Can consumption of antioxidant rich mushrooms extend longevity?: antioxidant activity of *Pleurotus* spp. and its effects on Mexican fruit flies' (*Anastrepha ludens*) longevity

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Abstract The variability of antioxidant capacity of 14 strains of the edible oyster mushroom *Pleurotus* spp. was determined, and the effect of selected mushroom supplements on the longevity of the Mexican fruit fly, *Anastrepha ludens*, was evaluated. The antioxidant capacity of the fruiting bodies was determined by three different methods, measuring the free radical scavenging activity of methanolic extracts, the OH radical scavenging capacity, and the total phenol content. The inhibition percentage of the DPPH radical varied between 32.6 and 85.7 % and total phenols varied between 30.6 and 143.3 mg/g. The strains with the highest (*Pleurotus djamor* ECS-0142) and lowest (*Pleurotus ostreatus* ECS-1123) antioxidant capacity were selected to study their effect on the survival, life expectancy, and mortality of the Mexican fruit fly *A. ludens*. The results demonstrated differing responses between male and female flies. High concentrations of mushrooms (5 and 20 %) in the diet resulted in a decrease in life expectancy. However, flies on the diet with 1 % *P. djamor* ECS-0142 showed slightly but significantly greater survival than those on the control diet. The possible adverse effect of protein content in mushroom extracts is discussed.

Keywords Mushrooms · Nutraceutical compounds · Life expectancy · Mortality rate · Edible fungi

Introduction

The edible oyster mushrooms *Pleurotus* spp. grow in tropical and subtropical zones and are easy to cultivate due to the diversity of substrates on which they can grow (Royse and Sánchez 2002). These mushrooms are considered of high nutritional value. Protein content varies between 20 and 40 % (d.w.), fibre content between 7.7 and 14.2 %, and fat content between 3 and 7 %. They are a significant source of vitamins (Valencia del Toro and Aguilar 2000). Their ability to regulate the immune system, reduce lipid concentrations in the blood, and prevent high arterial pressure and sclerosis suggest that they have nutraceutical properties. These nutraceutical benefits are attributable to active biological compounds that frequently act as antioxidants (Alam et al. 2009, 2011).

Pleurotus sajor-caju and *Pleurotus florida* showed antioxidant activity on rats with hypercholesterolemia and provided protection against oxidative stress (Khan et al. 2011). In addition, *Pleurotus ostreatus*, *Pleurotus cornucopiae*, and *Pleurotus salmoneostramineus* extracts proved to be effective in inhibiting HT-29 cells (cancerous cells in the human colon, Kim et al. 2009) that are associated with ageing. However, it is unknown whether antioxidant compounds of *Pleurotus* spp. can promote longevity.

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The antioxidant properties of fungi are derived from their secondary metabolism. Compounds such as ergothioneine, gallic acid, ascorbic acid, tocopherols, and selenium have been reported as antioxidants found in fungi (Teissedre et al. 2000; Cheung and Lo 2005). In *P. ostreatus*, the highest level of antioxidant activity occurs in the fruiting body and this activity could be attributed to the fiber content of this particular mushroom (Riberio et al. 2008).

A high protein concentration in the diet adversely affected the longevity of *Anastrepha ludens* fruit flies (Carey et al. 2008) and *Drosophila melanogaster* flies (Lee et al. 2008). Also, extracts from *Ganoderma lucidum*, *Lentinula edodes*, *Agaricus blazei*, and *Auricularia auricularia-judae* have an effect on the life expectancy of *D. melanogaster*, depending on dose and the sex of the flies (Huang et al. 2011). These authors indicate that this effect could be partially due to the antioxidant properties of the mushrooms. However, these pro-longevity effects could be diminished by the protein content of the mushrooms.

The administration of *P. ostreatus* extracts reduced the levels of malondialdehyde and increased the antioxidant activity of the principal organs in old rats as an effect on lipid peroxidation (Jayakumar et al. 2007). These authors concluded that the antioxidant activity of *P. ostreatus* extracts improved the antioxidant state during ageing. This suggests that the fruiting bodies of *Pleurotus* spp. are characterized by antioxidant activity that is capable of promoting longevity.

