学 位 論 文 要 旨

Assessment of Allelopathy Activity of Mushrooms in Japan: Proteinogenic and Non-proteinogenic Amino Acids Allelochemicals

日本に生育するキノコのアレロパシー活性の評価：
タンパク質性および非タンパク質性アミノ酸アレロケミカルについて

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Allelopathy through the release of allelochemicals is one of the ecological interactions which effects the growth and development of forest ecosystems. Allelochemicals are directly and indirectly involved in seedling growth disturbances, including the delay and reduction of germination and restriction of root development. Therefore, a total of 670 mushroom fruiting bodies belonging to 289 species of 16 orders of higher macro-fungi (Basidiomycete and Ascomycete) were collected from forests in Japan. Mushroom species were identified and screened for allelopathic effects on the growth of lettuce seedlings (*Lactuca sativa* L. Great Lakes 366, Takii Seed Co. Ltd, Kyoto, Japan) using Sandwich Method. Normally distributed modified sandwich result of current dissertation indicates that mushrooms (R = 41.1%; H = 76.8%; growth average of 289 mushrooms) are remarkably stronger growth inhibitors than higher plants (R = 67.3%; H = 109%; growth average of 660 plants in literatures).

With reference to radicle growth, *Calocybe gambosa* (2.8%), *Cortinarius violaceus* (4.3%), *Xeromphalina tenuipes* (5.1%), *Clavaria miyabeana* (7.6%), and *Heimiella japonica* (10.2%) showed the highest levels of inhibition of radicle elongation. Moreover, it was observed that *X. tenuipes* (11.4%), *Leucopaxillus septentrionalis* (21.4%), *Pholiota spumosa* (25.1%), *C. violaceus* (27.4%), and *Entoloma clypeatum* (28.4%) exhibited the highest levels of hypocotyl elongation inhibition. To illustrate the ranking of all data on strong mushrooms the criteria indices calculated and indicate that the radicle and hypocotyl growth rate data can be combined as a unique index. The standard deviation

variance analysis suggested that the 54 species of 289 are standing higher than a total average for all screened species. Conversely, the results based on the total estimation of criterion (99% confidence) showed that *X. tenuipes, C. violaceus, C. miyabeana, C. gambosa*, *E. clypeatum*, and *P. spumosa* are the strongest mushrooms respectively.

As mushrooms are rich in amino acids and some released non-proteinogenic amino acids into environment showed impact on neighbouring plant by fairy ring phenomenon, the plant growth inhibitory bioassay conducted with 10 mg/L, 50 mg/L, 100 mg/L, 150 mg/L concentrations of 20 proteinogenic and 36 non-proteinogenic amino acids (all commercially available) to measure the range of induced stimulating or inhibiting response on *Lactuca sativa* var. Great lake 366 seedlings growth. L-lysine, L-tryptophan, L-leucine, L- threonine exhibited 62.0, 78.4, 86.4, and 89.6 mg/L respectively, the lowest EC_{50} to radicle growth inhibition. L-DOPA, L-ethionine, 4-hydroxy-L-proline, and sarcosine demonstrated the lowest EC_{50} to radicle growth inhibition by 4.00, 5.00, 10.0, and 42.0 mg/L respectively. The results showed that treatment of exogenous amino acids and their derivatives have impacted on radicle and hypocotyl of lettuce seedlings.

Moreover, *Pleurotus ostreatus* among commercially available edible mushrooms showed the strongest activity, therefore, its free proteinogenic and non-proteinogenic amino acid profile provided by using Ez: faast free amino acid analysis procedure with gas chromatography-mass spectrometry. Sarcosine (4^{th} strongest growth inhibitor) was more abundant than the highest proteinogenic amino acids which were L-alanine and L-glutamic acid (an origin for L-agaritine).

Furthermore, the high-performance liquid chromatography derivatization of non-proteinogenic amino acids of *C. miyabeana*, the third strongest allelopathic mushroom in Japan, visualized that L-azetidine-2-carboxylic acid exists among many other free non-proteinogenic amino acids in this mushroom.

In conclusion, all mushrooms show a level of allelopathic activity to their environment and the Sandwich Method screening can provide a reliable data for that. And mushrooms are the abundant source of proteinogenic and non-proteinogenic amino acids as allelochemicals.