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Original Research Paper

Studies on Phytochemical Screening and Enzyme Activities of Composted and Uncomposted *Pleurotus pulmonarius* at various Fruit bodies and Substrate Stages

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ABSTRACT

The intracellular enzyme activities and some secondary plant metabolites of *Pluerotus pulmonarius* (fries) singer were determined at different stages of sporophore development using different substrates containing sawdust (86%) + rice bran (10%) + cassava peel (4%). Enzyme activities increased from very young to mature fruit body of composted (cellulase, mg Glucose/min/g protein 17.39, 26.20 and 48.65, Total (α and β), amylase mg maltose/min/g protein 18.27, 18.58 and 95.36, α-Amylase, mg maltose/min/g protein 11.99, 18.88 and 69.80, Proteinase, mg maltose/min/g protein 6.00, 6.82 and 9.16 and Lipase,ml0.02/NaOH/min/g protein 0.72, 1.18 and 5.36) substrate and uncomposted (cellulase, mg Glucose/min/g protein 19.97,25.38 and 36.37, Total (α and β), mg Glucose/min/g protein 33.86, 37.01 and 64.37, α-Amylase, mg Glucose/min/g protein mg Glucose/min/g protein 20.05, 31.32 and 61.37, Proteinase, mg Glucose/min/g protein 3.47, 4.23 and 5.23 and Lipase, ,ml0.02/NaOH/min/g protein 0.88, 1.27 and 1.36) substrate respectively with the preponderance of total amylase activities of both composted and uncomposted substrate. Saponins and anthraquinones were absent all various fruit bodies of both composted and uncomposted substrates, equal concentration of alkaloids were detected in both composted and uncomposted *Plerotus pulmonarius* substrate. The presence of intracellular enzymes helps to convert insoluble substrate to soluble component that could be utilized nutritionally by the growing sporophore. Phytochemical detected indicated that *Pleurotus pulmonarius* can be used or serves as a novelty medicine.

Key word: Compost Substrate, Uncompost Substrate, Enzyme activities, Phytochemical, *Pleurotus pulmonarius*

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INTRODUCTION

Species of the genus *Pleurotus* are importance industrially occupying third place in worldwide production of edible mushroom (i.e. 16.8% of the total production) (Chang, 1996). In Mexico, *Pleurotus* cultivation was introduced in 1974 and production of this important mushroom increased four-fold from 1990 to 1997 (Martinez-Carrera, 2000). A particularly, attractive feature for the commercial production of this mushroom is that it produces large amount of protein on substrates consisting primarily of waste materials (Poppe, 2000). Furthermore, degradative capacity and enzymatic activity have been well documented for *Pleurotus* spp. (and other edible fungi) when cultivated on straw and other waste pulps (Pal et al., 1995; Alarcon, 1997; Geetha and Swaprakasam, 1998). Enzymes had been detected in mushroom fruit bodies and mycelia. Among these enzymes is polyphenol oxidase, also called O-diphenol oxidase or tyrosinase, the enzyme responsible for browning in mushrooms.

Biochemical changes in edible mushroom are brought about by oxidizing and hydrolytic enzymes produced by the developing sporocarp. (Norkrans, 1967; Yamaguchi et al., 1979; Parangpe and Chem, 1979). Enzyme converts insoluble substrate into soluble component that could be utilized nutritionally by the growing sporocarp (Jabloskey, 1981). Kadiri and Fasidi (1990) have also shown that the activities of amylase, proteinase, cellulase and poylphenol oxidase increased from very young to

mature sporophores in *Pleurotus tuber-reguim* and *Tricholoma lobeyensis*. Polyphenol oxidase activity has the characteristic ability to break down lignin (Rajarathnam and Zakia, 1988). A number of phenol oxidase involved in lignin degradation are reported to be secreted by these mushroom species (Rajarathnam et and Zakia 1998).

Mushrooms are also known to possess medicinal value. Oyster mushroom and Shitake have been reported to contain substances which lowered the cholesterol level in serum and liver of rats (Oei, 1996). Species of mushrooms contain substrates, which suppress the growth rate of tumors like Lentinan from shiitake (*Lentinula edodes),* Schizophylla from *Schizophyllum* commune and Grifola (from *Grifola frindosa*). An extract of the monkey head mushroom *(hericium erinaceus)* is used to enhance the effect of chemo – or radiotherapy and well being of the patients (Oei, 1996). *Boletus edulis* and *L. edodes* have been reported to be antitumor and hypocholestronic agent respectively (Lucas et al., 1957; Suzuki and Oshima, 1976). *Faumulina velutipes*, *Volvariella volvacea* are known to possess anti-cancerous properties and to lower blood pressure (Bahl. 1998).

