Antioxidant activity as indicator of UV radiation and other abiotic stress factors on *Agaricus bisporus* (Lange/Imbach) and *Sedum hybridum* (L.)

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90

Abstract. Investigation of stress level might be facilitated also in plant and horticultural sciences, but currently mainly morphological parameters are in use. Antioxidant activity routinely measured in food-oriented researches and several studies indirectly indicated that stress factors can influence this parameter. Our aim was to assess the potential direct indicator role of antioxidant activity in stress conditions. We measured the effects of UVB and soil-delivered stress on *Agaricus bisporus* and *Sedum hybridum*. Our results indicate that UVB slightly decreases, while the inadequate soil conditions increase antioxidant activity; hence these measurements are suitable for determining the level of stress in different living samples.

1 Introduction

Antioxidant activity is a parameter that describes the capacity of a sample to neutralize the free radicals. It has got a crucial role in human health, as many diseases (e.g. malignant tumours, non-alcoholic fat liver, autoimmune diseases etc.) (*Felton et al.*, 1994; *Geösel et al.*, 2011; *Ozzard et al.*, 2008) can be developed by the damages in DNA and the structural and functional proteins evoked by these reactive substances (*Bagchi et al.*, 2000). Because of this, many studies investigated how efficient the different foods are (e.g. fruits and vegetables) in delivering protective antioxidants to the human body.

In plants, the role of the antioxidant type of characteristic substances is the same as in the human body: protect the structure and function of plant cell organelles from free-radical-caused damages, and thus maintain homeostasis.

In mammalian, plant, and fungal cells, the antioxidant capacity mainly comes from two systems: the first one is the non-enzymatic, which utilizes organic substances (e.g. ascorbic acid, lycopene, a-tocopherol, glutathione) to quench the Reactive Oxygen Species (ROS), while the enzymatic antioxidant system contains enzyme proteins, such as Glutathion-S-transferase, Ascorbate-peroxidase, to facilitate electron or hydrogen atom transfer (*Edwards et al.*, 2000; *Venisse*, 2003).

Most of the non-enzymatic participants are represented in a relatively small amount in the different samples. Increasing of their levels can be influenced by many factors, e.g. the concentration of lycopene levels depends on temperature (*Helyes et al.*, 2007). Polyphenols are expanded by the photo-oxidative UV-stress in many fruits, e.g. *Sambucus sp.*, *Fragaria sp.* (*Asami et al.*, 2003; *Murugesan et al.*, 2012). The joint feature of these induction factors is that they both mean stress to the plants.

Many horticultural products have been tested for this phenomenon with different methods (*Csambalik et al.*, 2014; *Geösel et al.*, 2011; *Sipos et al.*, 2013). Most of the studies investigate the effect of environmental factors on the health-promoting molecule contents. Some studies suggested that stress factors indicate the elevated synthesis of carotenoids (*Kim et al.*, 2012). Others show that polyphenol content increases during UV-stress to protect the plant tissues from damages (*Ozzard et al.*, 2008). Similar results were obtained from measurements performed on fruits exposed to insect invasions (*Felton et al.*, 1994; *Hemingway & Laks*, 1992). Other abiotic stress factors, such as water shortage or chilling injury, also alter the bioactive compound levels (*Esteban et al.*, 2001). The availability of mineral elements determine the redox status as well (*Tewari et al.*, 2006). Competition with weed also means relevant stress to the plants.

The antioxidant-producing ability of the different species describes the capacity of the plants to adapt to these stress factors and hence contribute to the viability characteristics. As measurements of antioxidant activity can be performed by rapid and economical methods, another paradigm also arises (*Huang et al.*, 2005). It is hard to deceive the effect of stress factors to the investigated species. Most of the agricultural and food engineers assess the optimal condition by the quality parameters e.g. size, yield, growing rate and dynamics, ornamental value (*VanWoert et al.*, 2005). These are indirect parameters.

As antioxidant capacity measures the total ROS elimination capacity of the processed tissue, and these radicals formed during the exposition to the stress factors, it is possible that the antioxidant capacity indicates the intensity of stress itself.

Because of the aforementioned points, we came up with a novel approach to determine the connection of antioxidant activity with the different stress factors in mushrooms, as a relevant part of human nutrition, and in *Sedum-s* as an ornamentally interesting species.

