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

氏名：
Name KAWSAR HOSSEN

学位論文題目：
Title of Dissertation

Allelopathic activity and identification of allelopathic compounds from Bangladeshi medicinal plants for the development of bioherbicides to control weeds
（生物除草剤の開発のためのバングラデシュ産薬用植物のアレロパシーとアレロパシー物質に関する研究）

学位論文要約：
Dissertation Summary

Background of the study

By the year of 2050, the global population might reach at 9.20 thousand million (s), an increase of 30% compared with the present population. Due to the continuous increase in the world population, demand for food is also increasing. To feed such a huge number of people, crop production needs to increase 80% (FAO, 2016). From the mid-nineteenth century, crop yields have increased as a result of major developments in agriculture through the use of chemical fertilizers, irrigation, pesticides, synthetic herbicides and modern varieties of different crops. Land cultivation for growing crops started about ten thousand years ago, and since then, weeds have been a familiar problem to the farmers. Weeds are defined as any unwanted crop or plant growing in the cultivated fields, and they are considered a significant restriction to crop production (Jugulam, 2017). To reduce the disruptive effect of weeds in crops fields, applying appropriate weed control practices is essential. Hand weeding, mechanical, cultural, physical, chemical, biological, and combined methods of controlling weeds are the basic weed control practices executed by farmers to reduce the deleterious effects of weeds (Chauvel et al., 2012). Despite all of the problems and issues associated with the use of synthetic herbicides, chemical control is still regarded as the most common and successful weed control method throughout the globe.

The extensive use of most herbicides in the agriculture is a major concern among the public due to their harmful effects on the surrounding environment and to public health. On the other hand, presently, 266 species of weeds have developed herbicides resistant throughout the world (Heap, 2022). Therefore, the search for environment-friendly alternate methods for controlling weeds by using biodegradable and natural products including allelopathy (allelochemicals) has rapidly expanded in recent times (Pinto et al., 2018; Grulova et al., 2019). Using the phenomenon of allelopathy to suppress weeds is one of the most effective weed management methods (Jabran and Farooq, 2013). Allelopathy is also considered as a biological process encompassing the liberation of substances that might have a stimulative influence, but commonly show an inhibitory influence, on the survival, growth, emergence, and reproduction of other plants (Aslam et al., 2017; Yuan et al., 2018). Allelopathic weed management is regarded as an effective approach because it is environmentally safe. Plants contain phytochemicals in different parts such as the roots, leaves, bark, stem, and flowers (Hussain and Reigosa, 2021), and released into the environment via various processes such as leaching, volatilization, decomposition,

and root exudation (Durán et al., 2019). The medicinal plants are well recognized as a promising source of phytochemicals or secondary metabolites. Several researches have been conducted to investigate the allelopathic activity of medicinal plants and numerous natural compounds have also been identified from different medicinal species and documented their allelopathic activity (Pukclai et al., 2010; Boonmee and Kato-Noguchi, 2017; Suwitchayanon et al., 2017a, 2017b; Hossen et al., 2020; Aniya et al., 2020). However, several medicinal species remain in different countries including Bangladesh to investigate their inhibitory activity and isolation of allelopathic compounds for the development of bioherbicides.

The medicinal plants are regarded as a vital source of bioactive substances with different biological activities. Although, it has been documented that different medicinal species contain allelopathic compounds, but there is no evidence on allelopathic effects and identification of allelopathic compounds from the *Acacia catechu* (L.f.) Willd, *Albizia richardiana* (Voigt.) King & Prain and *Elaeocarpus floribundus* Blume plants. Therefore, this research was carried out with the following objectives;

1. To investigate the allelopathic effects of *Acacia catechu*, *Albizia richardiana* and *Elaeocarpus floribundus* against examined plant species
2. To isolate and identify potent compounds from these medicinal plant extracts
3. To determine the allelopathic activity of the identified compounds against the examined plant species

Experiment 1: Determination of allelopathic properties of *Acacia catechu* (L. f.) Willd.

Materials and methods

Plant materials

The leaves of *Acacia catechu* (Figure 1) were collected from NSTU, Bangladesh. The collected leaves were washed in running water to clear debris. The leaves were then dried until reached in constant weight. Finally, the dried leaves were ground into powder, which were kept in a polyethene bag, and stored until use. Six test plant species were selected for this experiment: alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv), and timothy (*Phleum pratense* L.).

Extraction and growth bioassays

The leaf powder (50 g) of *A. catechu* was extracted with 450 mL of 70% (v/v) aqueous methanol for 48 h and filtered and re-extracted with same amount of methanol for 24 h and filtered. Both filtrates were then mixed and evaporated until to dryness. The crude extracts of *A. catechu* were dissolved in 150 mL of methanol to make six bioassay concentrations. The seeds, and sprouted seeds were placed on the Petri dishes (28 mm), and the seeds or sprouted seeds were also placed on a filter paper soaked with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 only as a control and kept in the growth chamber. After 48 h of incubation in the dark at 25°C the growth seedling of the tested plants was estimated.

Figure 1 *Acacia catechu* (Khair) tree

Statistical analysis

All the bioassay experiments were undertaken in a completely randomized design with three replications and repeated twice. The resulting data were analyzed using SPSS software version 20.0. The data obtained were subjected to analysis of variance (ANOVA), and the significant differences between the mean of treatments and control were calculated using Tukey's HSD test at the 0.05 probability level.

Results

Effect of aqueous methanol extracts of *Acacia catechu* on the growth of the test plants

The aqueous methanol extracts of A. catechu significantly inhibited the seedling growth of the tested plants (Figure 2). At a concentration of 0.1 g dry weight equivalent extract/mL, the seedling growth of the tested plants was inhibited more than 90% by the extracts, except barnyard grass (Figure 2). With exposure to the concentration of 0.3 g dry weight equivalent extract/mL, the growth of the tested plants was completely inhibited, except barnyard grass. In addition, the decrease in root growth of the tested plants was greater than that of the shoots.

