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Determination of Phytohormones Level in Some Dried and Fresh Macrofungi Taxa

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Summary

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In the present work the level of Indole-3-acetic acid (IAA), *trans*-Zeatin (*t*-Z) and Gibberellic acid's equivalents (GAs) were determined in dried and fresh specimen of 18 macrofungi taxa by High Performance Liquid Cromatograpy (HPLC). The phytohormones were extracted from fresh and dried samples of *Cortinarius* Fr. sp., *Lentinus tigrinus* (Bull.: Fr.) Fr., *Coprinus atramentarius* (Bull.: Fr.) Fr., *Boletus impolitus* Fr., *Suillus granulatus* (L.: Fr.) O. Kuntze, *Leccinum scabrum* (Bul.: Fr.) S. F. Gray, *Pleurotus ostreatus* (Jacq.: Fr.) Kumm., *Agaricus bernardii* (Quél.) Sacc., *Ptychoverpa bohemica* (Krombholz) Boud., *Agrocybe dura* (Bolt.) Sing., *Lycoperdon molle* Pers.:

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Pers., *Volvariella speciosa* (Fr.: Fr.) Sing., *Pleurotus eryngii* (DC.: Fr.) Quél., *Morchella esculenta* Pers.: St. Amans, *Coprinus comatus* (Muell.: Fr.)Pers., *Inocybe* Fr. sp., *Hebeloma longicaudum* (Pers.: Fr.) Kumm. and *Amanita gemmata* (Fr.) Bertil.

The highest GAs level in dry fungi was found in *Ptychoverpa bohemica*, 444.08 µg/mg, whereas the lowest was found in *Coprinus atramentarius*, 22.13 µg/mg. In fresh samples the highest and lowest GAs levels were found in *Ptychoverpa bohemica*, 148.65 µg/mg and *Coprinus atramentarius*, 10.24 µg/mg respectively. The highest *t*-Z and IAA levels in dried samples were determined in *Agaricus bernardii*, 92.18 µg/mg and *Agrocybe dura*, 23.41 µg/mg, whereas the lowest *t*-Z and IAA levels in dried samples were determined in *Inocybe* sp., 2.65 µg/mg and *Pleurotus ostreatus*, 0.18 µg/mg respectively. Among the fresh samples, the highest level of *t*-Z was found in *Cortinarius* sp., 4.58 µg/mg and the lowest were detected in *Morchella esculenta*, 0.013 µg/mg. The highest and lowest IAA levels in fresh samples were found in *Agaricus bernardii*, 1.36 µg/mg and *Agrocybe dura*, 0.018 µg/mg respectively.

Zusammenfassung

Türker M., Demirel K., Uzun Y., Battal P. & Tilekliöglu B. 2005. Bestimmung von Phytohormongehalten in einigen getrockneten und frischen Makrofungi Taxa. – Phyton (Horn, Austria) 45 (2): 145 – 157. – Englisch mit deutscher Zusammenfassung.

In dieser Arbeit wurden die Mengen an Indol-3-Essigsäure (IAA), trans-Zeatin (t-Z) und Gibberiellinsäureäquivalenten (GAs) in getrockneten und frischen Exemplaren von 18 Makrofungi Taxa mittels Hochdruckflüssigkeits-Chromatographie (HPLC) bestimmt. Die Phytohormone wurden von frischen und getrockneten Proben von *Cortinarius* Fr. sp., *Lentinus tigrinus* (Bull.: Fr.) Fr., *Coprinus atramentarius* (Bull.: Fr.) Fr., *Boletus impolitus* Fr., *Suillus granulatus* (L.: Fr.) O. Kuntze, *Leccinum scabrum* (Bul.: Fr.) S. F. Gray, *Pleurotus ostreatus* (Jacq.: Fr.) Kumm., *Agaricus bernardii* (Quél.) Sacc., *Ptychoverpa bohemica* (Krombholz) Boud., *Agrocybe dura* (Bolt.) Sing., *Lycoperdon molle* Pers.: Pers., *Volvariella speciosa* (Fr.: Fr.) Sing., *Pleurotus eryngii* (DC.: Fr.) Quél., *Morchella esculenta* Pers.: St. Amans, *Coprinus comatus* (Muell.: Fr.)Pers., *Inocybe* Fr. sp., *Hebeloma longicaudum* (Pers.: Fr.) Kumm. and *Amanita gemmata* (Fr.) Bertil extrahiert.

