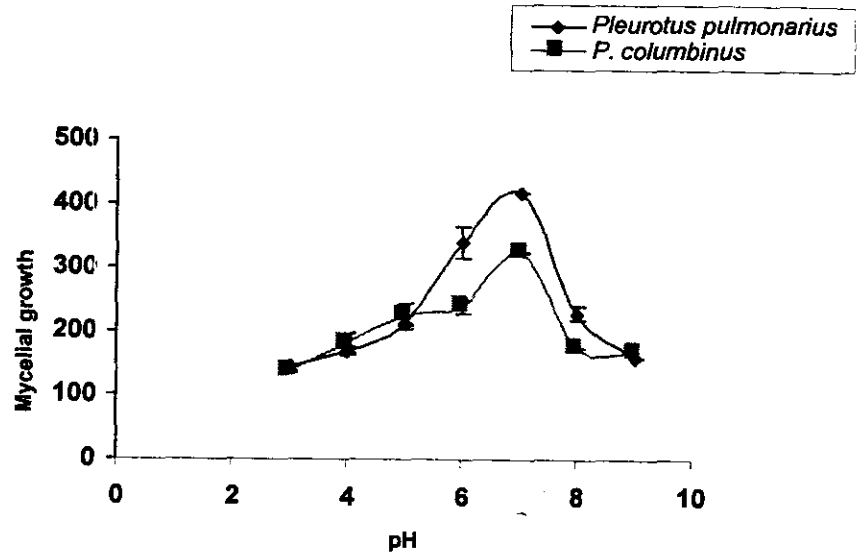

Fig .2. Effect of different pH values on mycelial growth of *P. pulmonarius* and *P . columbinus* in liquid medium .

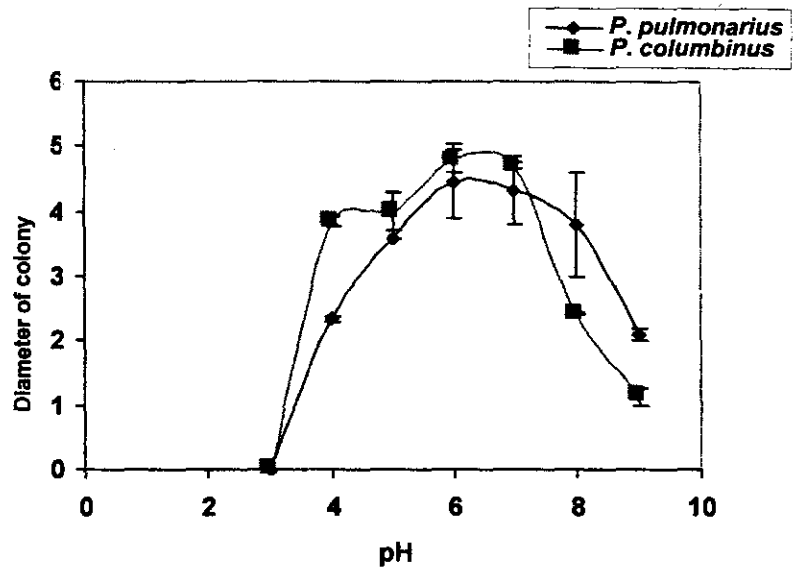

Fig . 3. Effect of different pH values on mycelial growth of *P. pulmonarius* and *P. columbinus* on solid media.

Asparagine greatly surpassed all other nitrogen sources tested in stimulating fungal mycelia growth. The differences between the other 6 sources of nitrogen were small and in most cases not significant (Fig. 4).

Within the range of nitrogen levels of asparagine tested, the growth response by *Pleurotus* spp. was strong and the differences were small. The data indicate that the upper limit of *Pleurotus* spp. tolerance to asparagine-N had not been approached at 4.8gN/l. There is no essential difference in growth value of each fungus at the range of 0.4-4.8 g N/l (Fig. 5). It can be concluded that *Pleurotus* spp. had an optimum nitrogen requirement in terms of asparagine-N between 0.4-4.8g N/l.