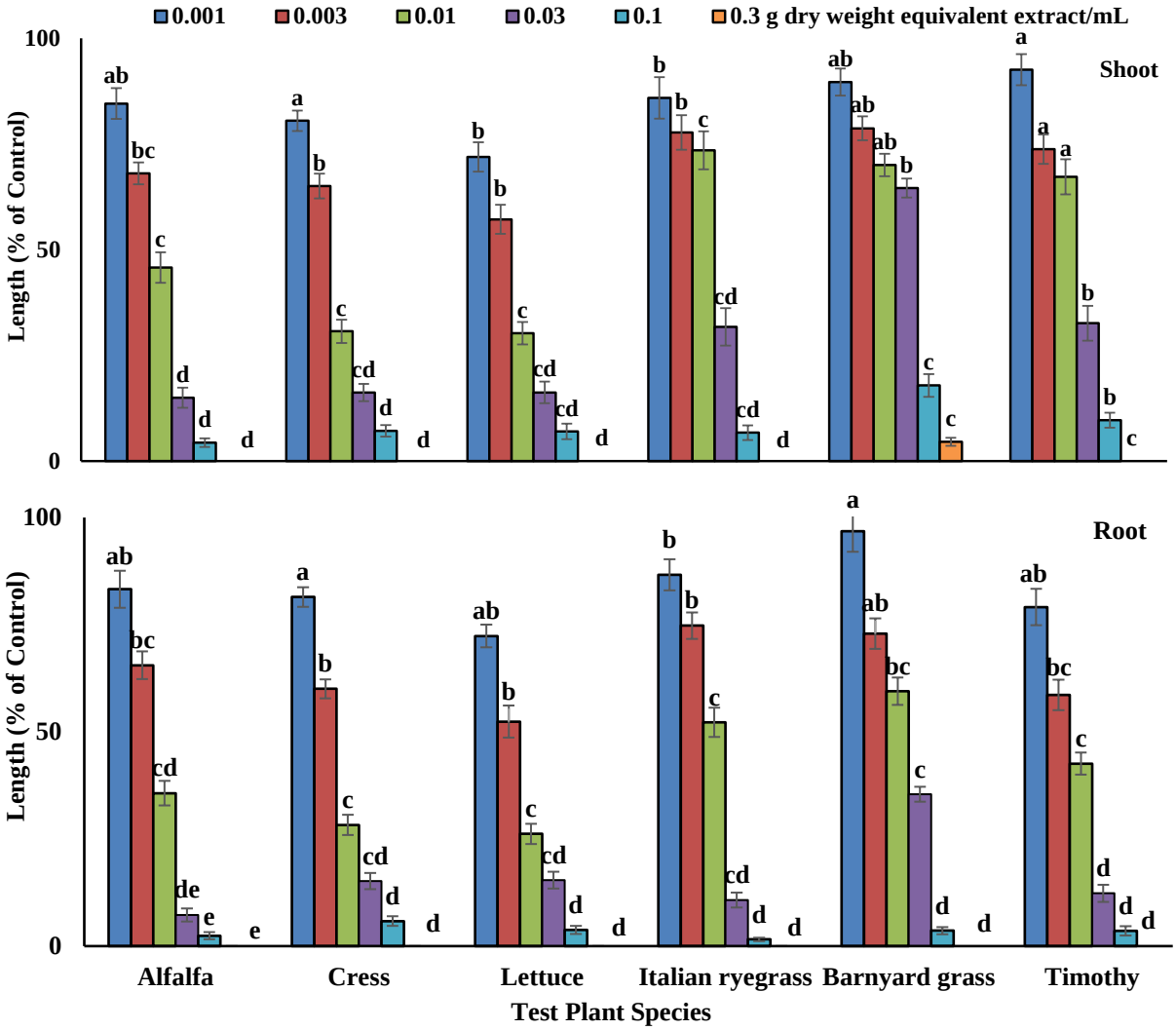


Figure 2 Effect of *A. catechu* extracts on the shoot and root growth of the six tested plants. Mean ± SE from 2 independent experiments with 3 replications for each treatment are presented. The different letters in the same panel indicate significant difference according to Tukey's HSD test at the 0.05 probability level.

Experiment 2: Allelopathic activity and growth inhibitory substances from *Albizia richardiana* (Voigt.) King & Prain

Materials and methods

Plant materials

Albizia richardiana (Figure 3) leaves were collected from BAU, Bangladesh. The leaves were processed by following same procedure as described in experiment 1 and same test plants also used for bioassay.

Figure 3 *Albizia richardiana* (Raj koroi) tree

Isolation and purification active substances

The detailed extraction and isolation procedures of the compounds are shown in the figure 4.

Growth bioassay of the isolated substances

The isolated compounds were dissolved in cold methanol to create different bioassay concentrations, and added in the Petri dishes. The growth inhibitory effects of the isolated substances were measured using the cress assay as previously mentioned.

Statistical analysis

The bioassay experiments were conducted using CRBD with three replications, and all the replications were repeated two times. The results are presented as mean ± standard error (SE). Analysis of variance (ANOVA) was determined by using SPSS statistical software, version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Significant differences were determined using Tukey's HSD test at the 0.05 level of significance.

