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Investigation of changes in antioxidant activity of some *Pleurotus* species by using composts including different metal salts

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Nowadays, high content of antioxidants in plants and mushrooms is the reason for choosing a healthy life. Is the change in antioxidant activity in mushroom cultivation related to the metal values found in the compost? In order to find out the answer to this question, antioxidant activities of *Pleurotus ostreatus* and *Pleurotus citrinopileatus* grown with the addition of different metal salts to cotton compost media were determined by using DPPH method and total phenolic content was determined using Folin-Ciocalteu method. In addition, metal analyzes of mushroom samples were determined using ICP-OES. Results showed that *P. citrinopileatus* had higher antioxidant activity than *P. ostreatus*. In both mushroom species, IC_{50} values antioxidant activities were found to be Fe > Ca > Pure Water > Na > K in terms of DPPH damping. In the same way, the total phenolic values were followed by Fe > Ca > Pure Water > Na > K order for both mushroom species. These results showed that divalent Fe and Ca metals are important for the development of antioxidant activities in mushroom cultivation.

Keywords: *Pleurotus ostreatus*, *Pleurotus citrinopileatus*, antioxidant activity, total phenolics.

Introduction

Mushrooms have been ingredient of the human diet for thousands of years, and their consumption has recently increased considerably. Mushrooms are important foods because of their low calories, fats and rich in protein, vitamins and minerals[1,2]. Mushrooms have many medicinal properties, such as lowering blood cholesterol concentrations, preventing or alleviating heart disease, and lowering blood glucose levels. Mushrooms also show cholesterol lowering, antitumor, antiviral, anti-thrombotic and immunomodulatory effects[3,4].

Genus *Pleurotus* have 40 species in the world and they are commonly known as oyster mushrooms are a type of mushrooms which is preferred by many people because of its delicious taste and unique aroma[1].

The popularity and consumption of *Pleurotus* species increased day by day due to their short planting time, medical and nutritional values and high yield potential compared to many mushroom species. For this reason, *Pleurotus* species is the second most common mushroom cultivated after *Agaricus bisporus* in the world[5–7].

The chemical composition of mushrooms causes some changes in their antioxidant properties, and their metal content is effective in changing these properties. However, there are very few studies showing the change in antioxidant properties along with the metal content. Minerals can accumulate in mushroom and this deposition is usually dependent on the metabolism of mushroom species and is also effected by the chemical composition of the substrate which mushrooms take their nutrients[8,9].

In this study, *Pleurotus ostreatus* and *Pleurotus citrinopileatus* were grown in cotton composts saturated with different metal solutions and changes in antioxidant and phenolic values were examined.

Material and methods:

All chemicals which were analytical grade provided from Sigma-Aldrich Co. LLC. In each stage deionized purity water

was used. Absorbents was measured using a Shimadzu the UVM-1240 UV-Visible spectrophotometer (Shimazu Corp., Kyoto, Japan manufactures) with a pair of identical quartz cuvette of 1 cm thickness at 517 nm. XRF measurements were made with X-Ray Fluorescence Spectrometer (Spectro Xepos II).

Preparation of compost:

Pleurotus ostreatus and *Pleurotus citrinopileatus* mycelles were added to composts with 5–7% rate. Mushrooms were then placed in growing chambers with 25°C and 85% humidity for one month. Then growing mushrooms were collected and dried at 38°C and pulverized.

Preparation of mushroom extracts:

Mushroom extracts were prepared according to a standard protocol[10] with minor modifications. The prepared mushroom material (2.5 g) dissolved in 20 ml of 80% methanol solution. The mixture was stayed at room temperature for 3 h. Then mixture was filtered. The resulting homogenate was centrifuged at 5000 rpm for 10 min (18°C). The supernatant from this process was again centrifuged at 7500 rpm for 10 min (at 4°C). The final supernatant was removed and DPPH was used for the measurement.

Determination of DPPH activity:

In this study two mushroom species were investigated by DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging method to evaluate antioxidant activity and the results are shown as percent of inhibition (%) for these molecules.

DPPH solution at concentration of 30 μM was used as control solution. The absorbance change in the solution was measured at 4 different concentrations for each added mushroom extract at 1.66–6.66 mg/mL.