Die höchste Menge an GAs wurde in trockenen Pilzen mit 444.08 µg/mg bei *Ptychoverpa bohemica* gefunden, die niedrigste bei *Coprinus atramentarius* mit 22.13 µg/mg. In frischen Proben wurden die höchsten Gehalte an GAs bei *Ptychoverpa bohemica* mit 148.65 µg/mg, die niedrigsten mit 10.24 µg/mg bei *Coprinus atramentarius* gefunden. Die höchsten t-Z- und IAA-Mengen wurden bei trockenen Proben bei *Agaricus bernardii* mit 92.18 µg/mg und *Agrocybe dura* mit 23.41 µg/mg gefunden, die niedrigsten t-Z- und IAA-Mengen hingegen bei *Inocybe* sp. mit 2.65 µg/mg und *Pleurotus ostreatus* mit 0.18 µg/mg. Unter den frischen Proben wurden die höchsten Mengen von t-Z bei *Cortinarius* sp. mit 4.58 µg/mg und bei *Morchella esculenta* mit 0.013 µg/mg bestimmt. Die höchsten und niedrigsten IAA-Mengen wurden bei *Agaricus bernardii* mit 1.36 µg/mg bzw. bei *Agrocybe dura* mit 0.018 µg/mg gefunden.

Introduction

Phytohormones play an important role in all phases of plant growth and development. They affect metabolism, including the activity of en-

zymes and the biosynthesis of some growth factors. They influence the appearance of organelles and nutrients throughout the plant (TREWAVAS 1981). They enhance plant resistance to adverse environments (ITEN & al. 1999). The environmental conditions may modify the hormonal balance in plants and fungi. These modifications include the rate of cell growth and differentiation acting in coordination with the vast range of environmental inputs. Plant hormones work to form defence mechanisms/metabolites and they are known transmitters of environmental changes in plant and fungi (KRAIGHER & al. 1991, 1993). Fungi have been reported to synthesize some plant hormones such as auxins, gibberellins, abscisic acid, and cytokinins (ÜNYAYAR & al. 1997, BATTAL & al. 2002). Auxins, cytokinins and gibberellins were found in vegetative mycelium, young stalk and in the cap of *Lentinus tigrinus* (RYPÁČEK & SLADKÝ 1972). GRUEN 1959 published a review on auxins and fungi. According to this paper a great number of lower and higher fungi synthesize auxins and release it into the culture medium. ÖZCAN TOPÇUOĞLU 2001 determined GA_3, ABA and cytokinin production in *Lentinus tigrinus* and *Laetiporus sulphureus* in free and bounded forms. The rapid growth of mycelium in fungi and the special way of their development as well as the comparatively high production of organic matter during the formation of fruiting bodies in higher fungi indicate that fungi also possess a regulation system in which growth substances take part. The participation of growth regulators in the differentiation process of fungi is also shown by the fact that fungi synthesize them in large quantities (RYPÁČEK & SLADKÝ 1972).

There are reports in the literature on phytohormon in plants. In addition to higher plants bacteria (TIEN & al. 1979), lichens (BATTAL & al. 2004) mosses (ERGÜN & al. 2002), algae (ZHANG & al. 1988) and fungi also synthesize plant growth regulators (GREENE 1980).