The medicinal values of the mushroom enumerated above could be due to their contents of secondary plant products.

The aim of this study was therefore to screen for the presence of plant secondary metabolites and to investigate the intracellular enzyme activities of *Pleurotus pulmonarius* at different fruit body stages and to know which parts that are metabolically most active.

MATERIALS AND METHODS

Fresh sporophores and substrate of *Pleurotus Pulmonarius* were harvested at three developmental stages according to the method used by Gruen and Wong (1982) and Hammond and Nicholas (1975). These developmental stages were designated as follows: very young stage (pileus, 0 -2cm), young stage (pileus, 2.1-4.0 cm) and mature stage (pileus, 4.1-9.0 cm).

Enzyme determination

Cellulase activity: The enzyme extract was prepared by grinding 1g of frozen pileus with 1/10M dibasic sodium phosphate (K_2HPO4) in a mortar maintained at 5^OC with crushed ice (Norkrans, 1957). The ensuring suspension was centrifuge at 18,000g for 30mins at 2^OC using the M.SE ultra high speed centrifuge. To 1 ml of the supernatant were added 1ml of 1% carboxymethyl cellulose in 0.05 M phosphate buffer (pH 5.0) and the mixture was allow to stand for 1h at 30^OC (Singh and Kunene, 1980). The enzyme action was stopped with 3, 5 - dinitrosalicylic acid (DNSA) reagent and the amount of reducing sugar formed was determined by taking the absorbance at 540nm against a blank containing 1 ml of boiled enzyme extract which was similarly treated. (Denison and Koelin, 1977).

Total amylase (α and β activity): Enzyme extract was prepared by grinding 1g of frozen pileus with sodium acetate buffer (pH 5.0) in a mortar maintained at 5^OC with crushed ice and the extract centrifuge at 18,000g for 30mins at 2^OC. 1ml of the supernatant was added 1ml of 1% soluble starch in 1/10M sodium acetate buffer and the mixture was incubated at 27^OC for 1h. The enzyme action was stopped with DNSA reagent and the quantity of reducing sugar formed was determined by taking the absorbance at 540nm against a blank containing 1ml of boiled enzyme extract treated similarly (Swain and Dekker, 1966).

Amylase activity: 5ml of the supernatant obtained for total amylase enzyme extract was heated at 70^OC for 15mins to denature the B-amylase.
To 1m of the heated extract was acted 1ml of 1% soluble starch in 1/10M sodium acetate buffer (pH 5.0) and the mixture was incubated at 27^OC for 1h. The enzyme activity was stopped with DNSA reagent and the reducing sugar formed was assayed for total amylase activity.

Proteinase activity: Enzyme extract was prepared in a manner similar to total amylase activity. Except that 0.05M sodium phosphate buffer (pH 6.0) was used as the extracting buffer proteinase activity in the enzyme was determined using the LOWRY FOLIN –CIOCALTEU method of McDonald et al., (1965).

Lipase activity: Enzyme extract was obtained in the same way for total amylase activity. Lipase activity in the enzyme extract was determined using the method of Young and Word (1977).

Test for phytochemicals

Alkaloids: This was done using mayer's and Dragendoff's reagents following the method described by kapor et al (1969) and Odebiyi and Sofowora (1978). One grain of powdered sample was extracted with 30ml boiling 95% ethanol in a soxlet extractor for 6h and the extract evaporated to dryness dry a vacuum evaporator.
The residue was re-dissolved in 5ml of 1% Hcl and the solution obtained was divided into equal parts. To one part seven drops of Mayer'sreagent were added and to the other, seven drops of Dragendoff's reagent. Turbidity or precipitation with both reagents was taken as an indication of presence of alkaloid.

Saponin: The persistent frothing test for saponin as described by kappor et al., (1969) and Odebiyi and

Table 1. Enzyme activities of *Pleurotus pulmonarius* at various fruit body stages produced on composted substrate.

Mushroom stage	Cellulase mg Glucose/min/g protein	Total ((α and β) Amylase mg maltose min/ g protein	α-amylase mg maltose/min/g protein	Proteinase mg maltose/ min/protein	Lipase ml ml 0.02/ NaOH/min/ g protein
Very Young	17.39	18.27	11.99	6	0.72
Young	26.2	18.58	18.88	6.82	1.18
Mature	48.65	95.36	69.80	9.16	5.36

Table 2: Enzyme activities of Pleurotus pulmonarius at various fruit body stages produced on uncomposted substrate.