Several studies have documented that antioxidants in diet have an effect on longevity. A cranberry polyphenol blend increased life expectancy and reduced age-related decline in the nematode *Caenorhabditis elegans* (Wilson et al. 2006). A diet of cranberry and oregano prolonged *A. ludens* longevity, and this effect depended on diet, age, and caloric restriction (Zou et al. 2010 and 2012). The effect of products recognized for their antioxidant capacities such as tocopherol (Zou et al., 2007a, b), resveratrol (Zou et al. 2009), and açai extracts (*Euterpe oleracea* Mart.) (Liedo et al. 2012) on the life expectancy of this species has also been documented. Due to their antioxidant capacities, regular consumption of *P. florida* and *P. sajor-caju* are efficient in protecting rats that suffer hypercholesterolemia and oxidative stress (Khan et al. (2011).

Studies that evaluate the pro-longevity effect of compounds with antioxidant capacities are complex. The pro-longevity effect depends on other variables such as

the amount of fat, sugars, and proteins in the diet; genetic background; age; and calorie intake (Liedo et al. 2012, Wang et al. 2013). Furthermore, the effect on longevity can be affected by reproduction. An antioxidant compound can favour fecundity, which in turn can result in a decrease in longevity, but the relationship between diet, fecundity, and lifespan is not clearly understood (Carey et al. 1998, 2001, 2008; Kulminski et al. 2009).

The Mexican fruit fly, *A. ludens*, has been used as a model organism to evaluate the longevity-promoting effects of nutraceutical compounds (Zou et al. 2007a and b, 2009, 2012; Liedo et al. 2012). The aim of this study was to determine the antioxidant capacity of fruiting bodies from 14 strains of *Pleurotus* spp. and evaluate the effect of the two extremes, which presented contrasting antioxidant capacity, on *A. ludens* longevity.

Materials and methods

Biological material

This study used strains of the edible mushrooms *P. ostreatus*, *P. pulmonarius*, and *P. djamor* obtained from the mycology strain collection of El Colegio de la Frontera Sur (ECOSUR) (Table 1). In order to evaluate the effect of antioxidant activity on longevity, the Mexican fruit fly *A. ludens* was used as an animal model. Individuals of this species were provided as pupae by the Moscafrut mass-rearing facility in Metapa, Chiapas, Mexico.

The production of fruiting bodies was carried out in the Edible Mushrooms Pilot Facility in ECOSUR. Inoculation was conducted using sorghum spawn, according to the guidelines provided by Quimio (2002). Pangola grass, *Digitaria decumbens*, was used as a culture substrate at 65 % humidity and with 2 % lime that had been pasteurized for 1 h at 90 °C. Incubation and fructification was carried out following the procedures suggested by Zadrazil and Kurtzman (1982). Only fruiting bodies from the first harvest were used. Once harvested, the mushrooms were processed according to Mourão et al. (2011), dried by lyophilization, and then ground to a fine powder.

Chemical analysis

Free radical scavenging activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) model

Table 1 Antioxidant capacity of the studied *Pleurotus* spp. strains (free radical scavengers identified using the DPPH method, scavenging of hydroxyl radicals and total phenols)

Species	Strains	DPPH (%)	OH (%) radical	Total phenols (mg/g)
<i>P. djamor</i>	ECS-0142	23.9 ± 0.9 b	66.8 ± 2.1 abcd	128.3 ± 2.9 a
<i>lmonarius</i>	ECS-0190	32.5 ± 5.0 a	45.4 ± 11.8 de	129.0 ± 5.3 a
<i>P. pulmonarius</i>	ECS-1312	7.9 ± 0.4 g	59.8 ± 0.5 bcd	143.3 ± 31.7 a
<i>P. pulmonarius</i>	ECS-1298	35.7 ± 0.3 a	85.7 ± 2.4 a	34.0 ± 5.3 f
<i>P. pulmonarius</i>	ECS-1297	15.8 ± 0.4 de	55.2 ± 0.7 cde	127.0 ± 1.0 a
<i>P. ostreatus</i>	ECS-1227	19.7 ± 1.8 cd	66.6 ± 7.2 abcd	94.0 ± 1.0 bc
<i>P. ostreatus</i>	ECS-1206	11.7 ± 0.7 efg	70.6 ± 3.8 abc	101.0 ± 1.7 b
<i>P. ostreatus</i>	ECS-1219	11.7 ± 0.2 efg	84.1 ± 11.2 ab	53.0 ± 2.6 e
<i>P. pulmonarius</i>	ECS-1303	10.6 ± 0.3 fr	83.0 ± 6.3 ab	54.0 ± 1.0 e
<i>P. ostreatus</i>	ECS-1225	7.8 ± 0.6 g	59.9 ± 3.6 bcd	91.6 ± 2.9 c
<i>P. ostreatus</i>	ECS-1224	12.2 ± 0.6 ef	64.5 ± 20.6 abcd	57.0 ± 1.7 e
<i>P. ostreatus</i>	ECS-1231	9.1 ± 0.8 fg	78.5 ± 9.8 abc	30.6 ± 1.1 f
<i>P. pulmonarius</i>	ECS-172	11.4 ± 0.2 fg	66.0 ± 1.0 abcd	50.0 ± 2.0 e
<i>P. ostreatus</i>	ECS-1123	20.6 ± 0.2 bc	32.6 ± 7.4 e	74.6 ± 0.5 d