UVB-treated white button mushrooms were chosen for the purpose of this study since nowadays they have become one of the novelty mushroom products with their increased vitamin D content, representing a natural source of this vitamin of high importance for the human nutrition (*Ozzard et al.*, 2008). The other object is *Sedum hybridum*, a commonly applied species on green roofs, especially extensive green roofs for its exceptional tolerance for climatic conditions and high decorative value (*Szőke et al.*, 2012).

2 Materials and methods

2.1 Investigated samples

Ultraviolet-B (UVB)-treated *Agaricus bisporus* samples were provided by the Department of Vegetable and Mushroom Growing. The biologically active, still developing fruitbodies were treated by a 15W output 290–315 nm wavelength VL-115 M lamp. 0, 5, 15, 20, 25, and 30 minutes of UVB radiation were applied on three consecutive days (a total of: 0, 15, 30, 45, 60, 75, 90 minutes). Samples were taken on 15$^{\text{th}}$ January 2015.

Sedum hybridum plants were propagated vegetatively. The samples were grown on a 3-year extensive green roof in four different substrate mixtures (S1: soil-zeolite-sand-based substrate, S2: riolite-based substrate, S3: brick-fracture- and ytong-based substrate and S4: soil) and in a 10-cm layer, respectively, as potential sources of substrate-associated stress factors. The control plants were grown on the ground in the original soil of the plot under the same ambient conditions. Samples were collected at the same phonological phase on the 10$^{\text{th}}$ of April 2015.

2.2 Sample preparation

Samples were homogenized with distilled water in a 1:1 ratio at 24000 min^{-1} RPM by a teflon homogenizer. This homogenate was kept in ultrasonic water bath for 15 min, and then spanned at 2000 g for 15 min. The supernatant was used for all of the measurements.

2.3 Antioxidant activity measurements

Ferric reduction antioxidant power (FRAP) was determined according to *Benzie & Strain* (1996). 100 µl of sample was added to pH $= 3.6$, 300 mM acetate buffer – 10 mmol/litre TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl – 20 mmol/litre FeCl$_3 \cdot$ 6H$_2$O, and then, after 5 minutes, absorbance was read at $\lambda = 593$ nm. Results were generated in ascorbic acid equivalence (*Benzie & Strain*, 1996).

DPPH (2,2-diphenyl-1-picrylhydrazyl) elimination assay was performed as described by *Brand-Williams et al.* (1995). 100 µl of sample was added to 3.9 ml of $6 \cdot 10^{-5}$ M methanolic DPPH solution, and then incubated for 20 minutes in dark. Absorbance readings were performed at $\lambda = 517$ nm, and inhibition % was calculated (*Brand-Williams et al.*, 1995).

CUPRAC assay was measured by the method of *Apak et al.* (2008). 1 ml

10^{-2} M CuCl$_2$, 1 ml $7.5 \cdot 10^{-3}$ M neocuproine solution, 1 ml pH $= 7.4$, 1 M
NH$_4$Ac buffer, 100 µl sample, and 1 ml distilled water was mixed and incuba-
ted for 30 minutes in dark at room temperature. After the absorbance reading
at $\lambda = 450$ nm, values were calculated to trolox equivalents (*Apak et al.*, 2008).

ABTS-assay was measured as described by *Huang et al.* (2005). Reaction
mixture contained 10 µl of sample; 20 µl of 3.50 mg/ml myoglobin in 50 mM,
pH $= 7.4$, 9% NaCl and 1% glucose containing potassium-phosphate buffer;
150 µl of 1 mg ABTS and 25 µl 3% H$_2$O$_2$ in 0.1 M pH5 citrate buffer. This
mixture was shaken for 5 minutes at 37 °C, then alkaline stop solution was
added and measured at $\lambda = 405$ nm against trolox calibration curve (*Huang
et al.*, 2005).

Total Phenolic Compound (TPC) was recorded as described by *Singleton*
and *Rossi* (1965). 1250 µl of 10-fold diluted Folin-Cioalteau reagent, 240 µl
methanol, 10 µl of sample, and 1 ml of 0.7 M NaCO$_3$ was mixed, and then
kept at 50 °C for 5 minutes, then measured at $\lambda = 765$ nm to calibration curve
set-up with gallic acid (*Singleton* & *Rossi*, 1965). All of the measurements
were carried out in five replicates.

