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学位論文題目	Study of regulation effect of bamboo vinegar and bamboo extractives on growth of seed plants (竹酢液と竹抽出物の種子植物成長への調節作用効果に関する研究)
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学位論文の内容の要旨

Recently, the researches of bioactivities of natural material from the plant on the people's life have been advanced. It is required to make safe food by promoting organic agriculture with accordance of the integrity of natural environment, healthy comfortable living space. Bamboo vinegar and bamboo charcoal are attracting more and more attentions as a natural substance in the action of decreasing the agricultural chemicals. Bamboo vinegar is a condensed liquor collected during the pyrolysis of bamboo, composed of more than 200 chemical components, with acetic acid being the main one. It can be used as a deodorizer, in food processing, and in soil improvement and disaffection. Bamboo extractive is a group of substances extracted from bamboo culms or leaves with water or organic solvents. Bamboo extractives involve many complex compounds with various bioactivities. Although at tiny quantity, bamboo extractives also contribute to the bamboo vinegar during the pyrolysis. Moreover, bamboo vinegar and bamboo extractives showed antioxidant activity as well as regulation effect on growth of seed plants. The bioassay results-induced method was used to investigate effective components and composition. At the other hand, the research about antioxidant activity of bamboo extractives identified and evaluated the effective fraction and components in the bamboo extractives with the result-induced method of DPPH scavenging activity.

Chapter 1 Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants

Two kinds of bamboo vinegar from madake bamboo (*Phyllostachys bambusoides*) and moso bamboo (*Phyllostachys pubescens*) were prepared to analyze their components by gas chromatography (GC). The original vinegar, distilled vinegar, ether-extracted vinegar, and three fractions including acidic, neutral, and phenolic fractions separated from ether-extracted vinegar were diluted with distilled water 10^2 to 10^7 times. These diluted vinegar solutions were used to investigate the effect of bamboo vinegar on the germination and radicle growth of seed plants. High concentrations of all kinds of treated bamboo vinegars (e.g., 10^2 of original vinegar and 10^3 of ether-extracted vinegar) showed strong inhibition against germination of the seeds. However, an appropriate dilution of bamboo vinegar showed an obvious promotional effect on germination and radicle growth for the four kinds of tested seeds (lettuce, watercress, honewort, and chrysanthemum).

Chapter 2 Composition of moso bamboo vinegar at different collection temperature and its effects on germination and radicle growth of seed plant

Moso bamboo vinegar was treated with extractive and separation methods. The acidic, neutral, and phenolic fractions separated from ether-extracted vinegar were analyzed by gas chromatography and gas chromatography-mass spectrometry to identify the major components in moso bamboo vinegar. The compositions of eight moso vinegar fractions collected over different temperature ranges from 100°C to 480°C were also analyzed and their effects on regulation of germination and growth were studied by bioassay with seeds of watercress and chrysanthemum.

The physical and chemical characteristics of moso bamboo vinegars from different collection temperatures reflected the changing composition of the vinegar during the pyrolysis. Although the vinegars from different collection temperatures showed almost the same composition, the relative contents of the main compounds in the vinegar appeared to vary with collection temperature, especially for acetic acid and the acetic fraction. The results of bioassays on two seed types with vinegars from eight collection temperature ranges showed the tendency of vinegars collected at 250°C - 400°C to inhibit radicle and hypocotyl growth of seeds. Chrysanthemum germination and initial growth of radicle and hypocotyl were markedly promoted with vinegar collected at 200°C - 250°C . Watercress was promoted less than chrysanthemum. In consideration of the bioassay results of two seeds, vinegar collected under 250°C had good regulation effect at 10^3 dilution for chrysanthemum and 10^4 dilution for watercress. Collection temperature could be an important factor for controlling the quality of bamboo vinegars in its production and to aid its further application.

Chapter 3 Regulation effect of bamboo extractives on growth of seed plants

The extractives from madake bamboo (*Phyllostachys bambusoides*) and moso bamboo (*Phyllostachys pubescens*) culms were investigated on the regulation effect on growth of seed plants by bioassay method. Methanol-extractives and its fractions separated by the solvents of n-hexane, diethyl ether, ethyl acetate were tested on seeds of lettuce, watercress, and chrysanthemum.

Methanol-extractives were found to have some regulation effects on the growth of seed plants tested. They showed an inhibition effect on the radicle growth and some promotion effects on the hypocotyl growth of seeds. Further fractionation of methanol extractives showed that diethyl ether soluble fraction, containing more phenolic substances, appeared as a strong inhibition on the growth of lettuce seeds. Only water soluble fraction showed a promotion effect on the hypocotyl growth of lettuce as well as an inhibition effect on radicle growth. Through heat treatment, the inhibition effect of water soluble fraction on the radicle growth was decreased and the promotion effect on the hypocotyl growth was increased at 0.1% concentration according to the bioassay results with lettuce seeds, suggesting that heat-treatment such as pyrolysis might induce some changes for regulation effect of bamboo extractives on initial growth of seed plants. Heat-treatment was thought to be a useful method for utilization of extractives from bamboo.