Mushroom stage	Cellulase mg Glucose/min/g protein	Total ((α and β) Amylase mg maltose min/ g protein	α-amylase mg maltose/min/g protein	Proteinase mg maltose/ min/protein	Lipase ml ml 0.02/ NaOH/min/ g protein
Very Young	19.97	33.86	26.05	3.47	0.88
Young	25.38	37.01	31.32	4.23	1.27
Mature	36.37	64.78	61.37	5.23	1.36

Sofowora (1978) was used. To 1g of powdered sample, 30ml of distilled water were added. The mixture obtained was vigorously shaken and warned frothing that persisted for 30mins was taken as an indication of saponin presence.

Tannin: Five hundred milligrams of powdered sample was shaken with 10ml of distilled water, the resulting mixture was vigorously shaken and filter. Seven drops of 10% $Fecl_3$ were added to the filtrate and the colour change to blue, blue-black, green or blue-green was taken as an evidence of tannin presence (Odebiyi and Sofowora, 1978).

Anthraquinone: One grain of powdered sample was shaken with 10ml of benzene and the mixture filtered. Ten milliliters 10% NH_4OH were added to the filtrate and the mixture transferred into a separately funnel to separate the two layers. The development of pink, red or violet colour in the ammoniacal layer indicates Anthraquinone presence (Odebiyi and Sofowora, 1978).

Results and discussion

Table 1 shows the enzyme activities at the various fruit body stages of *Pleurotus pulmonarius.* Cellulase, total (α and β) amylases, α - amylase, Proteinase an lipase activities were found to increase from very young to mature sporohores of composted substrate (Table 1). Similar result was obtained for uncomposted substrate (Table 2) were enzyme activities increase from very young to mature stages. The increase in enzyme activities with sporohore maturity is similar to the observed pattern of distribution of nutrients in the sporohores of these mushrooms (Fasidi and Kadiri 1990a Kadiri and Fasidi 1990b). A similar trend was detected by Paranjpe and Chen (1979)

Table 3a: Phytochemical screening of *P. pulmonarius* as very young, young and mature fruit body stages produced on composted substrate.

Mushroom Stages	Saponin	Tannin	Anthraquinone	Alkaloid
Very young	-	+++	-	+++
Young	-	+	-	+++
Mature	-	-	-	+++

+ to +++ indicate increasing concentration,– Indicate absence.

Table 3b: Phytochemical screening of P. *pulmonarius* as very young, young and mature fruit body stages produced on uncomposted substrate.

Mushroom Stages	Saponin	Tannin	Anthraquinone	Alkaloid
Very young	-	-	-	+++
Young	-	+++	-	+++
Mature	-	+	-	+++

+ to +++ indicate increasing concentration,– Indicate absence.

Table 4a: Phytochemical screening of *P. pulmonarius* at very young, young and mature Stages of composted substrate.

Mushroom Stages	Saponin	Tannin	Anthraquinone	Alkaloid
Spawn run	-	+	-	+
Very young	-	+	-	++
Young	-	++	-	++
Mature	-	+	-	++

+ to ++ indicate increasing concentration,- Indicate absence.

Table 4b: Phytochemical screening of *P. pulmonarius* at very young, young and mature Stages of uncomposted substrate.

Mushroom Stages	Saponin	Tannin	Anthraquinone	Alkaloid
Spawn run	-	+	-	++
Very young	-	+	-	++
Young	-	+	-	++
Mature	-	+	-	++

+ to ++ indicate increasing concentration,- Indicate absence.

for polyphenol oxidase and cytochrome oxidase activities of *Agaricus bisporus* sporohores. Saponin and Anthraquinone were absent all mushroom fruit bodies of both composted and uncomposted substrate (Table 3a, 3b and 4a, 4b). Tannin was detected in vary proportion of very young and young fruit body of composted substrate while it is absent at mature stage. For alkaloids the concentration was all the same at various fruit body stages of both composted substrate (Table 3a). Equal concentration of tannin was obtained at various substrate stages except for young stage that had increased. Similar result was obtained for alkaloids of both composted and uncomposted substrate except for spawn-run that had decreased in composted substrate (Table 4a). These findings were in line with Kuforiji, (2005) who worked on *V.volvacea* and *P.tuberegium.* This finding could explain the efficacy of *P. pulmonarius* in novelty medicines. Alofe, (1988), worked on Nigeria mushroom, obtained a high alkaloid concentration in C. molyditis, in alkaloid and tannin concentration in *L. subnudus* and *T. robustus* and absence of saponin in all mushroom tested.

Conclusion

The significance of the finding in that there were increased

in enzyme activities from very young stage to mature stage. The presence of intracellular enzymes helps to convert insoluble substrate to soluble component that could be utilized nutritionally by the growing sporophore. Alkaloid and Tannins were detected in various fruit body tested. Perhaps, the enumerated medicinal uses of these mushroom is due to presence of secondary metabolites.

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