Gibberellins were for a long time considered to be the products of fungi metabolism. The exogenously applied gibberellic acid inhibited the growth of mycelia of *Agaricus bisporus* and *Coprinus comatus* (SZABO 1969). It stimulates, however, the sporulation of yeast (KAMISAKA & al. 1967). Cytokinins produced by the fungus might act either directly on the physiological process of the organism or indirectly by influencing the production of other hormones. Cytokinins influence the morphological characteristics and distribution of minerals (KRAIGHER & al. 1991). Cytokinins play an important role in the establishment of the mycorhizal association (GOGALA 1970). This is a special adaptation to survival in unsuitable conditions and stress in the habitat (KRAIGHER & al. 1993, 1996). Hormones are produced by both partners, the fungus and the plant (KOVAČ & ŽEL 1994). High cytokinin activity in young fruiting bodies of *Coprinus micaceus* is reported by SZABÓ & al. 1970. Benzyladenin, proved to be effective in retarding cap opening, was suggested to have a role in vivo regulation of post

harvest morphogenesis (BRAAKSMA & al. 2001). Due to the discovery of plant growth regulators, it could be possible to control many processes related to the growth and development in fungi.

Macrofungi not only can convert the huge lignocellulosic biomass waste into human food, but also can produce notable mycomedical/nutriceutical products that have many health benefits. Because mushrooms lack chlorophyll and are therefore nonphotosynthetic organisms, they cannot use solar energy to convert carbon dioxide and water into complex organic matter, as do common green plants. However, they can produce several groups of enzyme complex, which can convert the huge lignocellulosic waste material into a wide diversity of products. These products have multibenefical effects on human welfare (CHANG 1999). Mushrooms have been shown to be promising immunomodulators and have demonstrated significant antitumor, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidibetic activities. Mushrooms useful against stomach, oesophagus, lungs, and other cancers are known in China, Russia, Japan, Korea, as well as in the Unites States and Canada (WASSER & WEIS 1999). Scientist at the Cancer Research Campaign used fragments of the plant hormones IAA in combination with the plant enzyme peroxidase, and they reported that this combination destroyed cancerous tumour without harming healthy cells (FOLKES & WARDMAN 2003). Jasmonates and salicylic acid which are considered to be plant hormones commonly found in small quantities in many edible plants induce apoptosis in several types of human cancer cells without harming healthy human cells (FINGRUT & FLESCHER 2002).

There are still unknown details of phytohormon production and of the effect of plant hormones on the development and the physiological process in fungi. For this reason, we studied and compared the level of IAA, GAs, and *t*-Zeatin in some taxa of fresh and dried macrofungi.

Material and Methods

Mature macrofungi samples were collected in April, May, June and July from different regions in Eastern Turkey. Semi terrestrial climate is dominant and the highest rainfall was recorded in the region during study period. Plant hormones in dried and fresh fruitbody of macrofungi were extracted from the same specimens. Fresh samples were stored in liquid nitrogen. The drying procedure was carried out in a well ventilated oven under low temperature. Samples were kept in deepfreeze until use. Dry and fresh weight of macrofungi were determined and water content was calculated as dry weight to fresh weight ratio in % (Table 1). Fungi were identified according to the relevant literature (PHILLIPS 1981, MOSER 1983, BREITENBACH & KRÄNZLIN 1984–2000, BUCZACKI 1989, DÄHNCKE & DÄHNCKE 1989 and DENIS 1995) and according the data provided during the field study. The habitats and harvesting time of macrofungi are given in Table 1.

Table 1. Classification, habitats and harvesting time of macrofungi.

