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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 Allelochemicals in Four Weed Species** (4種の雑草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

Since the ancient times of agriculture, manual, cultural and mechanical weeding, and later on, herbicide applications have been the most preferable weed control methods. But unavailability of labor, higher maintenance costs for mechanical implements, reduced effectiveness, and non-cost-effective weed controls ways are major problems associated with manual, cultural, mechanical weed control options (Riaz *et al.*, 2007; Hussain *et al*., 2008; Awan *et al*., 2015). Likewise, the most usage and reliable herbicidal weed control option is now facing some challenges. Herbicide resistance of weed-varieties, negative health effects to animal and human, weeds population shifts, environmental concerns are the major threats of using herbicides and reduce its efficacy. In addition, more importantly, application of herbicides is not possible in the fields where crops are being cultivated organically (Annett *et al*., 2014; Starling *et al*., 2014; Jabran *et al*., 2015). Thus, looking for a natural based approach, alternative to synthetic herbicides is necessary for weed management. The approach of using plant species having strong allelopathic potential is one of the alternative tactics to reduce the dependency on herbicides for weed management (Jamil *et al*., 2009; Duke *et al*., 2010; Chai *et al*., 2015).

In the plant communities, their interaction is either positive or negative. Usually plants which are in close proximity interact negatively, where their growth and emergence are inhibited. This effect is called interference (Foy and Inderjit, 2001). This plant interference can be explained by tow phenomena, allelopathy and resource competition. The word allelopathy has Greek roots, deriving from “Allelon” which means ‘of each other’ and

"Pathos" which means 'to suffer' or 'suffering'. The word demonstrates the damaging effect when there is chemical interference between plants under natural conditions other than nutritional crisis. On the other hand, competition is where resources are limited, such as nutrients, space, water, light (Rice, 1984; Qasem and Foy, 2001; Weston, 2005). The ability of a plant to have an influence upon a nearby plant through releasing chemicals into the environment has been known from approximately early as 370 BC. The Greeks and Romans, since 64 AD, used such knowledge in their agriculture. But the term "allelopathy" was coined in 1937, by Hans Molisch (Weston, 2005). To define allelopathy strictly, allelopathy makes reference to either a harmful or a beneficial effect that one plant has on another, including in both weed and crop species, by releasing allelochemicals from parts of the plant by leaching, residue decomposition, volatilization, root exudation, and other processes in agricultural and natural systems (Weston and Duke, 2003). Allelochemicals are secondary plant products which come from the plant's main metabolic pathways and are released into the environment (Putnam, 1988; Harun *et al*., 2014). Rice (1984) pointed out that allelochemicals have an effect on many biochemical and physical plant processes. Allelochemicals exist in many plants and organs, including buds, fruits, flowers, and leaves. In some circumstances, these compounds are released into the environment, this can be as exudate from living tissue or by decomposing plant residue in sufficient quantity to have an effect on successional or neighboring plants (Putnam and Tang, 1986; Einhellig, 1986).

In the past few decades, studies on allelopathy have increased, there is much literature which implicates that allelopathy is an important form of plant interference. Advancements in allelopathy occurred because of developments in techniques for identification, isolation, bioassay, and extraction of chemicals (Qasem and Foy, 2001; Weston, 2005). Recently, allelopathy's importance has grown as a potential biological weed control in the replacement of herbicidal weeds control (Inderjit and Keating, 1999; Qasem and Foy, 2001; Nishida *et al*., 2005). Studies have been carried out to search for allelopathic potential in different crops toward weed control, and vice versa. The scientists have suggested that allelochemicals can reduce the reliance on traditional herbicides. Allelopathy studies have focused mainly then on the isolation and identification of allelochemicals in both weed and crop plants (Macías *et al.*, 2007; Khanh *et al*., 2009; Tanveer *et al*., 2010; Weidenhamer and Callaway, 2010; Farooq *et al*., 2011; Harun *et al*., 2014; Kato-Noguchi *et al*., 2016).