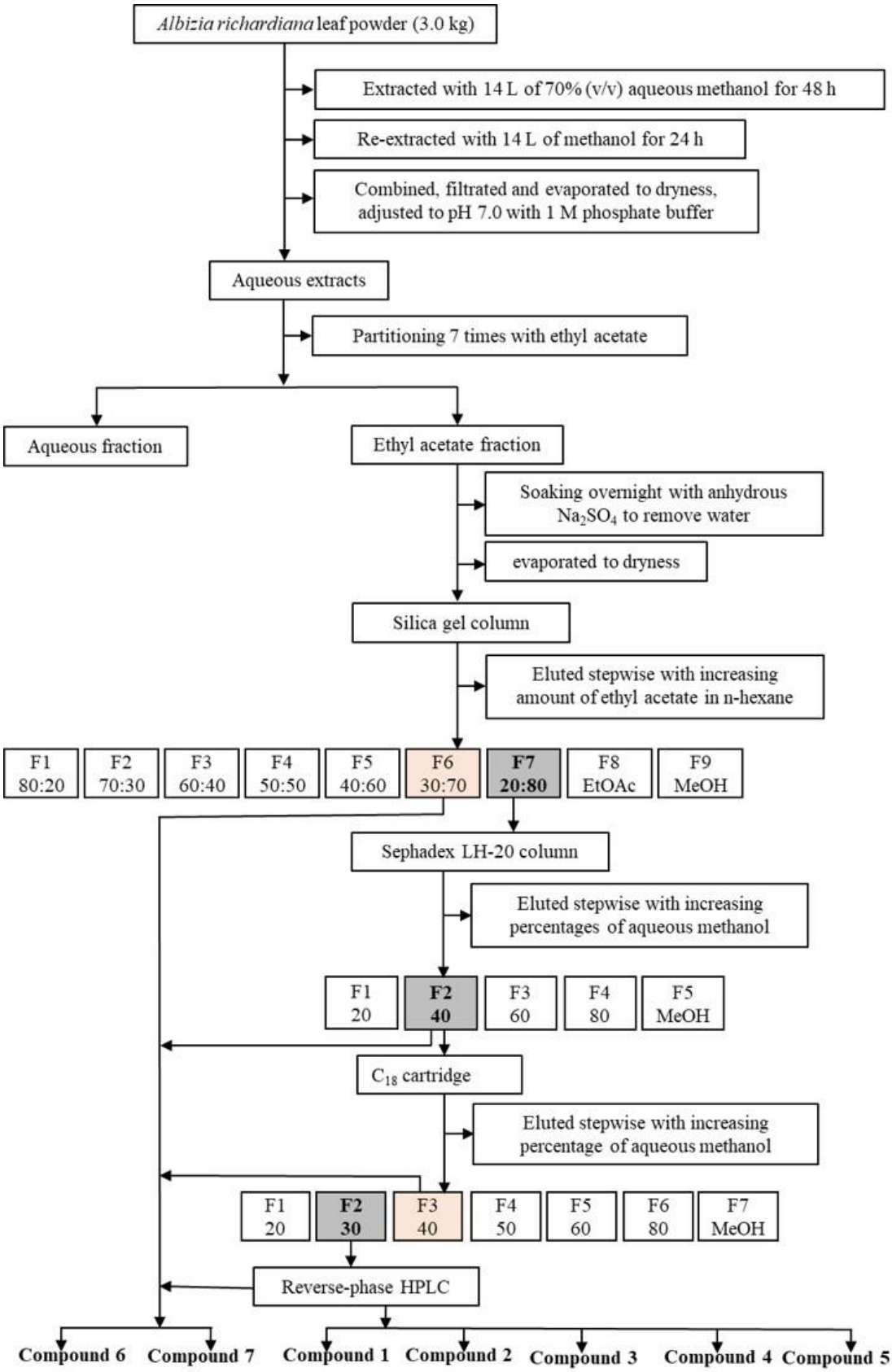


Figure 4 Extraction procedure and purification of compounds **1**, **2**, **3**, **4**, **5**, **6** and **7** (dehydrovomifoliol, loliolide, 4,5-dihydrovomifoliol,3-hydroxy-5α,6α-epoxy-β-ionone,3-(2-hydroxyethyl)-2,4,4-trimethyl-2cyclohexen-1-one 3-hydroxy-4-oxo-β-dehydroionol, and 3-oxo-α-ionone).

Results

Allelopathic effects of the *Albizia richardiana* extracts

The allelopathic effects of the aqueous methanol extracts of *Albizia richardiana* are displayed (Figure 5), and suppression increased with increasing extract concentrations. The aqueous methanol leaf extract of *Albizia richardiana* significantly restricted the seedling growth of the tested plants at the concentration of 0.01g and higher than the 0.01 g dry weight equivalent extract/mL. The seedlings of the test species were completely inhibited at the concentration of 1.0 g dry weight equivalent extract/mL, except the barnyard grass.

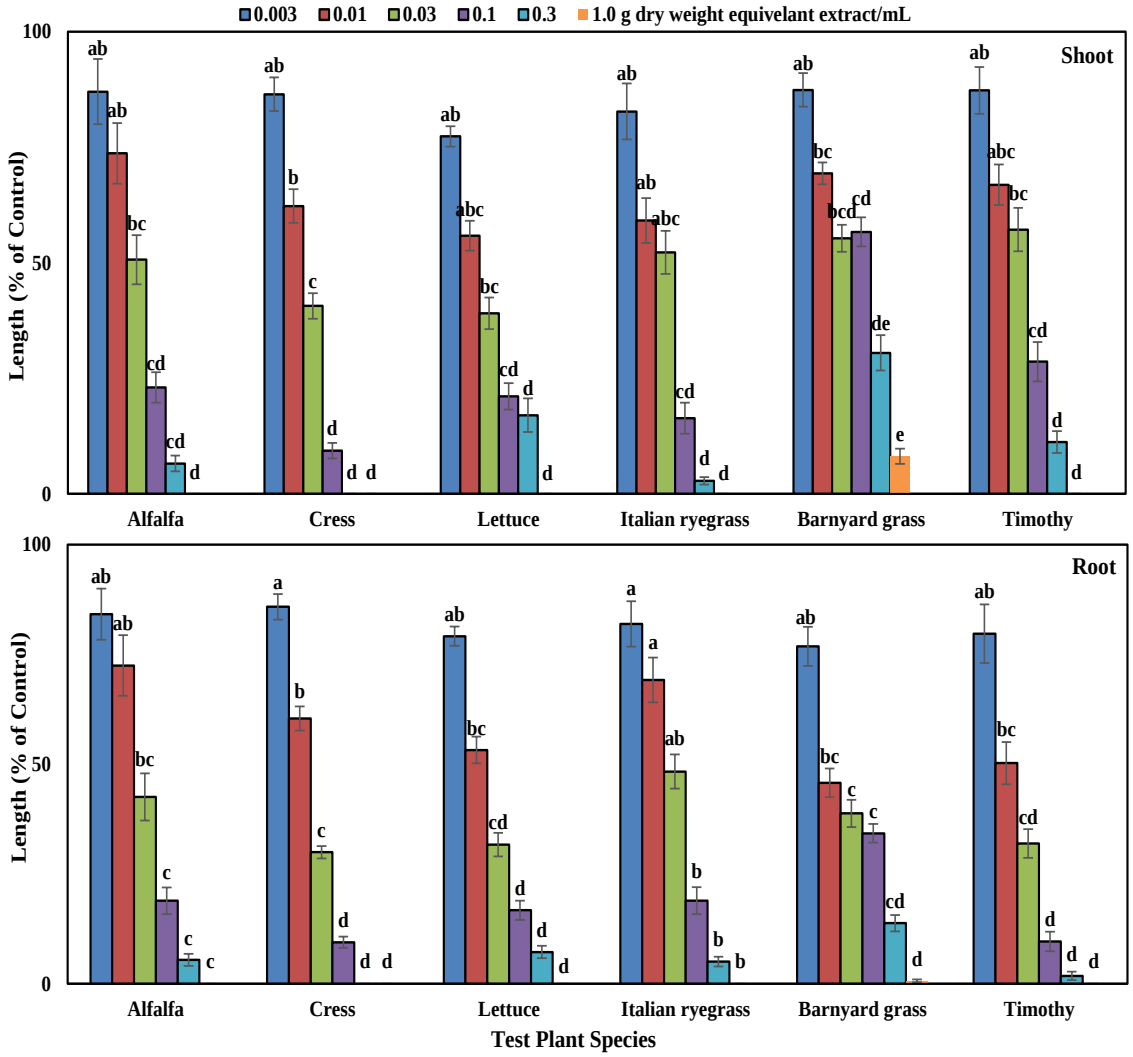


Figure 5 Phytotoxic effects of *Albizia richardiana* aqueous methanol extracts on the growth of test species. The mean ± standard error from the two independent experiments with three replications for every treatment are displayed. Various letters denote the significant differences according to Tukey's HSD test at a 0.05 probability level.