Families	Taxa	Habitat	Collection date	Water content (%)
Morchellaceae	*Morchella esculenta*	Batman (city), kozluk (town), kahveci (village) under broad leafy trees	26.04.2003	86.73±6.82
Morchellaceae	*Ptychoverpa bohemica*	Van (city), Edremit (town) on stumps of poplar tree.	24.05.2003	85.08±7.23
Pluteaceae	*Volvariella speciosa*	Van, Agricultural vocational school garden, meadow land	28.05.2003	88.34±5.64
Coprinaceae	*Coprinus atramentarius*	Van (city centre) under poplar trees	20.05. 2003	91.46±2.19
Coprinaceae	*Coprinus comatus*	Van, Agricultural Vocational School Garden, meadow land	28.05. 2003	89.66±3.35
Pleurotaceae	*Lentinus tigrinus*	Van, Edremit, on stumps of poplar tree.	24.05. 2003	78.57±6.88
Pleurotaceae	*Pleurotus eryngii*	Van, Erek mountain, on remnants of Ferula plant	26.06. 2003	84.26±6.74
Pleurotaceae	*Pleurotus ostreatus*	Van, Edremit, on stumps of poplar trees	24.05. 2003	86.14±5.04
Bolbitiaceae	*Agrocybe dura*	Van, University of Yüzüncü Yil, Campüs, under trees	17.05. 2003	77.83±6.43
Cortinariaceae	*Cortinarius sp.*	Kars (city), Sarikami? (town), Acisu region, Coniferous forest	14.07.2003	85.19±4.37
Lycoperdaceae	*Lycoperdon molle*	Kars, Sarikami?, Acisu region, Coniferous forest	14.07. 2003	89.27±4.52
Boletaceae	*Boletus impolitus*	Kars, Sarikami?, so?uksu region, Coniferous forest	15.07. 2003	85.56±5.75
Boletaceae	*Leccinum scabrum*	Kars, Sarikami?, so?uksu region, Coniferous forest	15.07. 2003	78.75±7.51
Boletaceae	*Suillus granulatus*	Kars, Sarikami?, so?uksu region, Coniferous forest	15.07. 2003	90.63±2.37
Agaricaceae	*Agaricus bernardii*	Kars, Sarikami?, acisu region, meadow land	14.07. 2003	86.42±3.38
Amanitaceae	*Amanita gemmata*	Kars, Sarikami?, Acisu region, Coniferous forest	14.07. 2003	77.91±7.64
Cortinariaceae	*Hebeloma longicaudum*	Kars, Sarikami?, Acisu region, Coniferous forest	14.07. 2003	81.77±6.38
Cortinariaceae	*Inocybe sp.*	Kars, Sarikami?, so?uksu region, Coniferous forest	15.07. 2003	82.26±7.36

The Macrofungi Taxa from which Phytohormones were analized

Identified macrofungi species were listed in Table 1. The phytohormones were extracted from fresh and dried fruitbody of *Cortinarius* Fr. sp., *Lentinus tigrinus* (Bull.:Fr.) Fr., *Coprinus atramentarius* (bull.: Fr.) Fr., *Boletus impolitus* Fr., *Suillus granulatus* (L.: Fr.) O. Kuntze, *Leccinum scabrum* (Bul.: Fr.) S. F. Gray, *Pleurotus ostreatus* (Jacq.: Fr.) Kumm., *Agaricus bernardii* (Quél.) Sacc., *Ptychoverpa bohemica* (Krombholz) Boud., *Agrocybe dura* (Bolt.) Sing., *Lycoperdon molle* Pers.:Pers., *Volvariella speciosa* (Fr.: Fr.) Sing., *Pleurotus eryngii* (DC.: Fr.) Quél., *Morchella esculenta* Pers.: St. Amans, *Coprinus comatus* (Muell.: Fr.)Pers., *Inocybe* Fr. sp., *Hebeloma longicaudum* (Pers.: Fr.) Kumm. and *Amanita gemmata* (Fr.) Gillet.