The two fungal species showed clearly differential growth responses to the 10 carbohydrate sources. The best growth values of *P. pulmonarius* were obtained with sucrose and glucose as carbon sources, whilst galactose supported the highest mycelia growth of *P. columbinus* followed by lactose (Fig. 6). In contrast, the least growth of *P. pulmonarius* and *P. coluinbinus* were actually obtained in lactose and glucose, respectively.

Galactose, maltose and arabinose supported fairly good mycelia growth of *P. pulmonarius*, while the two latter carbohydrates also supported good growth of *P. columbinus*.

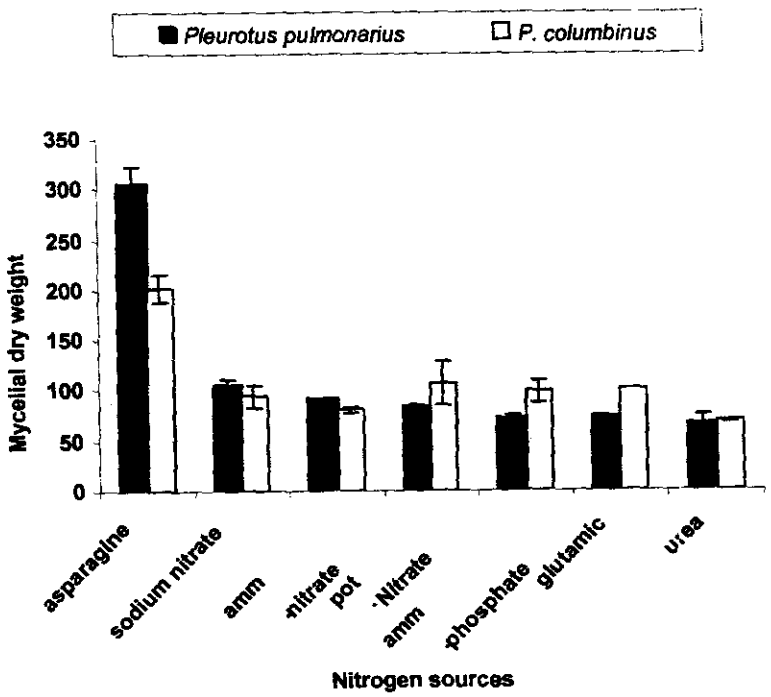

Fig .4. Effect of different nitrogen sources on mycelial growth of pleurotus spp.

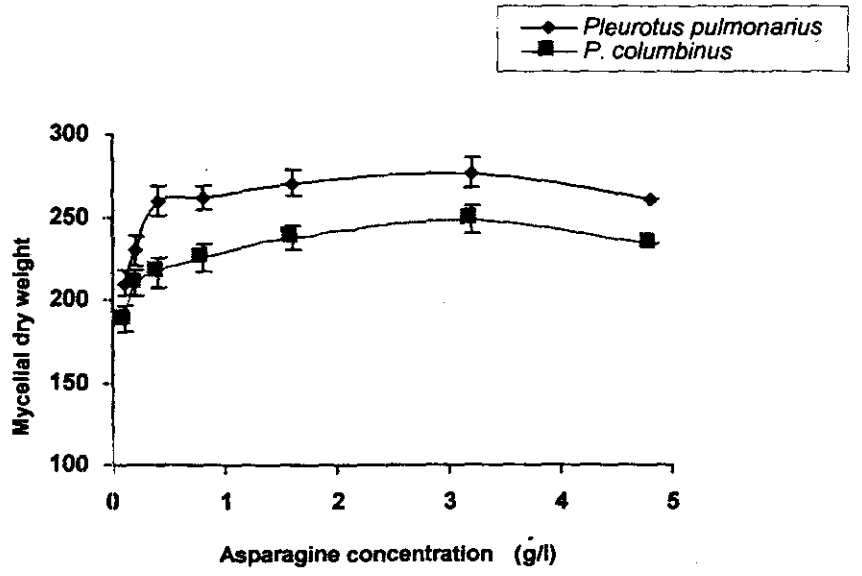

Fig .5. Effect of different asparagine concentratios on mycelial growth of pulmonarius and *P . columbinus* .