Determination of the structures of the allelopathic substances

The compounds (Figure 6 and 7) were identified by spectral analysis.

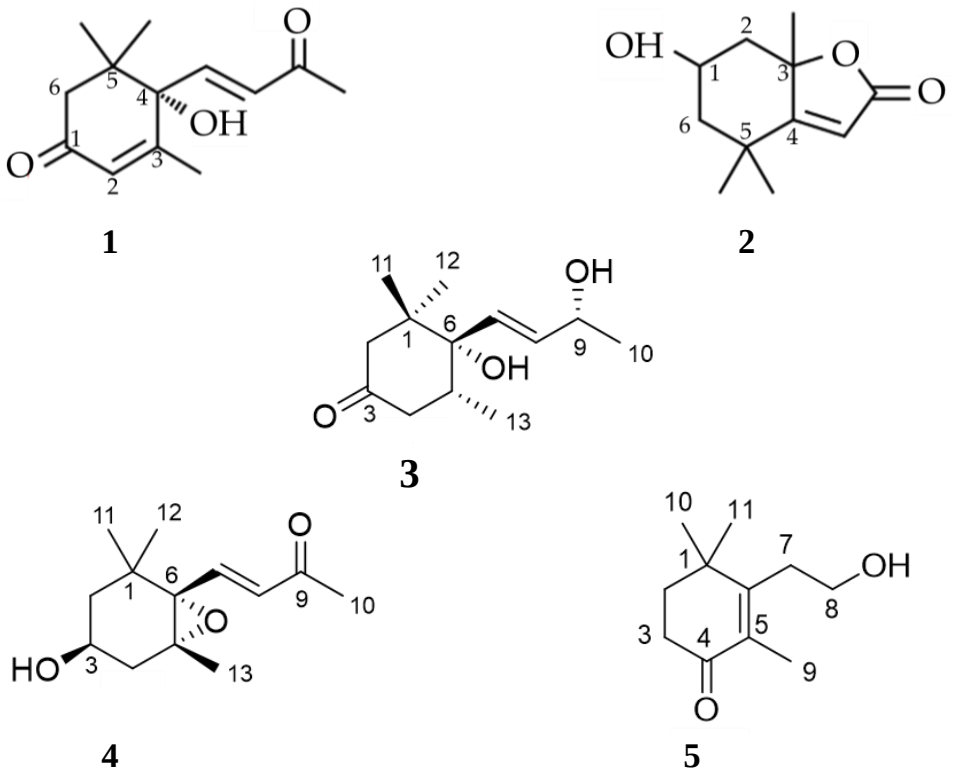


Figure 6 The chemical structures of the isolated allelopathic compounds **1**, **2**, **3**, **4**, and **5** (dehydrovomifoliol, loliolide, 4,5-dihydrovomifoliol, 3-hydroxy-5α,6α-epoxy-β-ionone, and 3-(2-hydroxyethyl)-2,4,4-trimethyl-2cyclohexen-1-one) from the extracts of *Albizia richardiana*.

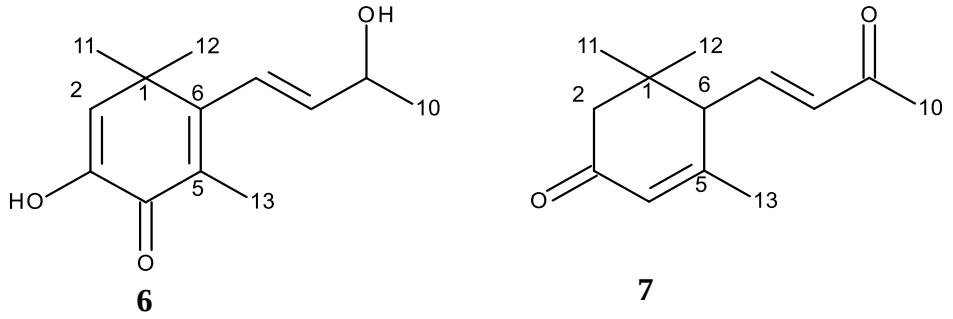
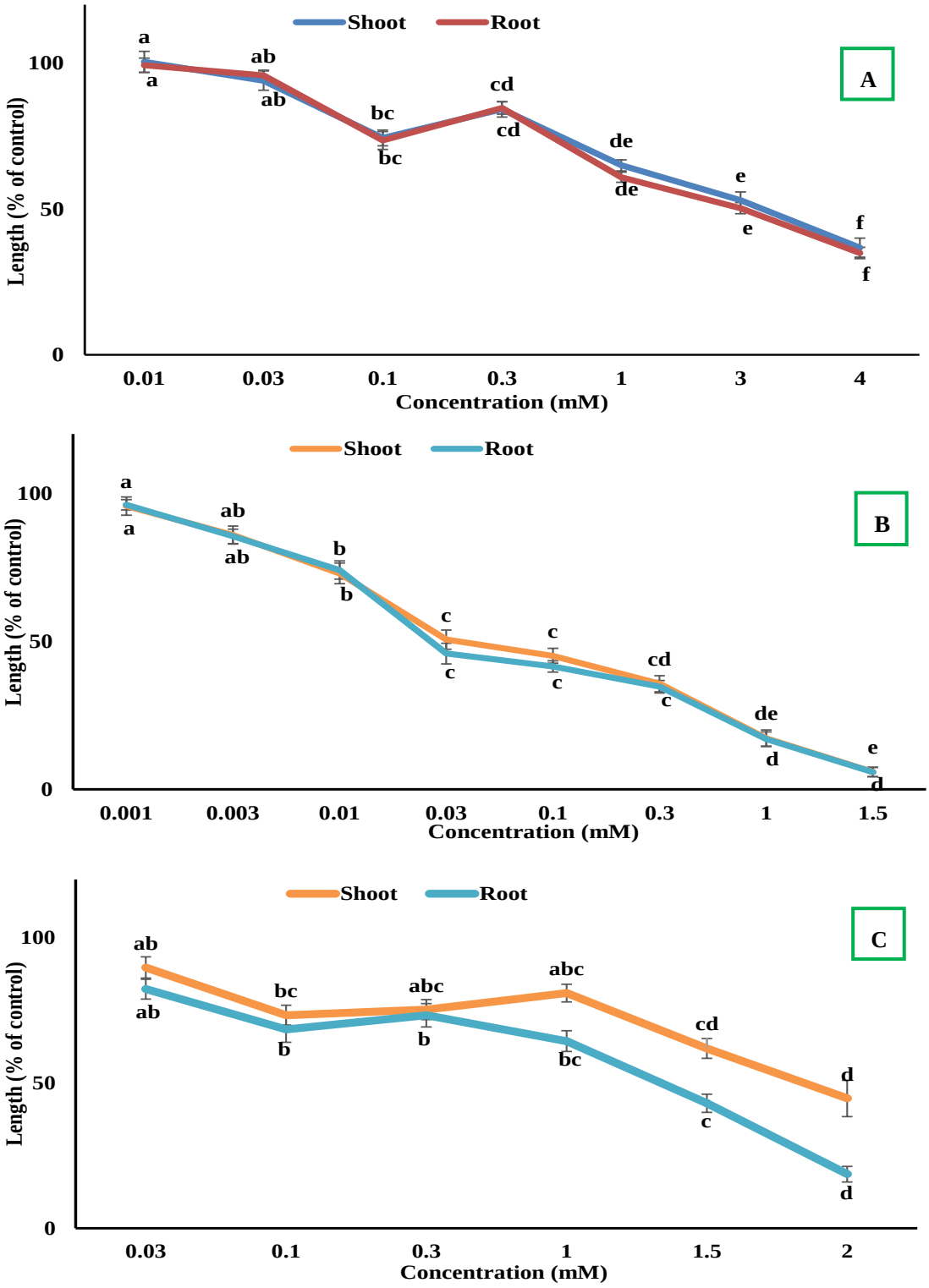