Extraction, Purification and Determination of Phytohormones

Extraction of t-Zeatin and Gibberellic acid Equivalents (GAs): Two grams of fruiting body from each species of fungi were ground into powder in liquid nitrogen. Then 3 ml of cold methanol was added and homogenized in an Ultra Tissue Lyser (Ultrasonic Processor Jenway LTD.) at 4 °C for 1 h. The homogenization process was continued at 4C for 24 h in dark. The samples were filtered through filter paper (Whatman No: 1) and the supernatant was transferred into clean vials. The residues were reprocessed and combined with the former supernatant. The supernatants were filtered through PTFE filters (0.45 µm) (Cutting 1991, Kuarishi & al. 1991 and Battal & Tilekliogl u 2001). The samples were evaporated under low pressure at 35 °C. For GAs, the aqueous residue was adjusted to pH 2.5 (2 M HCl). This solution was then partitioned with equal volumes of ethyl acetate and the combined organic phases were partitioned with 5% (m/v) sodium bicarbonate (3x1/5 volume) (Fujioka & al. 1986, Çakmak & al. 1989 and Wang & al. 2002). For *t*-Z the extracts were redissolved in KH_2PO_4 (potassium dihydrogen phosphate, pH 8.5) and centrifuged at 10.000 g at 4 °C for 1 h. Then, the supernatants were placed in flasks (25 cm^3), each containing 1 g polyvinylpolypyrolidone (PVPP, Sigma Chemical Co. UK), mixed and filtered through Whatman paper (No. 1) (Mooney & Staden 1984). The filtrates were passed through PVPP and Sep-Pak C_{18} (Waters, Hichrom Ltd. UK) cartridges (Machackova & al. 1993). Hormones adsorbed by the cartridge were eluted with 80% methanol and the extracts were collected in vials.

Extraction of Indole-3-Acetic Acid (IAA): The samples were homogenized in liquid nitrogen using a cold mortar and pestle. The homogenized material was then extracted with methanol containing 0.02% (w/v) diethylcarbamatic acid as an antioxidant for 5 h. Before reduction to the aqueous phase in a rotary evaporator, 10 ml of distilled water was added to the filtered extraction mixture. The aqueous phase was then adjusted to pH 2.7, and the sample was passed through a C18 solid-phase extraction column (Varian, Harbor City, CA) and eluted with 80% methanol (Ostin & al. 1998). For the HPLC analysis, the isocratic system was used. The extracts in the vials were injected into the HPLC equipped with the Waters 6000 A pump (Waters, Hicrom Ltd. UK); Ultraviolet detector (Unicam Analytical Systems, Cambridge, UK) and µBondapak C_{18} column (Waters, Hicrom Ltd. UK). Acetonitrile (12.00%; pH 4.98) was used as the mobile phase. The flow rate, pressure and wavelength were selected to be 2 ml min^{-1}, 2000 psi and 265 nm, respectively. Under these conditions the retention time of GA_3, *t*-Z and IAA was determined to be 2.54, 3.81 and 7.02 minutes for the standards, respectively. The use of the radioactive internal standard was

avoided due to health risk. Instead synthetic kinetin was used and the recovered amount was determined to be 88 %. All analysis was carried out at least in triplicate. Results were expressed as means of different experiments ± standard deviation.

Results and Discussion

The levels of endogenous phytohormones in dried and fresh macrofungi samples were determined by the HPLC and are presented in Table 2. The hormone levels were determined to be species-specific and therefore significantly varied in

Table 2. The level of phytohormones in dried and fresh macrofungi taxa.

