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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目： Allelopathic Potential and Allelopathic Substances from Leaves of Three Tree Species
Title of Dissertation (3 種の樹木の葉のアレロパシー活性及びアレロパシー物質)

学位論文要約：
Dissertation Summary

Weed management is one of the main challenging tasks for arable farmers as weed reduces crop yields by competing with crops for light, nutrients, water and space. Globally weeds caused higher amount of yield losses (about 34%) in major agricultural crops in compare to other pests (Jabran et al., 2015). In this sense, farmers rely on synthetic herbicides to control weeds in their crop fields because of easy accessibility and a more rapid return. Over reliance and continuation of synthetic herbicide to control weeds may cause negative impacts on environment as well as may change weed flora and development of herbicide-resistant weed biotypes (De Prado et al., 1997). Therefore, the research on allelopathy might be an alternative biological solution to help for improving weed control in sustainable crop production (Jabran et al., 2015).

The word 'allelopathy' was first initiated by Molisch (1937), and that word originates from two Greek words 'allelon' meaning 'mutual' or 'among each other' and 'pathos' meaning 'suffering' or 'feeling' which expressed as both beneficial and harmful effects of chemicals released from plants into environment (Willis, 2007). Allelopathy refers to the process where secondary metabolites produced by plants, microorganisms, viruses and fungi that impact on the growth, development and distribution of other plants, microorganisms, viruses and fungi in agricultural as well as biological systems (International Allelopathy Society, 1996). It has been noted that allelochemicals are present in many plants as well as many plant organs, such as roots, rhizomes, stems, leaves, flowers, fruits etc. (Weir et al., 2004). These substances are released into the environment under particular conditions through a number of physio-chemical actions viz. volatilization, foliar leaching, root exudation, residue decomposition or other processes in sufficient quantities. These allelopathic substances may interfere directly or indirectly with the growth and development of nearby plant species (Weir et al., 2004). This method of controlling weeds is considered environmentally friendly compared with commercial herbicides (Duke et al., 2000). Plants produce a diverse number of secondary metabolites, and some of them have been treated as allelopathic substances because of their potential phytotoxicity. Therefore, many researchers have been interested in isolating and identifying those allelopathic substances from different allelopathic plants because of their weed suppression ability, and these substances have been used for sustainable weed management systems (Kato-Noguchi et al., 2014; Miranda et al., 2015). Moreover, tree species with allelopathic potential may promote the stability of agro-forestry systems, especially for weed control (Chu et al., 2014). Many higher plants have been recognized as allelopathic plants as they exhibit allelopathic properties. Several researchers in this area have already checked the allelopathic effects of different tree species on different weeds and crop plants (Chu et al., 2014; Kato-Noguchi et al., 2014; Miranda et al., 2015). Thus, emphasis is given on tree species because of contain a myriad of bioactive substances.

Litchi (*Litchi chinensis* Sonn.), cannon-ball (*Couroupita guianensis* Aubl.) and mango (*Mangifera indica* Linn.) have been drawn the attention to many researchers because of their myriad of pharmacological properties. But very little information about the allelopathic properties of those tree species is available. Therefore, the aim of the study was conducted to check the allelopathic potential as well as isolate and identify allelopathic substances from leaves of these species.

Litchi leaves

Cannon-ball leaves

Mango leaves

The aqueous methanol extracts of litchi, cannon-ball and mango (cv. Khirshapat, Himsagor and Sinduri) leaves were examined at different concentrations against germination and growth of six test plants; cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.), Italian ryegrass (*Lolium multiflorum* Lam.) and barnyard grass (*Echinochloa crus-galli* L. Beauv) except litchi and canon-ball, where two test plants (lettuce and barnyard grass) were used for germination test. Among six test plants, the first three (cress, lettuce, alfalfa) were dicotyledonous, and rest of three (timothy, Italian ryegrass, barnyard grass) were monocotyledonous. Cress, lettuce, alfalfa and timothy were chosen as test plants for their known seedling growth pattern, whereas, Italian ryegrass and barnyard grass were selected because they are regarded globally as deleterious weeds. Bioassay technique is important to assess the allelopathic activity of plant extracts, and laboratory bioassays can allow to check biological activity of a large number of plant species at a time that would be helpful for selecting plants in field level to verify allelopathy (Hoagland and Williams, 2003).

Seed germination of all test plants were significantly inhibited by the extracts obtained from litchi, cannon-ball and mango leaves at concentrations ≥0.01 g dry weight (DW) equivalent extract mL^{-1}. The extracts of litchi and cannon-ball leaves completely inhibited germination of lettuce and significantly delayed germination of barnyard grass at the concentration of 0.1 g DW equivalent extract mL^{-1}. At the same concentration, extracts obtained from three mango cultivars (Khirshapat, Himsagor and Sinduri) showed a significant delayed or/and complete inhibition of germination of all test plants except for Sinduri extracts on barnyard grass. A similar pattern of inhibition of germination was reported by Escudero et al. (2000). The reasons for the inhibitory effects of germination caused by allelopathic substances could be i) disruption and impairment of mitochondrial respiration, ii) breakdown of the activities of metabolic enzymes (Weir et al., 2004), iii) breakage of cells leading to cell death (Lin et al., 2000), and iv) correlation with increased cell membrane degradation (Bogatek et al., 2006).