Figure 7 The chemical structures of the isolated allelopathic compounds **6**, and **7** (3-hydroxy-4-oxo-β-dehydroionol and 3-oxo-α-ionone from the *Albizia richardiana* extracts.

The Biological effects of the isolated compounds

The biological effects of dehydrovomifoliol, loliolide, 4,5-dihydrovomifoliol, 3-hydroxy-5α,6α-epoxy-β-ionone, and 3-(2-hydroxyethyl)-2,4,4-trimethyl-2cyclohexen-1-one) on cress and 3-hydroxy-4-oxo-β-dehydroionol and 3-oxo-α-ionone on cress and timothy were evaluated. The results from the bioassay showed that cress and timothy seedling growth were significantly suppressed by the substances (Figure 8, A–E). The inhibition level of the substances was raised by raising the concentration, indicating that suppression was dose-dependent. Dehydrovomifoliol and loliolide significantly inhibited the cress seedling growth in the concentrations of 0.1 and 0.01 mM, respectively (Figure 8, A–B). Dehydrovomifoliol and loliolide showed the highest inhibition of (36.7% shoot and 34.9% root) and (5.9% shoot and 5.8% root) at the highest concentration. The cress seedlings displayed the maximum restriction (shoot 55.35% and root 81.4%) with 4,5-dihydrovomifoliol, (shoot 76.9% and root

83.25%) with 3-hydroxy-5α,6α-epoxy-β-ionone, and (shoot 74.36% and root 80.00%) with 3-(2-hydroxyethyl)-2,4,4-trimethyl-2cyclohexen-1-one at the maximum concentration (Figure 8, C–E).