Macrofungi	GAs (µg/mg)		*t*-Zeatin (µg/mg)		IAA (µg/mg)	
	dried	fresh	dried	fresh	dried	fresh
Cortinarius sp.	188.03±12.40	80.24±12.53	67.05±7.23	4.58±0.40	0.68±0.14	0.43±0.03
Lentinus tigrinus	80.12±7.81	65.14±13.20	8.05±1.52	0.68±0.03	3.15±0.40	0.46±0.02
Coprinus atramentarius	22.13±3.42	10.24±3.23	17.1±3.30	0.62±0.03	9.48±0.82	0.23±0.01
Boletus impolitus	26.31±3.91	40.21±5.42	9.2±2.20	0.09±0.03	0.54±0.07	0.035±0.004
Suillus granulatus	35.21±4.34	28.41±6.40	6.65±1.13	0.04±0.004	0.85±0.09	0.035±0.006
Leccinum scabrum	267.22±16.2	37.3±4.84	4.36±1.22	0.04±0.01	2.83±0.55	0.065±0.008
Pleurotus ostreatus	30.18±4.21	25.02±2.94	4.43±0.70	0.42±0.03	0.18±0.02	0.024±0.003
Agaricus bernardii	45.86±6.34	28.37±4.32	92.18±11.03	0.89±0.06	0.23±0.04	1.36±0.06
Ptychoverpa bohemica	444.08±31.60	148.65±9.80	40.28±5.10	0.72±0.06	7.27±1.01	0.46±0.02
Agrocybe dura	250.4±11.93	20.18±3.50	32.99±5.33	0.3±0.04	23.41±1.8	0.018±0.006
Lycoperdon molle	376.29±29.62	103.06±10.63	6.39±0.74	0.42±0.05	1.49±0.30	0.16±0.02
Volvariella speciosa	372.02±17.80	96.24±7.23	11.2±2.04	0.042±0.001	12.24±1.01	0.02±0.001
Pleurotus eryngii	147.20±9.62	94.27±8.20	10.13±2.3	0.61±0.04	2.44±0.14	0.13±0.01
Morchella esculenta	76.21±4.40	18.26±2.62	8.14±1.10	0.013±0.001	1.24±0.08	0.06±0.01
Coprinus comatus	94.31±5.31	81.32±5.83	21.2±3.32	2.38±0.08	0.86±0.07	0.62±0.03
Inocybe sp.	425.16±29.70	12.06±1.51	2.65±0.61	0.16±0.02	2.6±0.42	0.03±0.008
Hebeloma longicaudum	80.24±4.63	19.24±3.20	4.41±0.62	0.18±0.03	1.13±0.20	0.074±0.001
Amanita gemmata	298.42±8.22	150.02±12.70	7.42±0.90	0.71±0.05	3.41±0.63	0.09±0.02

dry and fresh samples. The reason to the difference might be that the development of mushrooms is not regular throughout the year and the exact time of harvest is arbitrary. The hormonal levels may be dependent on the habitat, genetic characters, nutritional condition and the secondary products synthesized by fungi. The content of hormones in dried fungi was compared to that in fresh samples.

The highest GAs levels in dry samples were found in *Ptychoverpa bohemica*, 444.08 µg/mg, *Inocybe* sp., 425.16 µg/mg, *Lycoperdon molle*, 376.29 µg/mg, *Volvariella speciosa*, 372.02 µg/mg and *Amanita gemmata*,

298.42 µg/mg. The lowest GAs levels in dry samples were determined in *Coprinus atramentarius*, 22.13 µg/mg, *Boletus impolitus*, 26.31 µg/mg, *Pleurotus ostreatus*, 30.18 µg/mg, *Suillus granulatus*, 35.21 µg/mg. In fresh samples the highest GAs levels were detected in *Ptychoverpa bohemica*, 148.65 µg/mg, *Amanita gemmata*, 150.02 µg/mg, *Lycoprdon molle*, 103.06 µg/mg, *Volvariella speciosa*, 96.24 µg/mg, *Pleurotus eryngii*, 14.27 µg/mg. The lowest GAs level in fresh samples were noted in *Coprinus atramentarius*, 10.24 µg/mg, *Inocybe* sp., 12.065 µg/mg, *Morchella esculenta*, 18.26 µg/mg, *Hebeloma longicaudum*, 19.24 µg/mg, *Agrocybe dura*, 20.18 µg/mg, *Pleurotus ostreatus*, 25.02 µg/mg, *Agaricus bernardii*, 28.37 µg/mg, *Suillus granulatus*, 28.41 µg/mg. The planthormone with the highest level in dried and fresh samples was determined to be GAs. The reason could be that gibberellins stimulate cell division and cell elongation (Davies 1995). Fungi cells grow extremely fast and need a high amount of gibberellins. Interestingly it was observed that the macrofungi taxa in which GAs levels were found in lower concentration were edible and also the higher GAs levels were found mostly in inedible and poisonous taxa (Breitenbach & Kränzlin 1984 – 2000, Buczacki 1989). It can be speculated that there might be a relationship between the edibility and GAs levels produced by fungi. The triggering mechanism of the harmful products in fungi might be due to high GAs concentration. The high amounts of GAs determined in dried fungi, corresponded almost to the same taxa of fresh fungi. However the most degraded plant hormon was GAs when the ratio of lost water content was considered. GAs participation in fungi was higher than IAA and *t*-Z. It may be assumed that *Ptychoverpa bohemica*, *Volvoriella speciosa*, *Lycoprdon molle* and *Amanita gemmata* had a more efficient mechanism of GAs production compared to other fungi or they were more mature than all the rest. It has been suggested that when fungi completes the growth phase, GA_3 production is started. GA_3 synthesis was on the highest level in *Lentinus tigrinus* and *Laetiporus sulphureus* when food sources decreased in the medium. This might indicate a relationship between hormone synthesis and food expenditure (Özcan & Topçuoğlu 2001). GA_3 was reported to induce IAA formation and auxins and gibberellins to act synergistically (Rypáček & Sladký 1972). These results were not supported by the results of the present study because there was no correlation between GAs and IAA levels determined in same taxa.