2.4   Statistical analysis

Raw data were processed during statistical analysis. Since the resulting dataset
is considered to be low from statistical point of view (n < 13), the conditions
for parametric tests were not met. That is why the Kruskal-Wallis nonpara-
metric test (with the exact p-value calculation) was chosen (equivalent to
ANOVA parametric test), which is not sensitive either to the normality of our
data distribution or to the heterogeneity of its deviation (*Conover*, 1980).

Multiple pairwise comparisons using Dunn's procedure – as a suitable method
for detecting significant differences with Bonferroni correction – was applied.
The analyses were implemented by the XLStat-Sensory solution software, ver-
sion 2013.1.01 (Addinsoft, 28 West 27th Street, Suite 503, New York, NY
10001, USA).

To visualize and compare data in graphs, a 0 to 100 scaling method was
applied to normalize the data.

3   Results and discussions

Our results (*Table 1*) indicate that in the case of UVB exposition the an-
tioxidant activity does not alter. None of the applied methods show either
increasing or decreasing patterns via photo-oxidative stress.

Table 1: Antioxidant activity and phenolic compound alteration patterns via different stress factors

Species	Stressor	DPPH mean±SD	CUPRAC mean±SD	ABTS mean±SD	FRAP mean±SD	TPC mean±SD
	UVB-0	61.76±5.34[a]	100.00±0.80[b]	100.00±5.30[c]	99.53±2.86[b]	100.00±1.54[b]
	UVB-15	96.48±2.61[b]	81.66±1.76[b]	48.09±0.38[ab]	68.56±4.16[ab]	85.88±1.34[ab]
	UVB-30	79.50±0.00[ab]	66.50±0.69[ab]	47.96±0.29[ab]	66.06±0.00[ab]	74.92±0.00[a]
Agaricus	UVB-45	97.98±2.33[b]	77.45±1.59[ab]	47.99±0.67[a]	75.13±0.25[ab]	81.47±1.56[ab]
bisporus	UVB-60	82.19±1.38[ab]	50.19±0.00[ab]	53.85±0.03[abc]	100.00±4.79[b]	84.81±4.58[ab]
	UVB-75	98.67±2.70[b]	65.55±1.11[ab]	54.17±0.17[abc]	95.36±0.11[b]	95.66±5.87[b]
	UVB-90	100.00±6.86[b]	50.94±0.61[a]	54.59±0.00[bc]	18.90±3.29[a]	71.09±2.46[a]
	Range	38.24	49.81	52.04	81.10	28.91
	Control	9.29±0.00[a]	69.65±0.26[ab]	100.00±0.00[ab]	61.20±4.72[ab]	29.34±0.88[ab]
	S1	85.76±5.90[ab]	69.70±1.51[ab]	55.15±13.86a	61.48±0.00[a]	27.65±1.33[a]
Sedium	S2	85.91±0.43[ab]	68.61±0.00[a]	97.53±11.69[b]	65.62±0.88[ab]	28.45±0.86[a]
hybridum	S3	79.50±0.78[a]	100.00±0.13[b]	52.28±23.39[a]	100.00±3.75[b]	100.00±22.27[b]
	S4	100.00±0.92[b]	80.82±1.71[ab]	68.03±21.32[ab]	72.01±7.00[ab]	32.87±0.00[ab]
	Range	90.71	31.39	47.72	38.80	72.35

Data is given in mean ± SD format obtained from five parallel measurements. **a**, **b**, and **c** indicate homogeneous groups, **ab** and **abc** indicate heterogeneous groups. Homogeneity and heterogeneity are indicated by methods and species, respectively.