The hypocotyl/coleoptile and root growth of all test plants were significantly inhibited by extracts obtained from three species at concentrations ≥0.01 g DW equivalent extract mL^{-1}. The inhibitory activities were dependent on test plants and extracts concentration. The observed different inhibitory activities on different test plants indicates that the variation in selectivity of allelopathic substances was specific against target plants. The possibility of such unequal susceptibility to extracts may be due to the involvement of biochemicals with their different inherent responses. The variable sensitivities to allelopathic substances on various test plants have been reported in another study (Hussain and Reigosa, 2011). The extracts obtained from litchi leaves completely inhibited hypocotyl growth of cress, lettuce and alfalfa, and root growth of all test plants, while 87, 90 and 62% coleoptile growth inhibition was found on timothy, Italian ryegrass and barnyard grass, respectively at the concentration of 0.1 g DW equivalent extract mL^{-1}. At the same concentration, the hypocotyl/coleoptile and root growth of all test plants were inhibited by leaf extracts of cannon-ball to more than 90%, except for hypocotyl/coleoptile growth of alfalfa and barnyard grass. On the other hand, three mango extracts showed more than 70% hypocotyl/coleoptile and root growth inhibition of all test plants except barnyard grass at the concentration of 0.1 g DW equivalent extract mL^{-1}. The leaf extracts of Khirshapat, Himsagor and Sinduri leaves inhibited total mean hypocotyl/coleoptile growth of all test plants to 48, 45, and 36%, respectively, and inhibited total mean root growth of all test plants to 60, 60 and 42%, respectively. The concentration required for 50% growth inhibition (I_{50}) for hypocotyl/coleoptile and root growth of all test plants ranged from 0.002-0.079, 0.001-0.010 and 0.003-0.103 g DW equivalent extract mL^{-1} for litchi, cannon-ball and mango, respectively. Considering the hypocotyl/coleoptile and root growth of all the test plants, root growth was more sensitive to the extracts than hypocotyl/coleoptile growth. The causes of high sensitivity in the roots to allelopathic substances may be due to i) the involvement of root growth in both cell expansion and cell proliferation (Nishida et al., 2005), ii) higher permeability of allelopathic substances in root surfaces than the hypocotyl/coleoptile (Yoshimura et al., 2011), and iii) direct proximity between the roots and the extracts (Qasem, 1995).

As no significant differences among three mango cultivars were found for their inhibitory activities on germination and growth of hypocotyl/coleoptile as well as root of all test plants, all of them could be candidates for isolation and identification of allelopathic substances. Therefore, further isolation and identification of these substances from the extracts of Khirshapat leaves was first proceeded. The aqueous extract of mango leaves (cv. Khirshapat) was then adjusted to pH 7.0 with 1.0 M phosphate buffer and partitioned three times against an equal volume of ethyl acetate. As greater inhibitory activity of ethyl acetate fraction than that of aqueous fraction, the further purification process was proceeded with the ethyl acetate fraction. The ethyl acetate fraction was purified through several chromatographic runs, such as silica gel column, Sephadex LH-20 and reverse-phase C_{18} cartridges. Finally, a growth inhibitory substance was achieved after purification of reverse-phase HPLC. The compound was characterized by high-resolution ESI-MS, ^{1}HNMR (400 MHz, CD_3OD) and ^{13}CNMR spectra (100 MHz, CD_3OD), and an allelopathically active substance was identified by spectral data as methyl-3,4,5-trihydroxybenzoate (methyl gallate). Some researchers from different regions in the world used similar bioassay-guided fractionation strategy to isolate allelopathic substances from different plant species (Rimando et al., 2001; Queiroz et al., 2012). The inhibitory effects on seedling growth of garden cress and foxtail fescue were observed at concentrations ≥1 mM of methyl gallate. The I_{50} values of methyl gallate on garden cress root and hypocotyl were 4.9 and 3.4 mM, respectively, whereas those on foxtail fescue root and coleoptile were 1.4 and 10.1 mM, respectively. Comparing I_{50} values, effectiveness of methyl gallate on hypocotyl was 1.4-fold greater than that of root of garden cress and on root was 7.2-fold greater than that of coleoptile of foxtail fescue, respectively. These results indicate that methyl gallate may contribute an important role in allelopathic activities of mango.