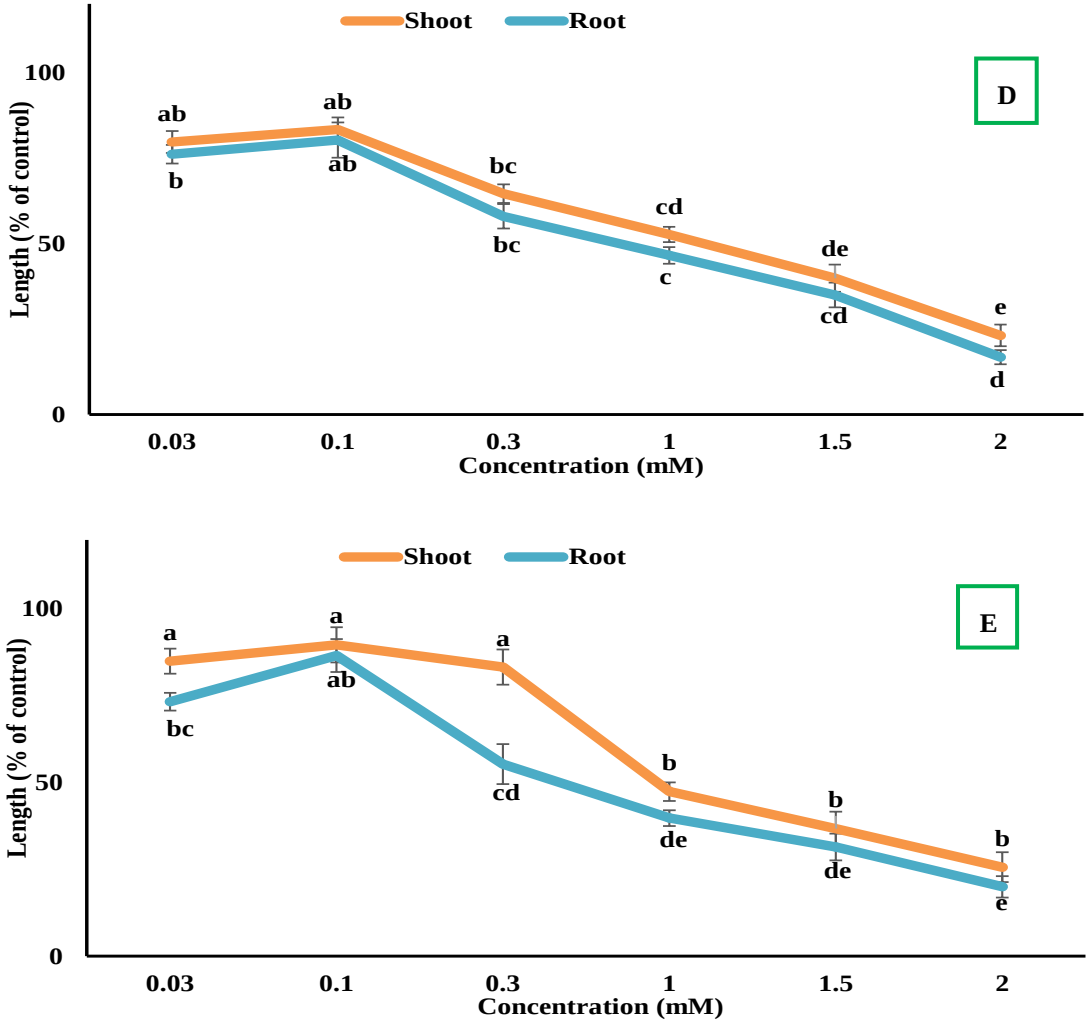


Figure 8 Phytotoxic potential of (dehydrovomifoliol (A), loliolide (B), 4,5-dihydrovomifoliol (C), 3-hydroxy-5α,6α-epoxy-β-ionone (D), and 3-(2-hydroxyethyl)-2,4,4-trimethyl-2cyclohexen-1-one (E)) against the growth of cress. The mean ± standard error (SE) from two different studies with 10 seedlings for each treatment. Different letters indicate significant variations according to Tukey's HSD test at the 0.05 level of significance.

The 3-hydroxy-4-oxo-β-dehydroionol and 3-oxo-α-ionone compounds inhibited the shoot growth and root development of cress at concentrations that was higher than 0.01 and 0.1 mM, respectively (Figure 9, A and B). The cress seedlings exhibited the highest inhibition (shoot 100% and root 98.9%) at the concentration of 1.5 mM for 3-hydroxy-4-oxo-β-dehydroionol and for 3-oxo-α-ionone (shoot 84.6% and root 88%) at the concentration of 3 mM. Similarly, timothy seedlings displayed the highest inhibition (shoot 97.62% and root 98.5%) at the concentration of 1.5 mM for 3-hydroxy-4-oxo-β-dehydroionol and for 3-oxo-α-ionone (shoot 89.7% and root 96.32%) at the concentration of 3 mM.

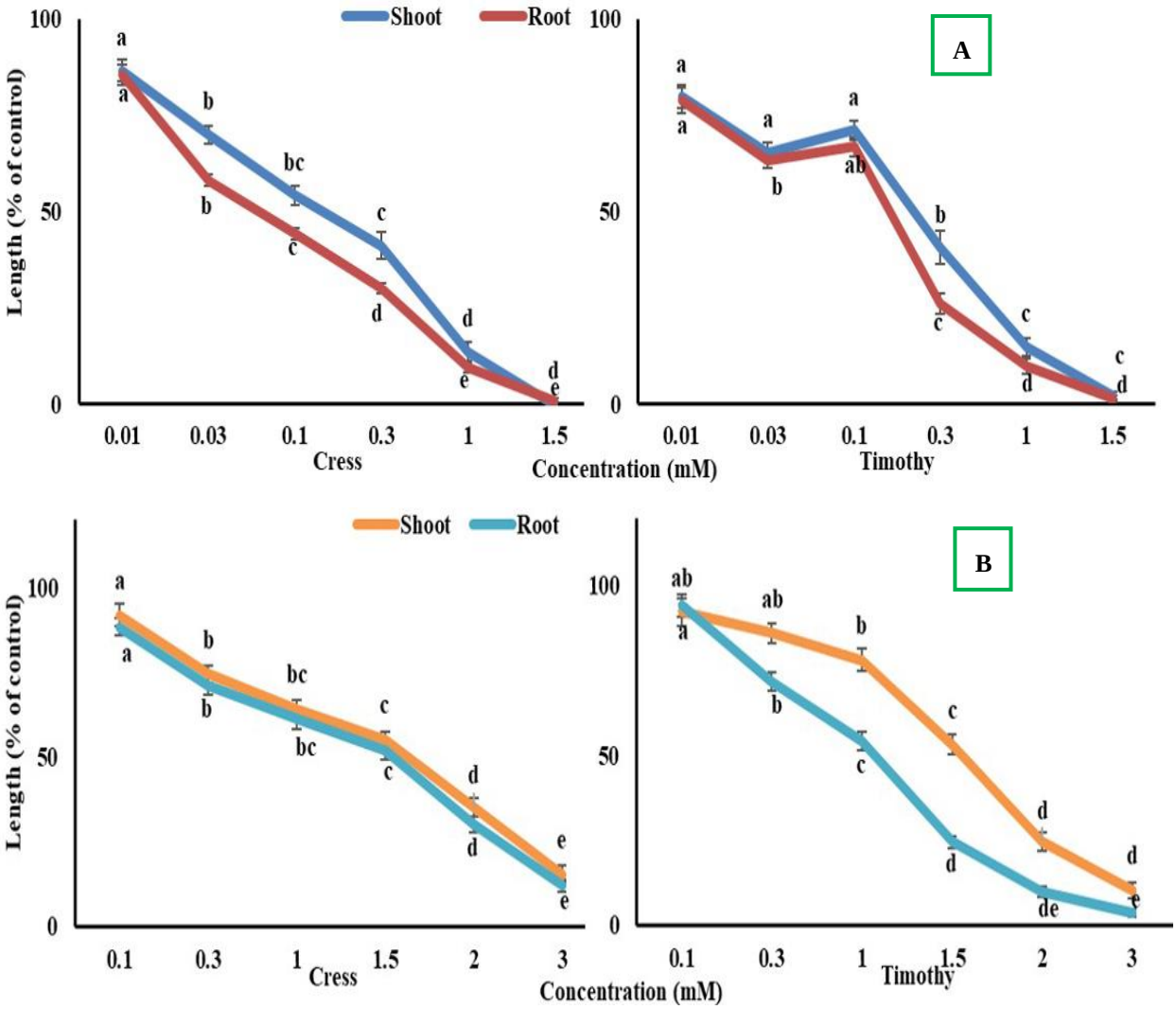


Figure 9 Phytotoxic activity of 3-hydroxy-4-oxo-β-dehydroionol (A), and 3-oxo-α-ionone (B) against the growth of cress and timothy. The mean ± standard error (SE) from two separate studies with 10 seedlings for each treatment. Different letters represent significant differences according to Tukey's HSD test at the 0.05 level of probability.