Although the size of changes is not meaningful, there was a slight loss of radical-eliminating molecules during the treatments. The highest values were observed in the control samples. The total phenolic compound changes also demonstrate the decreasing pattern observed in the case of antioxidant activity (*Figure 1*).
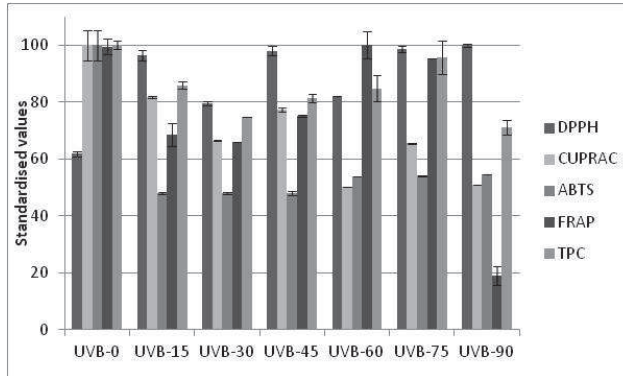

Figure 1: Antioxidant activity and phenolic compound characteristics of UVB-treated white button mushroom samples (mean, SD, n=5)

In the case of soil-derived stress, we observed the organic-poor substrate-derived stress, as the highest increase in the antioxidant activity was manifested in the S3 growth sample. Values of high-soil-content S1 and S4 samples show similar patterns in the case of each of the methods (*Figure 2*).
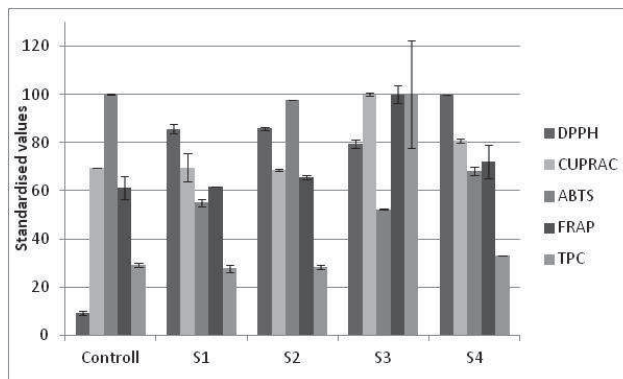

Figure 2: Antioxidant activity and phenolic compound characteristics of *Sedum hybridum* samples (mean, SD, n=5)

Range values show that the sensitivity of the applied 5 methods is different. In the case of *Agaricus*, the increasing order of ranking is TPC<DPPH< CUPRAC<ABTS<FRAP. In the case of Sedum, the following ranking applies: CUPRAC<FRAP<ABTS<TPC<DPPH.

4   Conclusion

In this study, we assessed the potential role of antioxidant-activity assays in the monitoring of photo-oxidative UVB- and substrate-associated stress factors on a nutritionally relevant edible mushroom and on an ornamentally valuable plant. Phenolic compound levels were also determined. During the measurements, we utilized the most common methods that can be easily adapted by any laboratory without the need of expensive instrumentation (*Huang et al.*, 2005).

Our results indicated that UVB stress did not cause an increasing pattern in mushroom samples, which is similar to what was observed in shiitake mushrooms by *Jiang et al.* (2010). It is maybe due to the fact that mushrooms do not possess a photosynthetic apparatus. There is evidence that the thylakoid membrane-bound enzymes of the photosynthetic system are the most sensitive proteins in the plants to the ROS damages. Avoiding the function loss can be achieved by the enhanced antioxidant capacity (enzymatic and non-enzymatic as well). The difference between *Sedum* and *Agaricus sp*-s may come from the lack of chloroplast, whence the necessity of fast-reacting antioxidant systems (*Halliwell*, 1989). The absence of phenolic content changes is due to the fact that mushrooms are not considered as a good source for these molecules (*Wong et al.*, 2013).

The results also indicated that alteration in antioxidant activity is a hallmark of the response to the related stress, as in the case of *Sedum hybridum* species the soils with the hypothesized different adequacy levels. The highest values measured in S3 substrates verified this assumption. This result is in line with others, who also highlighted the relevance of adequate salinity or other soil-delivered necessary compounds (*Esteban & Villanueva*, 2001; *Sairam et al.*, 2005).

Alteration in phenolic compound levels was also significant, which is in agreement with the results obtained from test performed on berries (*Asami et al.*, 2003; *Murugesan et al.*, 2012).

Our different range/rank/order results between the *Sedum* and *Agaricus sp*-s are also in line with others' conclusion that the simultaneous utilization of

different antioxidant activity methods is necessary as their sensitivity differs due to their different chemical background (*Huang et al.*, 2005).

On the basis of our results, we can conclude that antioxidant activity is a suitable derived parameter for the assessment of stress level.

Acknowledgements

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