Identical letters in the same column indicate that there are no statistical differences between strains according to the Tukey test ($\alpha = 0.05$)
 Analysis on lyophilized carpophores from strains grown on pangola grass. Mean of three replicates

according to Vamanu (2012) with a methanolic solution consisting of 2.9 ml of DPPH (60 μ M) and 0.1 ml of carpophore extract. After 30 min, absorbance was observed at 517 nm. Readings were repeated 5 times and free radical scavenging activity was determined using the following formula:

$$\text{Activity}(\%) = \left[1 - \left(\frac{\text{Ab}_{517\text{sample}}}{\text{Ab}_{517\text{control}}} \right) \right] \times 100\%$$

Hydroxyl radical scavenging activity was determined according to the method described by Ghiselli et al. (1998) and Fan et al. (2012). Specifically, 0.1 ml GLP at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/ml was mixed with 0.8 ml of 200 mmol/l phosphate buffer (pH 7.4), 1.75 mmol/l deoxyribose, 0.1 mmol/l ferrous ammonium sulphate, and 0.1 mmol/l EDTA. The reaction was initiated by adding 0.1 ml of 1.0 mmol/l ascorbic acid and 0.1 ml of 10 mmol/l H_2O_2 . This solution was incubated and subsequently reacted with thiobarbituric (TBA) and trichloroacetic acid. Absorbance was measured at 532 nm. The following equation was used to calculate the percentage of hydroxyl radical scavenged.

$$\text{OH}^{\cdot}\text{scavenged}(\%) = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \cdot$$

Total phenol content of the fruiting bodies methanolic extract was determined using the Folin-Ciocalteu

method (Cheung and Lo 2005). One millilitre of sample was mixed with 1 ml of Folin-Ciocalteu and incubated for 3 min at room temperature. Subsequently, 1 ml of Na_2CO_3 (35 % aqueous solution) was added to the mixture followed by 7 ml of distilled water. The sample was maintained in darkness for 90 min and then absorbance was read at 725 nm. A calibration curve was used for quantification, presenting gallic acid concentrations between 0.01 and 0.4 mM. The content of total phenols in the samples was expressed as gallic acid (EGA) equivalents.

Bioassays

Bioassays were used to ascertain the effect of mushroom-enriched diets on fruit fly longevity. Pupae were placed in containers inside the cages. After 48 h, all empty pupal cases and non-emerged pupae were removed. Individual fly age ranged from 0 to 48 h. Approximately 2200 *A. ludens* adult flies of both sexes were placed inside 90 × 45 × 15 cm cages. Three to four cages were set per treatment.

Adult flies were fed on a standard solid diet with sugar and enzymatically hydrolysed yeast (MP Biomedicals LLC) in a 3:1 ratio. The medium used for the test was based on the standard diet with the corresponding specific concentration of each lyophilized

mushroom powder. Three concentrations were evaluated: high (20 %), medium (5 %), and low (1 %) for two strains: one with a high antioxidant capacity (*P. djamor* ECS-0142) and the other with low capacity (*P. ostreatus* ECS-1123), based on the data from the chemical analyses reported in the “Results” section. The treatments were designated as follows: ECS-1123-20 %, ECS-1123-5 %, ECS-1123-1 % and ECS-0142-20 %, ECS-0142-5 %, ECS-0142-1 %.