Leaves of litchi, cannon-ball and mango inhibited seed germination and seedling growth of different test plants including weeds. These results suggest that these three tree species have allelopathic potential and may possess allelopathic active substances. Therefore, these three species could be candidates for isolation and identification of those active substances to serve as weed inhibiting agents for sustainable crop production. An allelopathic substance was found as methyl gallate in mango leaves and the substance showed inhibitory activities on test plants at concentration dependent manner. However, further isolation and identification of allelopathic active substances from leaves of litchi and cannon-ball species should be investigated for assessing their effects on weeds.

References:

Bogatek, R., Gniazdowska, A., Zakrzewska, W., Oracz, K. and Gawronski, S.W. (2006). Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth. Biol. Plantarum 50:156–158.

Chu, C., Mortimer, P.E., Wang, H., Wang, Y., Liu, X. and Yu, S. (2014). Allelopathic effects of eucalyptus on native and introduced tree species. Forest Ecol. Manag. 323:79–84.

De Prado, R., Jorrin, J. and Garcia-Torres, L. (1997). Weed and crop resistance to herbicides. Kluwer Academic Publishers, Dordrecht, The Netherlands, ISBN-13: 9780792345817, Page: 340.

Duke, S.O., Dayan, E.F., Romagni, J.G. and Rimando, A.M. (2000). Natural products as sources of herbicides, current status and future trends. Weed Res. 40:99−111.

Escudero, A., Albert, M.J., Pita, J.M. and Garcia, F.P. (2000). Inhibitory effects of *Artemisia* herba-alba on the germination of the gypsophyte *Helianthemum squamatum*. Plant Ecol. 148:71–80.

Hoagland, R.E. and Williams, R.D. (2003). Bioassays: Useful tools for the study of allelopathy. In: Allelopathy: Chemistry and Mode of Action of Allelochemicals (ed. Macias, F.A., Galindo, J.C.G., Molinillo, J.M.G. and Cutler, H.G.). Boca Raton, FL: CRC Press. pp. 315–351.

Hussain, M.I. and Reigosa, M. J. (2011). Allelochemical stress inhibits growth, leaf water relations, PSII photochemistry, non-photochemical fluorescence quenching, and heat energy dissipation in three C_3 perennial species. J. Exp. Bot. 62:4533–4545.

International Allelopathy Society (1996). Constitution and bylaw of IAS. IAS Newsletter, Cadiz-Spain.

Jabran, K., Mahajan, G., Sardana, V. and Chauhan, B.S. (2015). Allelopathy for weed control in agricultural systems. Crop Prot. 72:57–65.

Kato-Noguchi, H., Salam, M.A., Ohno, O. and Suenaga, K. (2014). Nimbolide B and nimbic acid B, phytotoxic substances in neem leaves with allelopathic activity. Molecules 19:6929–6940.

Lin, Y.C., Chang, J.H., Cheng, S.Y., Wang, C.M., Jhan, Y.L., Lo, I.W., Hsu, Y.M., Liaw, C.C., Hwang, C.C. and Chou, C.C. (2015). New bioactive chromanes from *Litchi chinensis*. J. Agric. Food Chem. 63:2472–2478.

Miranda, M.A.M., Varela, R.M., Torres, A., Molinillo, J.M.G., Gualtieri, S.C.J. and Macias, F.A. (2015) Phytotoxins from *Tithonia diversifolia*. J. Nat. Prod.78:1083−1092.

Molish, H. (1937). Der Einfluss einer Pflanze auf die andera-Allelopathie; Fischer: Jena, Germany.

Nishida, N., Tamotsu, S., Nagata, N., Saito, C. and Sakai, A. (2005). Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. J. Chem. Ecol. 31:1187–1203.

Quasem, J.R. (1995). The allelopathic effect of three *Amaranthus* spp. (Pigweeds) on wheat (*Triticum durum*).

Weed Res. 35:41–49.

Queiroz, S.C.N., Cantrell, C.L., Duke, S.O., Wedge, D.E., Nandula, V.K., Moraes, R.M. and Cerdeira, A.L. (2012). Bioassay-directed isolation and identification of phytotoxic and fungi toxic acetylenes from *Conyza Canadensis*. J. Agric. Food Chem. 60:5893–5898.

Rimando, A.M., Olofsdotter, M., Dayan, F.E. and Duke, S.O. (2001). Searching for rice allelochemicals: an example of bioassay-guided isolation. Agron. J. 93:16–20.

Weir, T.L., Park, S. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opin. Plant Biol. 7:472–479.

Willis, R.J. (2007). The history of allelopathy. Springer, Dordrecht, the Netherlands.

Yoshimura, H, Sawa, Y., Tamotsu, S. and Sakai, A. (2011). 1, 8-cineole inhibits both proliferation and elongation of by-2 cultured tobacco cells. J. Chem. Ecol. 37:320–328.