Experiment 3: Allelopathic activity and characterization of allelopathic substances from *Elaeocarpus floribundus* Blume leaves for the development of bioherbicides

Materials and method

Plant materials

Fresh and mature leaves of *Elaeocarpus floribundus* Blume (Figure 10) were gathered from the NSTU, Bangladesh. The leaves were processed by following same procedure as described in experiment 1. Two plant species were used barnyard grass, and cress.

Figure 10 *Elaeocarpus floribundus* (Jalpai) tree

Extraction, Isolation, purification, and bioassay of the substances and statistical analysis

The extraction, isolation, purification and bioassay procedures of the active compounds were same as the experiment 2. The statistical analysis also same as previous experiment.

Results

Phytotoxic activity of the *Elaeocarpus floribundus* extracts

The *Elaeocarpus floribundus* extracts were shown to have a considerable phytotoxic effect on the cress and barnyard grass. Growth inhibition increased with increasing extract concentration and also differed between the two species (Figure 11). At 0.1 g dry weight equivalent extract/mL, the growth of the cress shoots (0.38%) and roots (1.49%), and barnyard grass roots (4.16%) were less than 5% of the control treatment, whereas the barnyard grass shoot growth was 17.89% of control. However, at 0.3 g dry weight equivalent extract/mL, the *Elaeocarpus floribundus* extracts completely inhibited the seedling growth of cress and barnyard grass roots but not barnyard grass shoots.

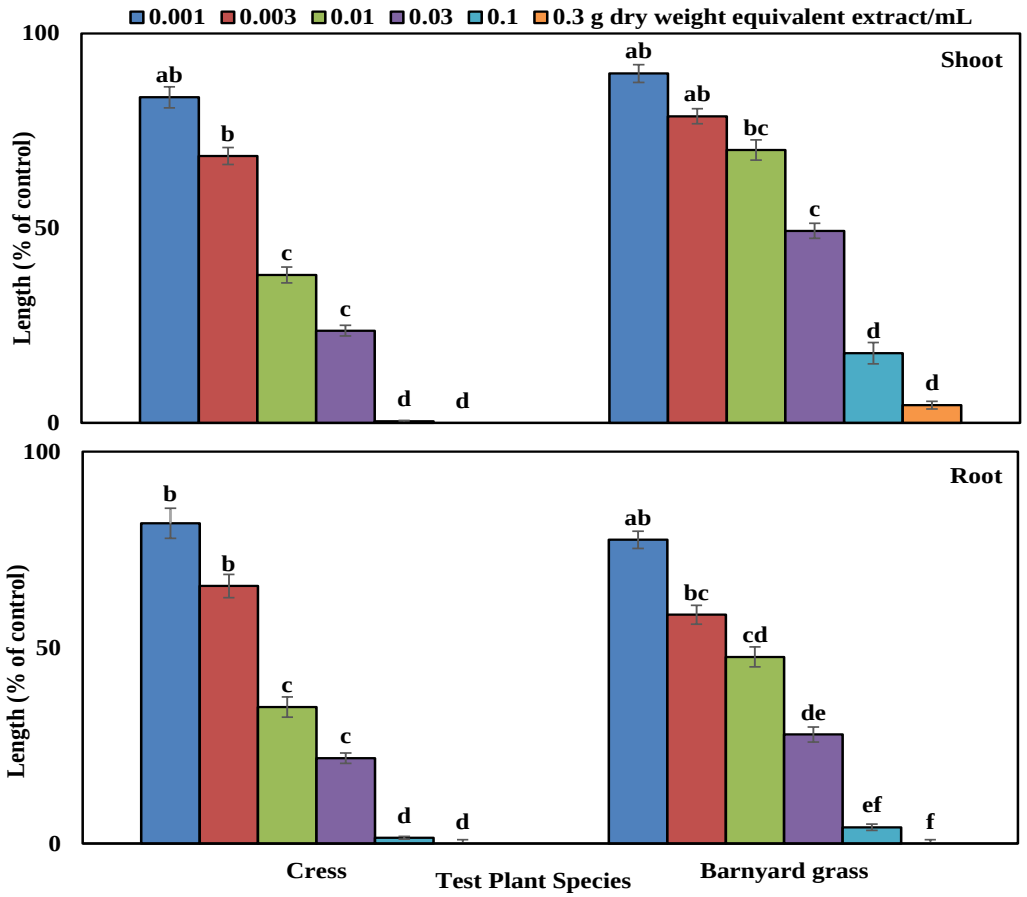


Figure 11 The phytotoxic potential of *Elaeocarpus floribundus* leaf (aqueous methanol) extracts against the growth of cress and barnyard grass. The mean ± standard error was determined from two different experiments. Various letters signify significant differences according to Tukey's HSD test at the probability level of 0.05.

Characterization of the allelopathic compounds

The compounds (Figure 12) were identified by spectral analysis.

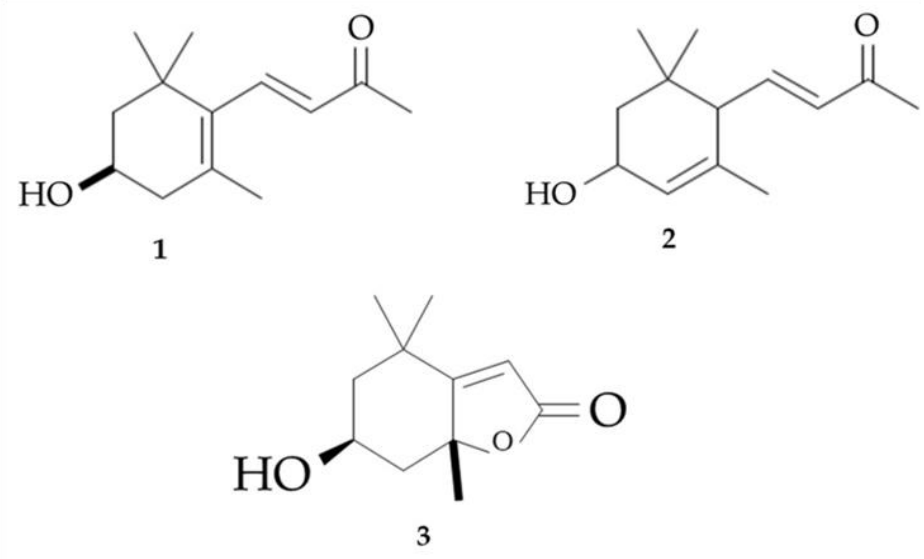


Figure 12 The molecular structure of the characterized allelopathic compounds from *Elaeocarpus floribundus* leaf extracts: 1. (3*R*)-3-hydroxy-β-ionone, 2. *cis*-3-hydroxy-α-ionone, 3. loliolide.