Weed studies are important to find species of weed which have allelopathic potential. Such weeds might play effective roles in controlling weeds rather than the application of chemical herbicides (Fujii, 2001). Thus, finding more weed species for allelochemicals and allelopathic potential offers a possible source of bioherbicides and insight into the interactions of weeds and crops. Worldwide there are approximately 1800 types of weed. Up to now, 240 species of weed have an allelopathic effect on neighboring plants. Many allelochemicals include phenolics, terpenes, flavonoids, sesquiterpenes and alkaloids which show strong allelopathic activity have been identified in various weeds (Qasem and Foy, 2001). Allelochemicals have been extracted from weeds, purified and applied as bioherbicides, such as Tricin from *Echinochloa colona* (L.), parthenin from *Parthenium hysterophorus* L. (Batish *et al.*, 1997) and artemisinin from *Artemisia* sp. (Dayan *et al.*, 1999; Duke *et al.*, 2000; Gomaa and AbdElgawad, 2012). Subsequent many experiments on weed allelopathic potential on crops have been carried out. The results demonstrated that different species of weed inhibited different crop growth (Batish *et al*., 2007; Netsere, 2015, Mubeen *et al*., 2012; Abbas *et al*., 2016; Joshi and Joshi, 2016).

Four species of weed, namely *Fimbristylis dichotoma* L., *Cyperus difformis* L., *Marsilea crenata* Presl. and *Rumex maritimus* L. were selected in the current research to find out their allelopathic potential and to search allelochemicals. *Fimbristylis dichotoma* (Cyperaceae) is one of the most widespread weedy species of *Fimbristylis*. It is one of the main weeds in rice fields and is distributed widely in Asia and Africa, as well as in other parts of the tropics (Nemoto *et al*., 1987; Gupta and Thacker, 2013). *Cyperus difformis* (Cyperaceae) is an annual tufted herb, which usually grows in very wet or flooded soil. It is distributed widely through Africa, America, Asia, and Europe (Holm *et al.*, 1977; Gupta and Beentje, 2017). *Marsilea crenata* (Marsileaceae) is a perennial, aquatic weed and particularly found in rice fields in South-East Asia (Bangladesh, Sri Lanka, Thailand, Myanmar) and Australia (Nagalingum *et al*., 2007; Zhuang, 2011; Bely *et al*., 2016). On the other hand, *Rumex maritimus* (Polygonaceae) is a stout, erect, annual herb, up to 1.2 m tall which is distributed widely as a weed in the crop fields of Bangladesh, North India, the Himalayas, and also European and North American countries (Figure 1) (Chopra *et al*., 2002; Sinha *et al*., 2007; Rahman *et al*., 2014). However, as far as we know, no research has been conducted until now on *Fimbristylis dichotoma* L., *Cyperus difformis* L., *Marsilea crenata* Presl. *Rumex maritimus* L. to evaluate its allelopathic potential.

Fimbristylis dichotoma L. *Cyperus difformis* L. *Marsilea crenata* Presl. *Rumex maritimus* L.

Figure 1 Weed species used in the study

The plant parts (leaves, stems, roots) of all selected weed species were collected from Bangladesh. The plant powder of each species was extracted with 70% aqueous methanol and methanol. An aliquot of the extract of each plant material was then evaporated to dryness at 40°C and dissolved in methanol to prepare four assay extract concentrations of 0.01, 0.03, 0.1 and 0.3 g dry weight (DW) equivalent extract/mL. Cress, lettuce, alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and foxtail fescue were selected in the study to check the biological activity of four weed species. Among these, cress, lettuce, alfalfa, rapeseed, and timothy were chosen because of their known seedling growth characteristics, whereas Italian ryegrass, barnyard grass, and foxtail fescue were chosen because of their availability as weeds in crop fields. The percentage length of seedlings was calculated by the reference to the seedlings length of control. The concentrations required for 50% growth inhibition (defined as I_{50}) of the test plant species in the assay were determined by a logistic regression equation of the concentration-response curves.