Chapter 4 Identification and evaluation of antioxidant activities of bamboo extracts

The antioxidant activity of solvent extracts from two main bamboo species, moso bamboo (*Phyllostachys pubescens*) and madake bamboo (*Phyllostachys bambusoides*) in Japan, was first evaluated by scavenging free radical of 1,1-diphenyl-2-picrylhydrazyl (DPPH), the inhibition activity for peroxidation of linoleic acid, and the reduction power. The methanol-extracts of moso bamboo culms and madake bamboo leaves presented stronger antioxidant activity compared with DPPH scavenging activity. Methanol-extract of moso bamboo culms was further fractionated by different solvents and *n*-butanol soluble fraction exhibited the most significant activity in the DPPH scavenging assay. The fractionation of *n*-butanol soluble extract was isolated by silica gel column with the gradient mixture solvent of chloroform and methanol. The isolated fractions were directed by the antioxidant activity measured by scavenging the stable DPPH free radical. It was observed that most of the eluted fractions showed the antioxidative activity. Fractions acquired from elution with the mixture solvent of chloroform:methanol/ (10:1-5:1) showed stronger antioxidant activity than the other fractions.

論文審査の結果の要旨

最近、植物由来の天然物質の生理活性に関する研究が注目を集めるようになった。安全快適な自然の生活空間を求めて、有機農法による安全な食物が求められており、竹酢液や竹炭は、減農薬のための資材として活用され始めている。さらに、竹酢液は脱臭剤、食品加工剤、土壌改良剤その他にも活用されている。しかし、竹酢液は木酢液ともども、科学的実証が少ないまま、官能評価による人気が先行している。それ故、今後の発展のため、正確な実験に基づいた資料を必要としている。

竹酢液は竹熱分解時の燻煙からの凝縮液であり、酢酸を主とする 200 種類以上の化学成分から成っている。一方、竹抽出物は竹の幹や葉から水または有機溶媒による抽出により得られる。竹抽出物は生理活性を有する数多くの複雑な化合物を含んでいると考えられている。竹抽出物は微量にもかかわらず、熱分解中に竹酢液成分として寄与する。さらに、竹酢液や竹抽出物は種子植物成長への制御効果とともに抗酸化活性を示す。

本研究では、再現性を得るために温度プロファイルを正確に制御した熱分解炉を用いて、孟宗竹および真竹からの竹酢液を得、原竹酢液、蒸留竹酢液およびエーテル抽出竹酢液を準備した。エーテル抽出竹酢液を、さらに酸性、中性およびフェノール性成分に分画した。これらを 10^2 から 10^7 倍に希釈し、レタス、クレソン、春菊等の種子植物の発芽、幼根成長試験を行った。高濃度 (10^2 倍、エーテル抽出液では 10^3 倍) 条件で強い成長抑制が認められた。しかし、適切な濃度に希釈すると成長促進効果が現れた。

熱分解温度の影響を検討するため、捕集温度別に 100°C から 480°C にかけて 50°C ずつ孟宗竹酢液を捕集した。竹酢液はエーテルで抽出し、エーテル抽出酢液を得た。さらに、酸性成分、中性成分とフェノール性成分を分画し、GC、GC-MS で化学成分を分析した。熱分解過程によって竹酢液の物理的、化学的な特性は変動した。捕集温度別孟宗竹酢液の構成成分は類似していたが主成分の含有量が異なった。特に、酢酸と酸性成分の変化が大きかった。春菊およびクレソン種子での生物検定の結果、 $250-400^{\circ}\text{C}$ で捕集した竹酢液は種子の幼根成長と胚軸成長を最も抑制した。 $200-250^{\circ}\text{C}$ で捕集した酢液は春菊種子の発芽と幼根成長および胚軸成長を著しく促進した。春菊種子よりクレソン種子に対しての促進作用が弱かった。 250°C まで捕集した酢液は春菊種子の場合に 10^3 倍、クレソン種子の場合に 10^4 倍希釈して、良好な促進効果が現れた。

竹酢液製造に用いたのと同じ孟宗竹および真竹からのメタノール抽出物を成分分析するため、n-ヘキサン、エーテル、酢酸エチルで分画した。各分画成分について、生物検定を行った結果、ほとんどすべての成分で、種子植物成長に対して抑制効果のみが現れた。エーテル可溶成分にフェノール性成分が多く含まれ、特に強い抑制効果が現れた。水可溶成分のみが、幼根成長抑制にもかかわらず、レタス胚軸成長を促進した。これらの抽出物を熱処理することにより成長促進が現れた。熱処理すなわち熱分解は必要な操作であることが明らかとなった。

孟宗竹および真竹からの抽出物について抗酸化能を求めた。孟宗竹幹および真竹葉からの抽出

物に高い抗酸化能が見られた。孟宗竹幹メタノール抽出物を分画すると、n-ブタノール可溶成分に抗酸化能が強く現れた。その中のクロロホルム：メタノール(10:1-5:1)可溶画分の抗酸化能が最も強かった。

以上のように、本研究は竹酢液の利用濃度条件により、種子植物の成長について、抑制あるいは促進されることを数値的に明らかにした。また、一部の促進成分についての解明にも成功した。種子植物の成長のためには、竹抽出物の熱変性が必要であることが明らかとなり、竹酢液の妥当性を明らかにした独創的な研究であり、学位論文として十分な価値を有するものである。