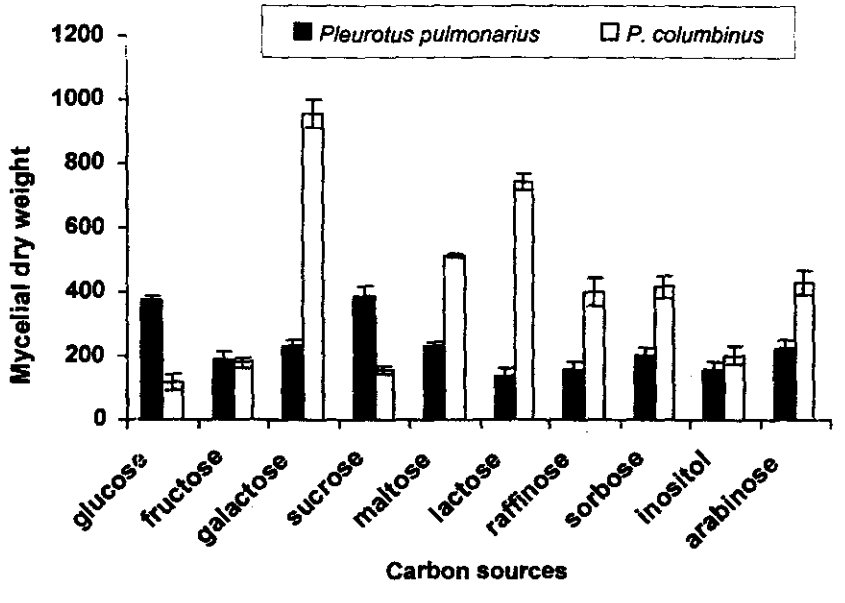

Fig . 6. Effect of different carbon sources on mycelial growth of both *Pleruotus* spp .

Discussion

The optimum temperatures range of *Pleurotus* spp. mycelial growths of 25-30°C, reflect its natural environment in tropical and subtropical countries. The data agreed with those of Hosni (1996) and Zervakis *et al.* (2001) who reported 25°C for mushroom production and 30°C for mycelia growth of P. *pulmonarius* as optima, respectively.

Both *P. pulmonarius* and *P. columbinus* appear to be able to grow over a wide range of pH values from 3-9, with pH 6-7 as an optimum for both species. Hashimoto and Takahashi (1976) found that pH range (6.5-7) was optimum for *Pleurotus ostreatus* growth. However, other mushroom species, namely *Agaricus campestris, Coprinus comatus, Pleurotus sp, Podaxis pistillaris* and Volvariella volvacea grow well at pH 5.5 (Chandra & Purkayastha, 1977; Child *et al.,* 1974; Rao, 1983; Ofosu *et al.,* 1984 and Phutela *et al.,* 1998), while Volvariella volvacea grew best at pH 6-8 (El-Fallal, 1990).

Asparagine sustained the greatest growth among the nitrogen compounds tested ($p < 0.05$). Hashimoto and Takahashi (1976), Khanna and Garcha (1985) and Madunagu (1988) obtained similar results on different *Pleurotus* spp. Asparagine also supported high growth of *Podaxis pistillaris* (Phutela *et al.,* 1998). Results from other basidiomycotina including the cultivated species are similar to *Pleurotus* spp. Nearly all show optimal mycelia growth on asparagine. Species studied include *Agaricus bisporus* Treschow, 1944, and Fermor & Wood, 1981), *Lentinus edodes* (Leatham, 1985) and *Pholiota destruens* (Krishna & Sharma, 1987).