The results of the study showed that, the aqueous methanol extracts of *F. dichotoma*, *C. difformis*, *M. crenata* and *R. maritimus* had significant growth inhibitory effects on the shoot and root growth of cress, lettuce, alfalfa, rapeseed, barnyard grass, Italian ryegrass, timothy and foxtail fescue. A complete inhibition of lettuce seedling was found at 0.1 g DW equivalent extract of *F. dichotoma*/mL, and also the seedling growth of cress, alfalfa and foxtail fescue was inhibited completely at the concentration of 0.3 g DW equivalent extract of *F. dichotoma*/mL. In addition, total inhibition of seedling growth of cress, alfalfa, lettuce, timothy and foxtail fescue was also found at concentration obtained from 0.3 g DW equivalent extract of *C. difformis*/mL, whereas others test species showed inhibition over 10% of control growth. The aqueous methanol extracts of *M. crenata* showed growth inhibitory effects on the seedling growth of test plants at the concentration greater than 0.01 g DW equivalent extract/mL. Different levels of inhibition were also discovered by the extracts of *M. crenata* when the test plants were exposed to other treatment concentrations. On the other hand, the extract obtained from 0.3 g DW of *R.* maritimus/mL completely inhibited the shoot and root growth of cress, lettuce, alfalfa, rapeseed, Italian ryegrass, foxtail fescue, and the root growth of timothy, whereas the shoot growth of timothy inhibited by 8.9% of control shoot growth. At identical concentration, the extract of *R. maritimus* also significantly inhibited the shoot and root growth of barnyard grass by 8.1 and 1.9% of control growth, respectively. The inhibitory effects displayed by the *F. dichotoma*, *C. difformis*, *M. crenata* and *R. maritimus* were concentration-dependent. A number of previous studies suggested that the stronger inhibition was found with the increased concentration of extracts (Bich *et al*., 2012; Miranda *et al*., 2015). Inhibitory effect by the weed species extracts was also reported by the Usuah *et al.*

(2013), where the inhibitory effects of six weed extracts on several crops increased with increasing its extract concentration, and these extracts inhibited growth by 10-100% of the crops tested. Additionally, I_{50} values for seedling growth of all the tested plants differed. This is also an indication that inhibition is dependent on the species. The concentration- and species-dependent inhibitory activities were also reported by Wang et al. (2015), Kato-Noguchi et al. (2016). The allelopathic potential of the extracts of *F. dichotoma*, *C. difformis*, *M. crenata* and *R. maritimus* on the tested plants indicates that the extracts might possess allelochemicals. Therefore, these species of weeds could be used as candidates for isolating and identifying allelochemicals.

The extracts were then adjusted to pH 7.0, partitioned against an equal volume of ethyl acetate. The ethyl acetate fraction was subsequently purified by several chromatographic steps such as silica gel column, Sephadex LH-20 column, C18-cartridges and HPLC. Two growth inhibitory substances were isolated and identified by HRESIMS, and ^{1}H- and ^{13}C NMR as loliolide and isololiolide from the extracts of *M. crenata* (Figure 2). Loliolide and isololiolide showed inhibitory effects on cress growth at concentrations greater than 10 and 30 μM, respectively, and inhibition began from 30 μM on barnyard grass seedling by loliolide and isololiolide (Figure 3). There was increased inhibition with increasing concentration of loliolide and isololiolide. The I_{50} values for both allelochemicals on cress and barnyard grass seedlings ranged from 32.1 –176.2 μM.