The cages were maintained at a 24 ± 2 °C, 65 ± 10 % RH, and 12 h/12 h light/dark. The food was covered with aluminium foil in order to avoid exposure of the diet components to light. The foil was replaced every 15 days to avoid oxidation. Dead flies were collected daily, sorted by sex, and recorded. In each cage, one treatment was evaluated and three or four replicates were made per treatment.

Data analysis

A randomized design with three repetitions was used for the assays on antioxidant capacity. An analysis of variance (ANOVA) and a Tukey means comparison test ($\alpha = 0.05$) were applied to the data. The strains with the highest and lowest antioxidant activity (AOC) were selected for fly bioassays by means of a weighted analysis (Table 2). In the first column, the same factor was considered for all three analyses, estimating the weighted antioxidant capacity as $AOC = 1 \text{ DPPH} + 1 \text{ OH} + 1 \text{ FT}$. In the second column, an unequal factor is considered where $AOC = 2\text{DPPH} + 2 \text{ OH} + 1\text{FT}$, taking into account that the free and OH radical analyses (free and OH) are expressed as percentages and total phenols expressed as concentration. A third analysis was conducted considering a second unequal factor with $AOC = 2\text{DPPH} + 1\text{OH} + 2\text{FT}$, to give more weight to the two analyses most frequently used for the estimation of the antioxidant capacity. This after Huang et al. (2005) and Halliwell (1995), who consider that the scavenging of hydroxyl radicals by antioxidants in biological systems, is unlikely.

To analyze the effect of mushroom diets on *A. ludens* longevity, life tables were constructed (Carey 1993). Life expectancy at adult eclosion was estimated for each cage (treatment–replicate) and means were analysed by ANOVA and Tukey multiple comparison test. LogRank analysis was used to compare survival curves of the different treatments (Hothorn et al. 2006). Smoothed (7-day geometric mean) age-specific mortality (q_x)

curves for males and females were done. The R Project for Statistical Computing program (<http://www.r-project.org/>) was used for all analysis.

Results

Antioxidant capacity

The values for antioxidant capacity, based on the DPPH method, varied between 7.8 % (*P. ostreatus* ECS-1225 strain) and 35.7 % (*P. pulmonarius* ECS-1298 strain). The statistical analysis resulted in the separation of 7 groups (a–g). Group a, which consisted of the strains that presented most antioxidant activity, included the *P. pulmonarius* ECS-01298 and ECS-0190 strains with 35.7 and 32.5 %, respectively. Group g was composed of the strains that presented less antioxidant capacity: *P. ostreatus* ECS-1219, ECS-1231, ECS-1206, ECS-1225 and *P. pulmonarius* ECS-172, ECS-1303, and ECS-1312 with values ranging from 7.8 to 11.7 % (Table 1).

The values obtained from the quantification of carophore capacity to scavenge OH radicals varied between 32.6 % (*P. ostreatus* ECS-1123) and 85.7 % (*P. pulmonarius* ECS-1298). The statistical analysis resulted in the strains being divided into 5 significantly different groups (a–e) ($\alpha = 0.05$). Group a contains strains that presented the highest values: *P. pulmonarius* ECS-1298, ECS-0172, ECS-1303; *P. ostreatus* ECS-1219, ECS-1224, ECS-1231, ECS-1206, ECS-1227; and *P. djamor* ECS-0142, with values ranging between 85.7 and 64.5 % (Table 1).

Total phenols, determined by the Folin-Ciocalteu method, varied between 30.67 mg/g (*P. ostreatus* 1231 strain) and 143.3 mg/g (*P. pulmonarius* ECS-1312 strain). Statistical analysis resulted in seven groups of strains (a–g). Group a comprises strains with the highest phenol content: *P. djamor* ECS-0142 (128.3 mg/g), *P. pulmonarius* ECS-1297 (127 mg/g), ECS-1312 (143.3 mg/g), and ECS-0190 (129 mg/g) (Table 1).

