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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：
Title of Dissertation
Allelopathic potential and allelopathic substances of three medicinal plants in Bangladesh
(バングラデシュ産薬用植物3種のアレロパシー活性とアレロパシー物質)

学位論文要約：
Dissertation Summary

Among all the pests, weeds are the hurdle barrier to crop production, always have a deleterious effect on crops, and causes about 34% yield losses of crops (Pitelli 1985; Jabran et al. 2015). In addition, weeds act as a shelter for other pests: insects, pathogenic fungi, and nematodes (Caballero-Lopez et al. 2012; Foerster et al. 2015). Riches (2001) reported that nearly 227 weed species are directly or indirectly involved in crop losses that might reach up to 90%. As weed causes significant loss in crop yields and quality, proper management of weed is an important and reclaim task in agriculture (Davies and Welsh 2002). In agricultural practices, applying synthetic herbicides to control weeds has long been an established and effective approach (Varshney et al. 2012; Tanveer et al. 2015). In this regard, farmers mostly depend on synthetic herbicides because of their availability, effectiveness, and rapid action, resulting in billions of dollars spent on herbicides every year (Aktar et al. 2009; Varshney et al. 2012; Kraehmer and Baur 2013). But indiscriminate and extensive use of synthetic herbicides now possesses a conceivable risk both to human beings and the environment (Daniel et al. 2013; Pimentel and Burgess 2014; Starling et al. 2014; Mahmood et al. 2016). To mitigate the hazardous effects of synthetic herbicides, alternative methods of weed control based on natural products is now a pressing issue. Natural products based alternative management options rather than the use of synthetic chemicals might be helpful to control weeds in sustainable agriculture (Khanh et al. 2007). Natural herbicides from allelochemicals or phytotoxic compounds have more benefits over synthetic compounds as they have less or non-toxic effect and short half-life, considered as safe weed management way for environment (Asaduzzaman et al. 2014; Nebo et al. 2014). Therefore, natural product based herbicide can be applicable as a component of sustainable weed management to ensure satisfaction of human needs for present and future generations (Chai et al. 2015; Das and Kato-Noguchi 2018). Therefore, many researchers have been interested in isolating and identifying those allelopathic substances from different allelopathic plants. Phytotoxic or allelopathic compounds have diversified modes of action and influence the growth of target plants (Lipkus et al. 2008; Ullah et al. 2015). Moreover, allelochemicals or phytotoxic substances have no residual toxic effects on the environment (Amb and Ahluwalia 2016).

Consequently, allelopathy may be an important consideration in replacing synthetic herbicides (Ullah et al. 2015). Allelopathy is a biological phenomenon, in which one plant directly or indirectly interferes with the growth and development of other plants through the release of allelochemicals (Rice 1984). Allelopathy has gained increased attention as an alternative tool for eco-friendly management of weeds in a sustainable way (Farooq et al. 2011; Nebo et al. 2014).

To explore allelopathic activity, much effort has gone into searching for potential growth inhibitory substances in controlling weeds from medicinal plants, which appear to be promising sources of secondary metabolites (Gilani et al. 2010; Alvin et al. 2014; Shakya 2016). In this regard, there has been much attention on finding potential allelochemicals from medicinal plants (Kuddus et al. 2011).

In the present experiments, we took plant parts of three medicinal plants from Bangladesh comprising *Swietenia mahagoni* L. (Meliaceae), *Coccinia grandis* L. (Cucurbitaceae) and *Cassia alata* Linn. (Caesalpiniaceae) for the assessment of allelopathic potential and identifying allelochemicals within them. These three plants are known as medicinal plants locally and as far we know, the allelopathic potential of these plants are not well documented. Therefore, the aim of the study was conducted to check the allelopathic potential as well as isolate and identify allelopathic substances from these species.



***Swietenia mahagoni* L.** ***Coccinia grandis* L.** ***Cassia alata* Linn.**

The aqueous methanol extracts of three medicinal plant species were examined at different concentrations against seedling growth of some test plants; cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus* L.), broccoli (*Brassica oleracea* Var. *italica*), cabbage (*Brassica oleracea* Var. *capitata*), radish (*Raphanus raphanistrum* subsp. *sativus*), barnyard grass (*Echinochloa crus-galli* L. Beauv), timothy (*Phleum pratense* L.), Italian ryegrass (*Lolium multiflorum* Lam.) and foxtail fescue (*Vulpia myuros* (L.).C.C.Gmel. The hypocotyl/coleoptile and root growth of all test plants were significantly inhibited by extracts obtained from three species and the inhibitory activities were dependent the extracts concentration. This may simply because of the greater amount of inhibitory substances present in the concentrated extracts and well agreed with the findings of Sarkar et al. (2012). In line with our results, several researchers also reported similar findings of concentration dependent activity of plant extracts having allelopathic potential (Ashrafi et al. 2008; Hassan et al. 2012; Salhi et al. 2012).