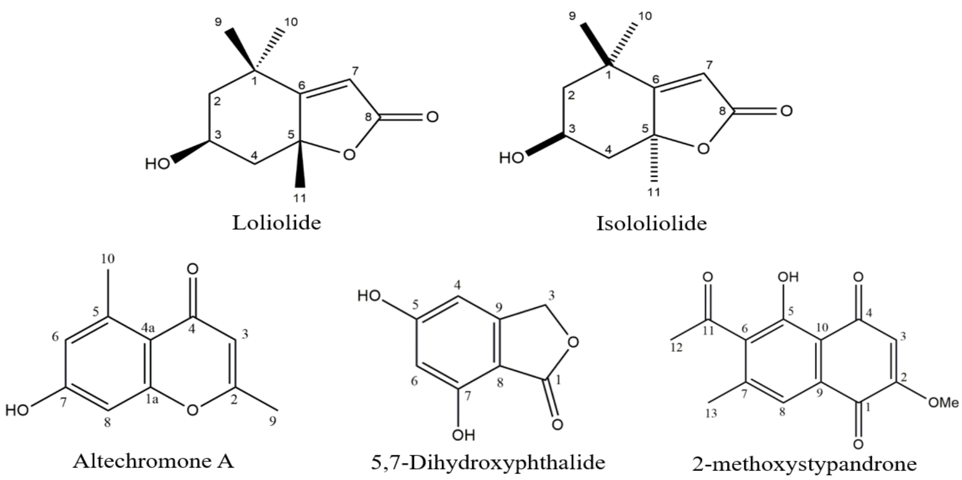

Figure 2 Chemical structure of the identified allelochemicals

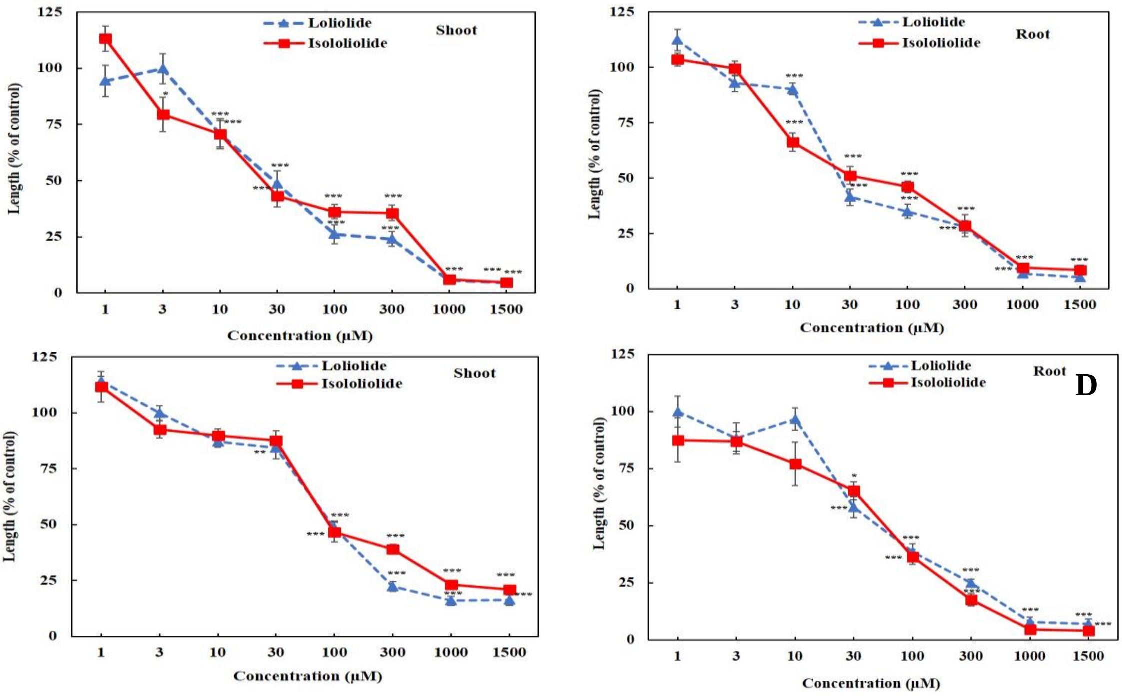

Figure 3 Effects of loliolide and isololiolide on the shoot and root growth of cress (**A–B**); and barnyard grass (**C–D**). Values given are means ± SE from two independent experiments with 10 seedlings for each treatment. Significant differences between treatments and control are indicated by asterisks: *p<0.05, **p<0.01, and ***p<0.001