Table 2 shows a weighted analysis of the three chemical analyses implemented on all the studied strains. The statistical analysis performed defined 6 significant different groups in each column ($\alpha = 0.05$), observing similarities between the three columns with regard to the groups of strains that demonstrated the highest and lowest antioxidant activity. Thus, the strains *P. djamor* ECS-0142 (representing statistical group “a” with the

Table 2 Weighted estimation for the classification of antioxidant capacity (AOC) of the evaluated *Pleurotus* spp. strains, based on the three chemical analyses carried out in Table 1

Species	Strains	Equal weighting (1:1:1)	Unequal weighting 1 (2:2:1)	Unequal weighting 2 (2:1:2)
<i>P. djamor</i>	ECS-0142	219.1 ± 3.5 a	310.9 ± 0 a	371.4 ± 5.4 a
<i>P. pulmonarius</i>	ECS-0190	207.0 ± 8.0 ab	285.0 ± 21.1 ab	368.6 ± 7.8 a
<i>P. pulmonarius</i>	ECS-1312	211.1 ± 31.7 ab	278.9 ± 31.7 abc	362.4 ± 63.8 a
<i>P. pulmonarius</i>	ECS-1298	155.4 ± 6.9 cde	276.8 ± 8.8 abc	225.1 ± 12.0 de
<i>P. pulmonarius</i>	ECS-1297	198.0 ± 1.0 ab	269.1 ± 1.2 abc	340.9 ± 2.2 ab
<i>P. ostreatus</i>	ECS-1227	180.3 ± 7.3 bcd	266.7 ± 14.1 abc	294.1 ± 7.5 bc
<i>P. ostreatus</i>	ECS-1206	183.3 ± 6.0 bc	265.7 ± 10.3 abcd	296.2 ± 8.3 bc
<i>P. ostreatus</i>	ECS-1219	148.9 ± 11.7 cdef	244.8 ± 22.8 bcde	213.6 ± 12.7 e
<i>P. pulmonarius</i>	ECS-1303	147.7 ± 5.9 def	241.5 ± 12.5 bcde	212.4 ± 5.7 e
<i>P. ostreatus</i>	ECS-1225	159.4 ± 6.2 cde	227.1 ± 9.5 cdef	258.9 ± 9.0 cd
<i>P. ostreatus</i>	ECS-1224	133.8 ± 19 ef	210.0 ± 39 def	203.1 ± 17.5 e
<i>P. ostreatus</i>	ECS-1231	118.3 ± 10.7 f	206.0 ± 20.3 ef	158.1 ± 11.7 f
<i>P. pulmonarius</i>	ECS-172	127.4 ± 2.8 ef	204.9 ± 3.6 ef	188.9 ± 4.7 ef
<i>P. ostreatus</i>	ECS-1123	127.8 ± 8.3 ef	181.0 ± 16 f	223.1 ± 9.1 de

Column 3, similar weighting for all three analyses (AOC = 1 DPPH + 1 OH + 1 FT); column 4, unequal weighting 1 (AOC = 2DPPH + 2OH + 1FT); column 5, an unequal weighting 2 (AOC = 2DPPH + 1OH + 2FT)

Identical letters in the same column indicate that there are no statistical differences between strains according to the Tukey test ($\alpha = 0.05$)

highest antioxidant capacity) and *P. ostreatus* ECS-1123 (representing group “f” with the lowest capacity) were selected for the subsequent study on the effect of antioxidant capacity on fly longevity.

Effect of antioxidant capacity on longevity

The longevity values for *A. ludens* adults fed on a diet consisting of 3 concentrations of both *Pleurotus* spp. strains were greater for males than females in all cases ($p = 0.001$). Differences in mean life expectancies were significant. Flies exposed to the high concentration of the ECS-1123 strain showed the lowest life expectancy (Table 3). Survival differences among extracts were highly significant ($p = 0.001$), both for males (Fig. 1) and females (Fig. 2). The 1 % *P. djamor* ECS-0142 strain was the only treatment that presented significantly greater survival than the control for both males and females ($p = 0.027$ and $p = 0.001$ for males and females, respectively).

When compared with the other treatments, the 20 % lyophilized extract ECS-1123 showed the highest fruit fly mortality during the first 40 days. The 5 % ECS-1123 treatment also resulted in high mortality during the first 30 days (Fig. 3a, b). When flies were fed on the *P. djamor* ECS-0142-1 % treatment, they showed the lowest mortality rate during the first 45 days, a similar pattern to the

control (Fig. 3c, d). This lower mortality rate resulted in a significantly greater survival (Figs. 1 and 2).