The extracts obtained from *S. mahaoni* seeds significantly inhibited the seedling growth lettuce, rapeseed, alfalfa, cress, barnyard grass, timothy and foxtail fescue by 5.3, 9.1, 9.5, 12.1, 12.9, 21.7 and 23.6 % of control and 0.0, 2.7, 8.9, 6.1, 3.0, 7.1 and 12.7 % of control, respectively at the concentration of 300 mg DW (Dry Weight) equivalent extract/mL. When the test plant species were subjected to a concentration of 300 mg DW equivalent extract/mL of *C. grandis*, the seedling growth of cress and lettuce was completely inhibited. At the same concentration, shoot and root growth of the other test species (alfalfa, rapeseed, barnyard grass, timothy, foxtail fescue, and Italian ryegrass) were significantly inhibited to 5.2, 1.8, 64.7, 36.0, 13.7, and 7.7% and 3.9, 0.6, 10.5, 8.3, 6.3, and 5.0% of control, respectively.

The concentration obtained from 300 mg dry weight equivalent extract of *C. alata*/mL completely inhibited (100%) the shoot and root growth of cress, lettuce, Italian ryegrass, and timothy. The shoot and root growth of alfalfa, rapeseed, barnyard grass, and foxtail fescue were inhibited by 4.1, 13.0, 5.6, and 0.5% of the growth of the control shoots and 2.9, 5.3, 1.5, and 0.4% of the growth of the control roots. To corroborate that findings, in another experiment we have checked the biological activity of the leaf extracts of *C. alata* using five species of Brassicaceae family. This is a large family with many plants of major economic importance, including many familiar vegetables, oil crops, ornamental plants, and weeds (Guarino et al. 2000). Here, Complete (100%) growth inhibition of cress was found at a concentration of 300 mg dry weight equivalent extracts of *C. alata* mL^{-1}. At the same concentration, the hypocotyl and root growth of broccoli, cabbage, radish, and rapeseed were inhibited by 9.2, 1.9, 9.1, and 13.0% of the control hypocotyl growth, respectively, and 3.6, 0.6, 6.1, and 5.2% of the control root growth, respectively.

The concentrations required for 50% growth inhibition (I_{50}) for hypocotyl/coleoptile and root growth of all test plants ranged from 0.7-34.8, 4.8-220.8 and 3.3-107.7 mg DW equivalent extract mL-1 for *S. mahagoni*, *C. grandis* and *C. alata*, respectively. Different species, varieties and types of plant having different genetic makeup may be responsible for species specific inhibitory effect (Rashed Mohasel et al. 2009; Prati and Bossdorf 2004).

Though all the plant materials showed the inhibitory activity, but considering the amount of plant material and more inhibitory activity, we took *Cassia alata* for further isolation and identification of allelochemicals. The extracts of *C. alata* leaves was purified by several chromatographic separations through bioassay-directed fractionation, and four allelopathically active substances were identified by spectral data as rutin, syringone, (+)-rhododendrol, and 3-hydroxy-α-ionone. The biological activities of rutin and syringone were measured by conducting bioassays with cress and foxtail fescue. The seedling growth of cress and foxtail fescue was significantly inhibited by rutin at concentrations greater than 3 µM. In contrast, syringone significantly inhibited the seedling growth of cress and foxtail fescue at concentrations greater than 100 and 10 µM, respectively. The I_{50} values of rutin for the shoots and roots of cress were 246.7 and 129.5 µM, respectively, and those of foxtail fescue were 417.8 and 320.4 µM, respectively. On the other hand, the I_{50} values of syringone for the shoot and root growth of cress were 223.8 and 160.1 µM, respectively, whereas those of foxtail fescue were 466.5 and 219.6 µM, respectively. As the amounts were not enough, only the cress bioassay was conducted to determine the biological activities of (+)-rhododendrol and 3-hydroxy-α-ionone. (+)-Rhododendrol significantly inhibited the seedling growth of cress at concentrations greater than 100 µM. In contrast, 3-hydroxy-α-ionone significantly inhibited cress seedling growth at concentrations greater than 30 µM. The I_{50} values of (+)-rhododendrol for the hypocotyl and root growth of cress were 296.1 and 192.0 µM, respectively, and the I_{50} values of 3-hydroxy-α-ionone for the hypocotyl and root growth of cress were 195.3 and 132.4 µM, respectively. These results indicate that rutin, syringone, (+)-rhododendrol, and 3-hydroxy-α-ionone, all these four allelopathically active substances may contribute the important role in allelopathic activities of *C. alata*.

The results of present research indicated that seeds of *S. mahagoni*, shoots of *C. grandis*, and leaves of *C. alata* inhibited seedling growth of different test plants including weeds. These results suggest that these three species have allelopathic potential and may possess allelopathic active substances. Four allelopathic substances were identified from the leaves of *C. alata* which were termed as rutin, syringone, (+)-rhododendrol, and 3-hydroxy-α-ionone. These substances inhibited seedling growth of the test plant species in concentration-dependent manner. These results suggest that the identified four compounds from *C. alata* leaves may contribute the important role on allelopathic activities, which may provide an insight into managing weeds to replace the common practice of using synthetic herbicides. Further research efforts, however should focus not only their efficacy in laboratory condition but also needed to find out the allelopathic activities of these species under the field and greenhouse condition for assessing their effects on weeds.

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