Biological potential of the compounds

The assay results showed that activity of the compounds against growth of cress varied significantly, and the phytotoxic activity increased with the increasing concentration of the compounds (Figure 13, A–C). Significant variation in the growth of cress occurred at the concentrations of 0.1 mM or more with (3*R*)-3-hydroxy-β-ionone, and 0. 03 mM or more with *cis*-3-hydroxy-α-ionone and loliolide. At the highest concentration (1.5 mM), (3*R*)-3-hydroxy-β-ionone inhibited the seedling growth 11.53 and 8.37% of control, respectively, *cis*-3-hydroxy-α-ionone to 20.80 and 17.19%, and loliolide to 12.14 and 9.50%.

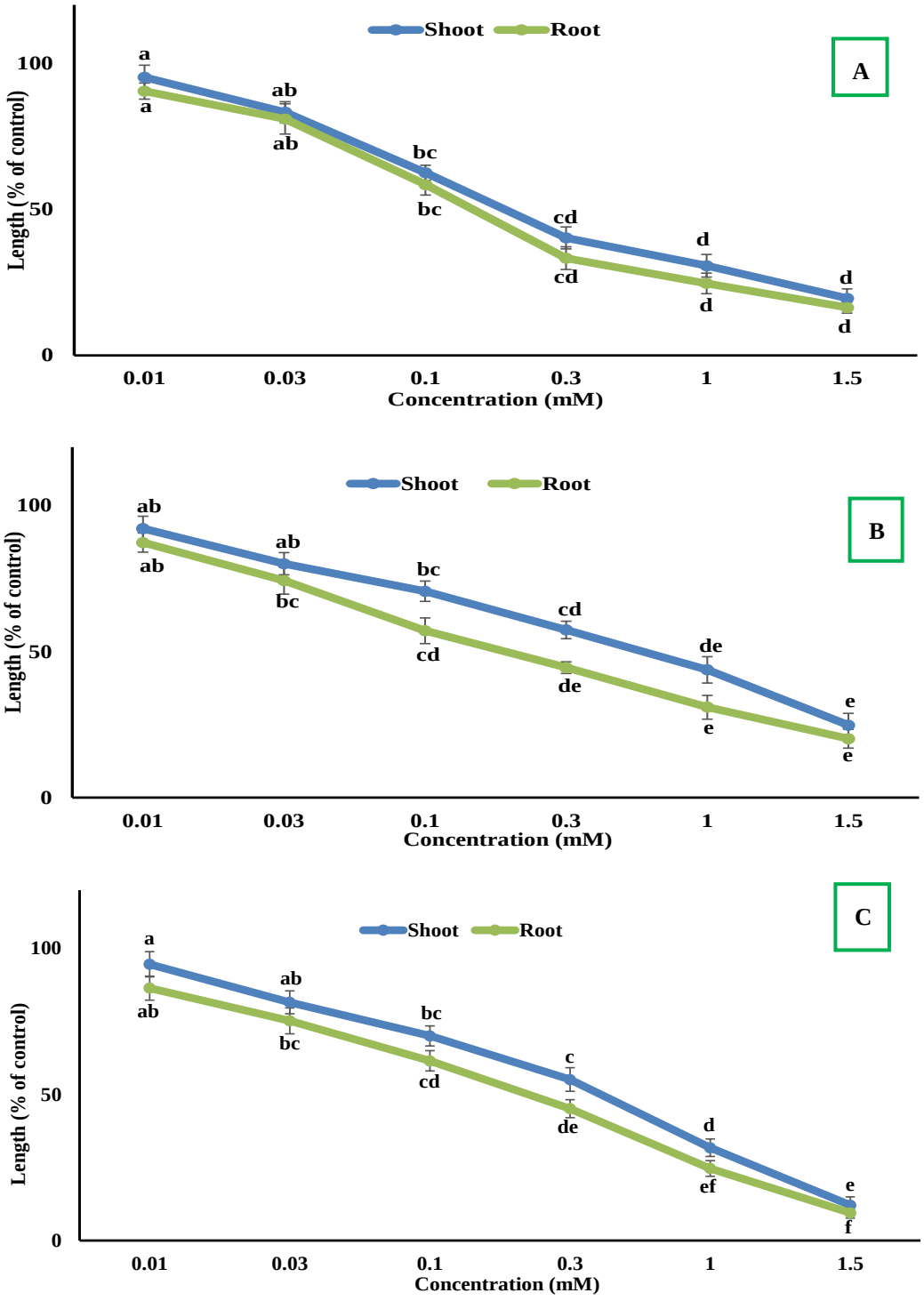


Figure 13 The phytotoxicity of (3*R*)-3-hydroxy-β-ionone (A), *cis*-3-hydroxy-α-ionone (B), loliolide (C) against cress. Values indicate means ± SE from three replications (n = 30). Significant variations between control and treatment are indicated by various letters ($p < 0.05–0.001$).

Summary

In sum, the findings of the present study showed that the aqueous methanolic extracts of *Acacia catechu*, *Albizia richardiana* and *Elaeocarpus floribundus* leaves suppressed the growth of the tested species in a concentration- and species- dependent way, which denotes that the three species have allelopathic activity and may possess allelopathic compounds. Total ten allelopathic compounds were isolated from *Albizia richardiana* (dehydrovomifoliol,loliolide,4,5-dihydrovomifoliol,3-hydroxy-5α,6α-epoxy-β-ionone,3-(2-hydroxyethyl)-2,4,4-trimethyl-2cyclohexen-1-one, 3-hydroxy-4-oxo-β-dehydroionol (novel compound) and 3-oxo-α-ionone) and *Elaeocarpus floribundus* ((3*R*)-3-hydroxy-β-ionone, *cis*-3-hydroxy-α-ionone, and loliolide) leaves. The results obtained from this research suggest that the identified compounds from *Albizia richardiana* and *Elaeocarpus floribundus* leaves might be helpful for the development of bioherbicides to control weeds as well as save the environment.

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