In general, the mushroom treatments resulted in greater mortality than the control during the first 50 days of adult life. Flies exposed to *P. djamor* ECS-0142 (higher antioxidant capacity) extracts showed lower mortality than flies exposed to *P. ostreatus* ECS-1123 (lower antioxidant capacity) (Fig. 3). The individual with the longest longevity (112 days) was a male from the ECS-0142-20 % treatment.

Table 3 Mean life expectancies (±SD) at adult eclosion of *A. ludens* males and females exposed to diets with extracts from different strains of *Pleurotus* spp.

Strain, concentration (%)	Males	Females
1123, 1	32.84 ± 1.62 a	30.62 ± 1.20 a
1123, 5	30.48 ± 2.98 ab	27.98 ± 2.21 ab
1123, 20	25.70 ± 1.84 b	23.17 ± 3.05 b
142, 1	34.29 ± 2.00 a	30.62 ± 4.25 ab
142, 5	32.16 ± 3.42 a	27.81 ± 4.61 ab
142, 20	30.99 ± 1.46 ab	24.03 ± 1.86 ab
Control	33.60 ± 1.87 a	30.22 ± 1.41 a

Identical letters in the same column indicate that there are no statistical differences between treatments according to the Tukey test ($\alpha = 0.05$)

Fig. 1 Survival of *A. ludens* males fed on a diet of three concentrations of the *P. djamor* ECS-0142 and *P. ostreatus* ECS-1123 strains, obtained by means of a regression tree using a logrank test for survival curve comparisons

Discussion

Antioxidant capacity

The present study discovered substantial differences in antioxidant capacity as well as phenol content of the 14 strains of *Pleurotus* spp. studied and thus allowed the classification of all strains according to their level of antioxidant capacity. Exposing fruit flies to diets with the lowest (ECS-1123) or highest (ECS-0142) antioxidant capacity showed significant effects on fruit fly longevity.

The free and stable radical diphenylpicrylhydrazyl (DPPH) is commonly used to precisely and directly evaluate antioxidant and free radical scavenging capacity and has become a reference point for the evaluation of in vitro antioxidant capacity (Brand-Williams et al. 1995). In this study, the antioxidant capacity measured using DPPH varied between 7.8 and 35.7 % among the evaluated strains. These values are lower than those reported by Soekwanto et al. (2012) for a pink strain of *P. salmoneostramineus* (46.7–78.3 %) cultivated on rice husk. In another study, Vamanu (2012) reported that

Fig. 2 Survival of *A. ludens* females fed on a diet of three concentrations of the *P. djamor* ECS-0142 and *P. ostreatus* ECS-1123 strains, obtained by means of a regression tree using a logrank test for the survival curve comparisons

Fig. 3 Smoothed (7-day geometric mean) age-specific mortality (q_x) of *Anastrepha ludens* flies exposed to lyophilized mushroom containing diets: **a** *P. ostreatus* ECS-1123 strain on male flies, **b** *P.*

djamor ECS-0142 strain on male flies, **c** *P. ostreatus* ECS-1123 strain on female flies, and **d** *P. djamor* ECS-0142 strain on female flies

the exopolysaccharides from a strain of *P. ostreatus* cultivated in a liquid medium for 10 days presented an antioxidant capacity of between 50 and 78 %, according to mycelium concentration within the medium (4–10 mg/ml). Alam et al. (2011) determined values that varied between 42.5 and 95.4 % for carpophore methanolic extracts at a concentration of 2 mg/ml. Xia et al. (2011) isolated peptidoglycans from the carpophores of a strain of *P. ostreatus* and using the DPPH method determined an antioxidant capacity of between 57.7 and 82.4 % at a concentration of 8 mg/ml. Ren et al. (2014) found that the aqueous (polysaccharide) extracts of a strain of *P. australis* had the highest DPPH scavenging activity among 8 basidiomycetes tested.