Urea supported some growth of both *Pleurotus* spp. Leung *et al.* (1965) also found that urea decreased the growth of *Psilocybe baeocystis* . However, urea has been reported to support good growth for many fungi (Pateman & Kinghorn, 1976) and for basidiomycetes *e.g .Volvariella volvacea,* Agaricus *campestris* and *Pleurotus tuber-regium* (El-Fallal, 1990; Casmir & Heinemarin, 1953 and Fasidi & Olorunmaiye, 1994).

Nitrate can be utilized by both fungi and supported greater growth as a nitrogen source than inorganic ammonium sources. Studies on higher fungi divided them into nitrate utilizers and nitrate-non-utilizers. *P. sajor caju* and

V. volvacea were suggested to be nitrate utilizers (Mention & Plassard, 1983; Plassard *et al.*; 1986 and El-Fallal, 1990). The carbon: nitrogen ratio affected mycelia growth of both *Pleurotus spp.* The Highest significant growth was achieved from 8:1 until, it reached the optimum at 4:1, but the difference was not statistically significant ($p < 0.05$). Growth at 32:1 is not significantly different from 2:1 indicating that *Pleurotus* spp. are able to utilize substrates that are rich in either carbon or nitrogen. These results agreed with those obtained by Fasidi & Olorunmaiye (1994) for *P. tuberregium,* Chandra & Purkayastha (1977) for *A. campestris* and Chang-Ho & Ho (1978) and El-Fallal (1990) for *V. vohacea.* The best growth of *Podaxis pistillaris* was observed at C/N ratio 20:1 (Phutela *et al.*, 1998)

The mycelia growths of both species were compared on 10 different carbon sources. Glucose efficiently supported the mycelial growth of *P. pulmonarius* and maltose supported good growth of both species. This is in agreement with the results of Hashimoto & Takahashi (1976), and Hong (1978) on *P. ostreatus.* It has been found that *P. ostreatus, Lentinus edodes* and *Agaricus bisporus* grew best in media containing glucose as the sole source of carbon (Labaneiah *et al.*, 1977). Cailleux *et al.,* (1976) studied six isolates including *P. pulmonarius* and *P. columbinus* and reported that all *Pleurotus* isolates grew well on maltose, sucrose, fructose and glucose. Galactose was preferable above glucose by *P. columbinus.* This agrees with results of Rypacek (1977) on *P. ostreatus.* Low efficiency of lactose as a carbon source was indicated by Abou El-Seoud (1962), Guha and Banerjee (1971) and Labaneiah *et al.,* (1977) and was attributed to its slow rate of assimilation. Inositole showed little effect on *P. pulmonarius* as reported by Voltz (1972) on *P. ostreatus.*

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دراسات على بعض متطلبات النمو الخضري لنوعين من فطرة بليوروتس

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في هذه الدراسة تم دراسة تأثير بعض العوامل الفسيولوجية على النمو لفطرتي بليوروتس كولمبينس وبليوروتس بالمونيرس واتضح أن درجة الحرارة المثلى للفطرتين المختبرتين كانت بين ٢٥-٣٠م وأن بليوروتس كولمبينس كان أكثر تحملاً لارتفاع الحرارة عن بليوروتس بالمونيرس وأن كلاً من الفطرتين فضلتا الوسط المتعادل ومقدره على استخدام الكربوهيدرات . ووجد أن السكروز والجلاكتوز هما أفضل المصادر الكربونية للفطرتين بالمونيرس وكولمبينس على التوالى . واتضح أن الأسبراجين كمصدر نيتروجين هو الأكثر فعالية لهما . وكانت استجابة النمو قوية عند تركيزات ٠,٤ - ٠,٤٨ نيتروجين في الوسط الغذائى والتى تكافىء ١:٣٢ - ٢,٦٣ : ١ من نسبة C/N.