The highest *t*-Z levels in dry macrofungi were measured in *Agaricus bernardii*, 92.18 µg/mg, *Cortinarius* sp., 67.05 µg/mg, *Ptychoverpa bohemica*, 40.28 µg/mg, *Agrocybe dura*, 32.99 µg/mg. The lowest *t*-Z levels in dry fungi were detected in *Inocybe* sp., 2.65 µg/mg, *Hebeloma longicaudum*, 4.41 µg/mg, *Pleurotus ostreatus*, 4.43 µg/mg, *Leccinum scabrum*, 4.36 µg/mg. In fresh samples, the highest *t*-Z levels were determined in *Cortinarius* sp., 4.58 µg/mg, *Coprinus comatus*, 2.38 µg/mg, whereas the

lowest levels were found in *Morchella esculenta*, 0.013 µg/mg, *Leccinum scabrum*, 0.04 µg/mg, *Volvariella speciosa*, 0.042 µg/mg (Table 2). High Z production was reported to be synthesized at the early growth phase of *Lentinus tigrinus* and *Laetiporus sulphureus* (ÖZCAN & TOPÇUOĞLU 2001). In this study *t*-Z levels were determined to be lower. *t*-Z metabolism could be less efficient or most part of the produced *t*-Z might be broken down in later developmental stages of fruiting body. Cytokinins have an inhibiting effect upon cap opening of *Agaricus bisporus*. The effect has been seen with different concentration of benzyladenine in different individuals. It has been hypothesized that the cap opening on the mycelium is initiated by inhibition of cytokinin supply from the mycelium towards the fruiting body (BRAAKSMA & al. 2001).

An inhibitive effect of cytokinins has been reported by RYPÁČEK & SLADKÝ 1972. Cytokinin activity in cap differentiation and basidiospor formation was reported by SZABO & al. 1970. They confirm the assumption that cytokinins are connected with nucleic acid and protein synthesis which precede the development of basidia. Cytokinins are potent growth factors necessary for cell growth and differentiation. They inhibit the breakdown of protein and nucleic acids, thereby causing inhibition of senescence, and they have the capacity to direct the flow of amino acids and other nutrient through the plant toward the point of high cytokinin concentration. Cytokinins act by preventing genes from being 'turned off' and by activating genes that have been previously 'turned off' (AGRIOS 1988). In the present study, *t*-Z was determined in different levels in all macrofungi taxa. This finding and abundant reports on cytokinin production in fungi reveal that cytokinins play an important role in all growth and developmental stages of macrofungi.