Hydroxyl is an extremely reactive free radical formed in biological systems that rapidly reacts with almost every type of molecule present in living cells including sugars, amino acids, phospholipids, DNA bases, organic acids, etc. Determining the degree of hydroxyl radical scavenging activity permits the estimation of antioxidant activity in a specific sample. Jayakumar et al. (2009) determined the antioxidant effect of *P. ostreatus* extracts against hydroxyl radicals and determined that a concentration of 10 mg/ml obtained 56.2 % activity. When testing antioxidant capacity against hydroxyl

radicals of a different strain of *P. ostreatus*, Xia et al. (2011) determined values between 44.4 and 78 % for peptidoglycans isolated from carpophores using 8 mg/ml concentrations. A methanolic extract of *Ganoderma tsugae* demonstrated an increase in OH radical scavenging activity with concentration until reaching a peak activity of 53.4 % at 5 mg/ml (Yen and Wu 1999). The above data falls within the range of values obtained in this study, where antioxidant activity against the hydroxyl radical fluctuated between 32.6 and 85.7 %.

Phenolic substances are widely distributed among macromycetes. Essentially, every phenolic substance plays an important role of response and resistance to pathogenic and infective agents. Del Signore et al. (1997) determined the phenol content of 75 macromycete carpophore samples. In the case of *Pleurotus* species, these authors determined values between 0.9 and 2.95 mg/ml of total phenols in an extract of 1 mg carpophore/ml. Kim et al. (2009) determined the polyphenol content of several coloured strains of *Pleurotus* spp., obtaining values that ranged between 21.2 and 39.3 mg/ml, indicating that the yellow strains presented higher values than those that were grey and pink. Several reports have provided convincing evidence of the relationship between antioxidant capacity

and phenolic content of samples (Duan et al. 2007; Pan et al. 2010; Zhao et al. 2006). The values obtained in this study ranged between 50 and 143.3 mg/ml. Soekwanto et al. (2012) established the content of total phenols from *P. salmoneostramineus*, observing values between 41.9 and 86.2 mg/ml, depending on the initial content of the rice husk in the substrate where the carpophores were cultivated. Using the high-performance liquid chromatography (HPLC) method, Alam et al. (2011) determined the content of total phenols in *P. nebrodensis* (298 µg/g) and concluded that due to its high phenolic content, the antioxidant activity of this species is of high interest.

Effect on longevity

There were significant differences in the survival and mean life expectancies of *A. ludens* fed on diets with *P. djamor* and *P. ostreatus*. The survival of flies exposed to a diet with a concentration of 1 % *P. djamor* ECS-0142 was slightly, but significantly, greater for both sexes when compared with the control. For the remaining diets and their corresponding concentrations, a negative effect was observed on survival and life expectancy. *P. ostreatus* (ECS-1123) at 20 % concentration showed a 24 and 36 % decrease in male and female mean life expectancy compared to the control, respectively. Life expectancy decreased as mushroom concentration increased. A possible explanation for this is the protein content. Carey et al. (2008) found that an increase in protein content decreases longevity in this same species. Similar results were reported by Lee et al. (2008) in *Drosophila*.

Age-specific mortality rates during the first 30 to 40 days of adult life were greater for flies exposed to the 5 and 20 % concentrations of both mushroom strains than those exposed to the 1 % concentration and the control (Fig. 3). This greater mortality could be attributed to a greater protein content that stimulates reproductive output, with an increase in mortality hazard. However, the ECS-0142 strain (the strain with the highest antioxidant activity and 72 % more phenol content) resulted in lower mortality rates than the ECS-1123 strain (least antioxidant activity) for all evaluated concentrations. The greater survival of flies exposed to *P. djamor* (ECS-0142) at a concentration of 1 % suggests that the antioxidant capacity had a pro-longevity effect that was not counteracted by the protein content.

Regarding the effect of phenols on insects, there is contradictory evidence. Zivanov-Curils et al. (2004) reported that a chronic intoxication by phenol in nourishing medium induced decrease of fertility and shortening of life span of *D. melanogaster*. Rampadarath et al. (2014) determined that the phenolic content of *Jatropha* spp. had antimicrobial activity and was toxic for the larvae of *Batrocera zonata* and *Batrocera cucurbitae* fruit flies. However, Weisman et al. (2012) found a pronounced protective effect and extended lifespan of *D. melanogaster* females but not males when exposed to phenolic antioxidant TS-13.

Our study confirms that oyster mushroom strains present inter-strain variation in antioxidant capacity, particularly those that belong to the *Pleurotus* genus and that this capacity can result in life span-extending effects. The possible effects of antioxidants, phenols, and protein content on fecundity and life expectancy as well as interactions among nutritional factors are important aspects that require further research.

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