The percent radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

A_0 is the absorbance in the presence of control absorbance and A_1 samples.

Determination of total phenolics:

The total phenolic component of methanol extracts was determined using the methods given in the literature[11] which included Folin-Ciocalteu reagent and gallic acid as standard.

The concentrations of the phenolic compounds were calculated according to the following equation, obtained from the standard gallic acid graph:

Absorption = 0.0264 gallic acid (mg) + 0.0462

$R^2 = 0.9891$

Study of metal content:

Metal analyzes of mushroom samples were determined using ICP-OES (SpectroBlue).

Statistical analysis:

The relationship between Trolox and antioxidant concent of mushrooms were calculated using descriptive statistical analysis with Microcal Origin Pro 8.5.1 (Origin Lab. Corp., Northampton, MA, USA). Statistically significant effects were investigated using SPSS software (SPSS Inc., Chicago, IL, USA) for Windows version 13.

Results and discussion

Pleurotus ostreatus and *P. citrinopileatus* were grown in cotton compost filled with four different metal solutions and antioxidant activities of mushroom samples were determined by DPPH method. The variation of the percent inhibition values with concentration for *P. ostreatus and P. citrinopileatus* was shown in Figs. 1 and 2, respectively. According to this, the highest increase in percent inhibition by concentration increase was observed in $FeCl_2$ solution.

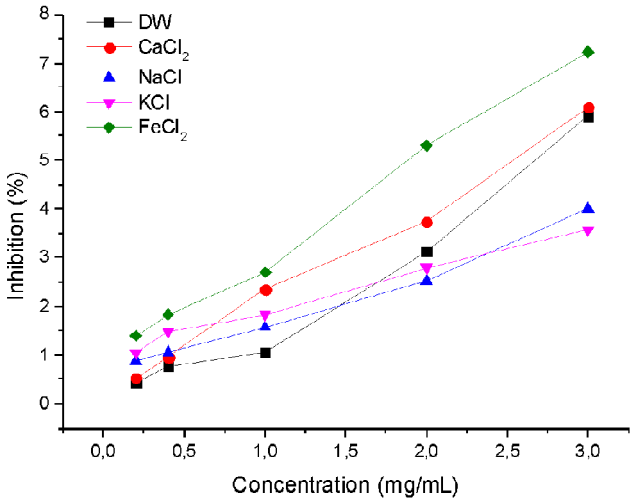

Fig. 1. Inhibition change of *Pleurotus ostreatus* depend on concentration.

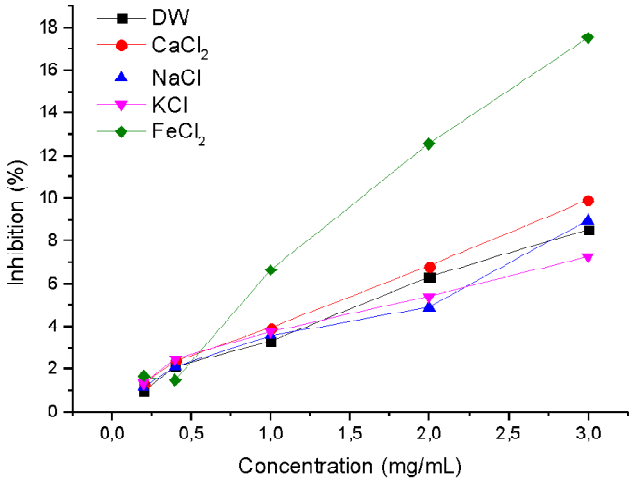

Fig. 2. Inhibition change of *Pleurotus citrinopleatus* depend on concentration.

are shown in Table 1. According to this, it was observed that *P. citrinopileatus* exhibited higher antioxidant activity and total phenolic values than, *P. ostreatus* in all composts. In their study, Kumamoto *et al.* revealed the effects of pH and metal ions on antioxidant activity[13]. The aim of this study was to investigate the change of antioxidant activity of mushrooms with the effect of metal ions. As a result of the study, the mushroom growing in each compost were found to have higher total phenolic and antioxidant properties in mushroom samples grown in $FeCl_2$ and $CaCl_2$ solutions.