The highest IAA levels in dry fungi were found in *Agrocybe dura*, 23.41 µg/mg, *Volvariella speciosa*, 12.24 µg/mg, *Coprinus atramentarius*, 9.48 µg/mg. The lowest IAA levels in dry fungi were detected in *Pleurotus ostreatus*, 0.174 µg/mg, *Agaricus bernardii*, 0.23 µg/mg, *Boletus impolitus*, 0.54 µg/mg, *Cortinarius* sp., 0.675 µg/mg. The highest IAA levels in fresh macrofungi were determined in *Agaricus bernardii*, 1.36 µg/mg, *Ptychoverpa bohemica*, 0.46 µg/mg, *Lentinus tigrinus*, 0.450 µg/mg, *Cortinarius* sp., 0.43 µg/mg. The lowest IAA levels in fresh samples were detected in *Agrocybe dura*, 0.018 µg/mg, *Volvariella speciosa*, 0.020 µg/mg, *Morchella esculenta*, 0.021 µg/mg and *Pleurotus ostreatus*, 0.024 µg/mg (Table 2). In general IAA levels were determined to be lower than GAs and *t*-Z levels in dried and fresh macrofungi samples. Low IAA and high GAs level were also reported by RYPÁČEK & SLADKÝ 1972. They speculated that the low content of endogenous auxins and high GAs could give an explanation for insignificant effects of IAA and the inhibitive effect of GAs on vegetative mycelium. The comparatively low content of IAA may presumably be due

to the small quantity synthesized or to the quick destruction of formed IAA. The high concentration of IAA is characterized by mitotic activity of mycelia and the primordial fruiting body (RYPÁČEK & SLADKÝ 1972). The phytohormonal levels of *Volvariella speciosa, Coprinus atramentarius, Coprinus comatus, Lentinus tigrinus, Pleurotus ostreatus* and *Agrocybe dura* collected from the urban area were lower than those collected from the rural area. It might be speculated that atmospheric pollution has adverse effect on phytohormon production in fungi. This was confirmed by BESSONOVA 1993, who found low auxin, cytokinin and gibberellin activities in plants grown in heavy metal polluted environments.

Some researches investigated hormonal relationship under some stress conditions in fungi. (ÜNYAYAR 2002). Hormonal interactions may affect hormonal levels in fungi as they do in higher plants (LOPEZ-CARBONELL & al. 1996).

Water content of macrofungi was determined between 77.83–91.46 % (Table 1). The increase in plant hormones content in dried macrofungi was expected theoritically similar to the lost amount of water content. However, hormone content in some macrofungi samples was found more than expected values. The reason was tought that the macrofungi samples maintained to synthesize plant hormones after they were harvested until cells loose viability. It was reported that plant produced more hormone to resist unfavourable environmental conditions (JESCHKE & al. 1997). Macrofungi may have received stress impulses when they lost connection from habitat. Therefore they may have produced more hormones to struggle surviving against the disorganized metabolic functions and the loss of water content.

In some dried macrofungi samples, hormone content decreased because of degridation during drying procedure.

From the above results it follows that endogenous growth regulators take part in the growth and differentiation process of fungi and that these processes are regulated by them. The hormonally controlled mechanism of growth regulation has many common features in fungi and in higher plants (RYPÁČEK & SLADKÝ 1972). There is much evidence in the literature that GAs, Z and IAA are synthesized by fungi. These studies were confirmed by the present study. However, further studies are needed for an integrative understanding of hormone metabolism in macrofungi.

Macrofungi has been used in folk medicine throughout the world since ancient times. However the active substances for the treatment have not been completely determined. Recently, IAA (FOLKES & WARDMAN 2003), methyl jasmonate and salyciylic acid (FINGRUT & FLESCHER 2002) which are considered to be phytohormones were tested in cancer research and found to be influential as antitumors. IAA has been determined in macrofungi. Methyl jasmonate and salyciylic acid are needed to be investigated in macrofungi.

The present study was aimed to supply useful information on the phytohormones potential of macrofungi and will provide further knowledge on their cultivation technology.

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Erratum to

„Determination of phytohormones level in some dried and fresh macrofungi taxa" by TÜRKER M., DEMIREL K., UZUN Y., BATTAL P. & TILEKLIOĞLU B. published in Phyton (Horn, Austria) **45 (2): 145–157**.

The units of phytohormone concentrations in the published article are erroneously reported as µg/mg and need to be replaced by µg/g throughout the entire article.