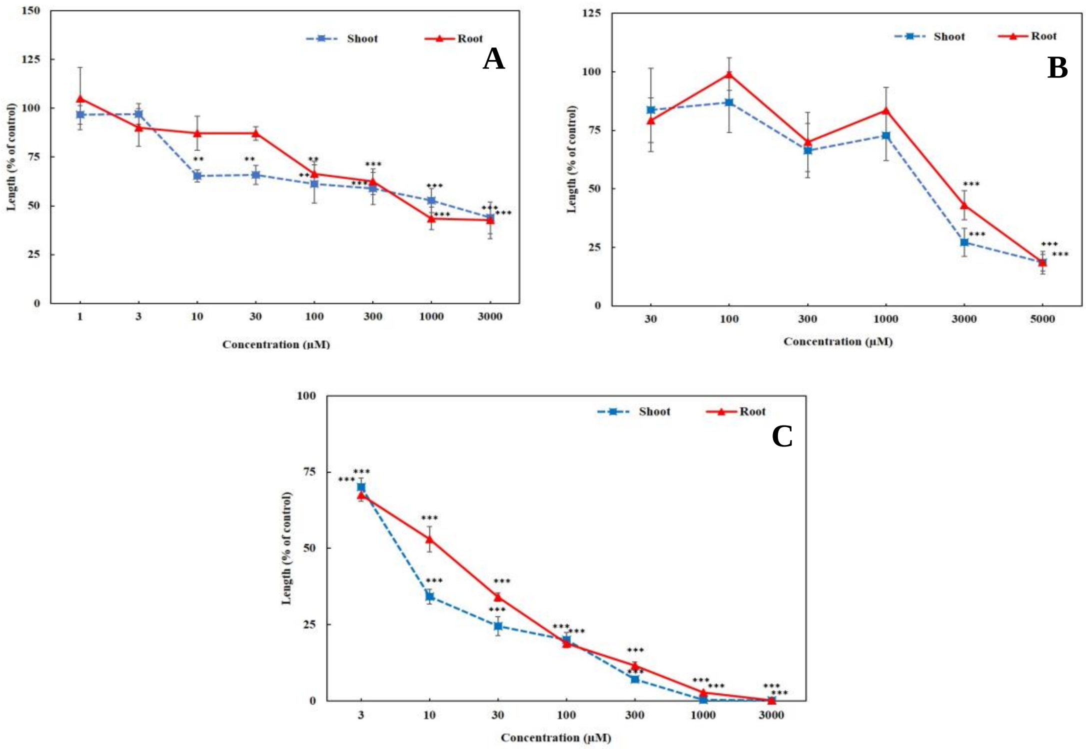

Figure 4 Effects of altechromone A (**A**), 5,7-dihydroxyphthalide (**B**), and 2-methoxystypandrone (**C**) on the shoot and root growth of cress. Values given are means ± SE from two independent experiments with 10 seedlings for each treatment. Significant differences between treatments and control are indicated by asterisks: **p<0.01, and ***p<0.001

Loliolide and isololiolide have also reportedly been found in other plant species; however, this is the first instance of the allelopathic properties of loliolide and isololiolide isolated from *M. crenata.* The results demonstrate that both loliolide and isololiolide could be responsible for *M. crenata* allelopathic potential.

On the other hand, three growth inhibitory substances were isolated and identified by spectral data as altechromone A, 5,7-dihydroxyphthalide and 2-methoxystypandrone from the aqueous methanol extracts of *R. maritimus* (Figure 2). Altechromone A, 5,7-dihydroxyphthalide and 2-methoxystypandrone had a significant effect on the inhibition of cress seedling growth at concentrations higher than 100, 3000 and 3 μM, respectively (Figures 4). The I_{50} values of the three substances ranged from 5.8-2481.2 μM. In studies, a large number of biological properties have been found in the literature for altechromone A, 5,7-dihydroxyphthalide and 2-methoxystypandrone (Yoshikawa *et al*., 2010; Liu *et al*., 2012; Kuang *et al*., 2014). This, however, is the first-time report of the allelopathic activities of altechromone A, 5,7-dihydroxyphthalide and 2-methoxystypandrone. The results indicate that altechromone A, 5,7-dihydroxyphthalide and 2-methoxystypandrone are potent allelochemicals and could be the cause of allelopathic activity in *R. maritimus*.

In the study, the aqueous methanol extracts *Fimbristylis dichotoma* L., *Cyperus difformis* L., *Marsilea crenata* Presl. and *Rumex maritimus* L. displayed strong allelopathic potential on the shoot and root growth of test plants, which indicates those weed may contain allelochemicals. Five allelochemicals were isolated and identified from *M. crenata* and *R. maritimus* and those allelochemicals also had concentration-dependent allelopathic effects on the growth of cress and barnyard grass seedlings. Collectively, the findings of the results might have a crucial role in understanding of crop-weed interference mechanism and to develop an alternative means of weed management.

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