There are many reports on the induced accumulation of phenolic compounds and peroxidase activity in plants that cultivated with high metal concentrations[14]. Jung *et al.* emphasized that the antioxidant effect of phenolic compounds

Table 1. Concentration equation and IC_{50} values calculated using the DPPH method and total phenolic values measured using Folin-Ciocalteu method for mushroom samples

		Concentration equation (0.5–7.5)×10^{-6} *M*	R^2	IC_{50} (mg/mL)	Total phenolics (mg/L)
	Gallic acid	$y = 33848.06.x - 0,012$	0.995	0.001478	–
		Concentration equation* (0.2–3) mg mL^{-1}			
Pleurotus citrinopileatus	Deionized water	$y = 2.651.x + 0,734$	0.99	18.58173	0.636364
	$CaCl_2$	$y = 2.992.x + 0,911$	0.997	16.40533	0.674242
	NaCl	$y = 2.561.x + 0,748$	0.948	19.23014	0.598485
	KCl	$y = 1.988.x + 1,399$	0.973	24.44075	0.484848
	$FeCl_2$	$y = 5.942.x + 0,118$	0.987	8.394765	2.037879
Pleurotus ostreatus	Deionized water	$y = 1.920.x - 0,270$	0.942	26.1747	0.409091
	$CaCl_2$	$y = 1.926.x + 0,193$	0.989	25.85683	0.484848
	NaCl	$y = 1.095.x + 0,558$	0.981	45.14443	0.371212
	KCl	$y = 0.870.x + 0,994$	0.987	56.29976	0.333333
	$FeCl_2$	$y = 2.123.x + 0,891$	0.992	23.13085	0.598485

Mushroom extract concentrations (c*): 0.2, 0.4, 1.0, 2.0, 3.0 mg mL^{-1}.

Previously, Rice-Evans *et al.* studied the antioxidant activity and structure relationships of phenolics and flavonoids[12]. In this study, metal analyzes of mushroom samples were done and evaluated together with total phenolic substances and antioxidant activities. The IC_{50} and total phenolic values of both mushroom species which grown in different composts is due to the tendency of metals to chelate and has hydroxyl and carboxyl groups that can bind Fe and Cu in particular[15]. When the content of metal in mushrooms is examined, it has been found that K has the highest value in all composts especially in compost containing KCl. Also it has been found that Fe has a high value in all composts.

Table 2. Metal contents of mushroom species grown in different compost

Compost	Mushroom species	Ca (ppm)	K (ppm)	Na (ppm)	Mg (ppm)	Cr (ppb)	Fe (ppb)	Ni (ppb)	Cu (ppb)	Zn (ppb)	Al (ppb)
Deionized water	*P. citrinopileatus*	7.303	44.861	11.367	13.439	< 0.475	92.478	< 0.568	7.579	63.431	6.706
	P. ostreatus	1.095	70.966	8.896	7.774	< 0.508	118.756	< 0.489	4.192	62.144	4.989
$CaCl_2$	*P. citrinopileatus*	3.338	92.100	6.170	10.529	5.043	97.776	0.703	13.653	47.513	9.894
	P. ostreatus	2.615	70.941	9.688	8.015	1.240	91.134	< 0.488	9.570	72.729	9.524
NaCl	*P. citrinopileatus*	0.124	107.797	9.182	9.101	< 0.662	100.818	< 0.624	6.214	52.557	8.997
	P. ostreatus	0.760	68.966	10.521	7.528	< 0.570	87.878	< 0.460	5.445	62.802	5.826
KCl	*P. citrinopileatus*	3.207	128.282	6.894	11.728	< 0.624	82.464	< 0.606	2.529	44.423	3.580
	P. ostreatus	1.958	106.134	7.993	7.994	< 0.403	80.533	< 0.513	3.762	54.297	2.914
$FeCl_2$	*P. citrinopileatus*	3.517	119.782	8.210	13.306	0.716	255.671	0.683	6.515	70.422	44.243
	P. ostreatus	1.429	95.655	11.598	8.916	< 0.569	127.592	< 0.577	5.500	66.600	7.169

Conclusions

There are no studies showing that antioxidant activity in mushrooms has changed by metal salts added to the compost.

According to these results, it has been shown in this study that gaining high antioxidant activity and phenolic content of mushroom are possible by using different salt solutions in compost.

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