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PHYTOTOXICITY ASSESSMENT OF *CYPERUS DIFFORMIS* (L.) TOWARDS A SUSTAINABLE WEED MANAGEMENT OPTION

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ABSTRACT

The present study was conducted to evaluate the allelopathic potentiality of *Cyperus difformis* from the Cyperaceae family. Aqueous methanol extracts from *C. difformis* were applied at four concentrations (0.01, 0.03, 0.1 and 0.3 g dry weight [DW] equivalent extract/mL) on the seedling growth of eight test species namely, cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus* L.), Italian ryegrass, (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* L.), timothy (*Phleum pratense* L.) and sand fescue (*Festuca megalura* Nutt.). A complete inhibition of seedlings of lettuce was found at concentration of 0.1 g DW equivalent extract/mL. At a concentration of 0.3 g DW equivalent extract/mL, complete inhibition was also found in cress shoot and root of cress, alfalfa, timothy and sand fescue, whereas others test species showed more than 90% inhibition. This inhibition was concentrations dependent and increased with increasing extracts concentrations. Considering concentrations required for 50% growth inhibition (I_{50}), rapeseed was the most sensitive to the extracts. The results indicate that, *C. difformis* may possess allelopathic properties and therefore, could be a candidate for the isolation and identification of allelopathic substances to develop environment friendly bio-herbicides.

Key words: allelopathy and allelochemicals, *Cyperus difformis*, aqueous methanol, I_{50}, bio-herbicides.

INTRODUCTION

Weeds are considered one of major obstacles for successful crop production as they compete with crops throughout the growing period and reduce crop yield and quality (Qasem and Foy 2001). About 34% of the yield loss of crops is due to the weed interference all over the world (Jabran *et al*. 2015). Proper weed management thus, is needed for increasing production of crops to make constant supply of food for growing population worldwide as well as to strengthen the security of foods. Since the ancient times of agriculture, manual, cultural and mechanical weeding, and later on, herbicide applications have been the most preferable weed control methods (Riaz *et al*. 2009; Rueda-Ayala *et al*. 2011; Chauvel *et al*. 2012). But unavailability of cheap labour due to industrialization, additional maintenance costs and reduced effectiveness of mechanical implements, and low cost-effective weed controls ways are major problems associated with manual, cultural, mechanical weed control options (Hussain *et al*. 2008; Awan *et al*. 2015). The most reliable weed control methods in current agriculture is herbicidal control. Farmers mostly rely on herbicidal control of weed due to its easy accessibility, rapid out returns and target oriented mode of actions of herbicides (Aktar *et al*. 2009). But, the evolution of herbicide-resistance weeds, negative health effects to human and animal, environmental concerns are the major threats of using herbicide to control weed (Derksen *et al*. 1996; Sandham *et al*. 2010; Annett *et al*. 2014; Starling *et al*. 2014). Due to the disadvantages of conventional methods and especially, the negative impacts of herbicides application, the researchers have been diversifying to different weed management options (Weston 1996; Macías *et al*. 2007).

Other weed control tactic such as allelopathy could be good option for reducing the dependency on chemical herbicides to control weeds (Rice 1984; Duke *et al*. 2000). Allelopathic plants expect to play a significant role to control weeds by releasing allelochemicals which have the potentiality to supress the growth and development of neighbouring plants (Rice 1984). These allelochemicals are regarded as the basis of allelopathic events (Babula *et al*. 2009) as well as have drawn attention as an alternative to conventional herbicides in crop protection (Nebo *et al*. 2014).

Cyperaceae (sedges) is the third largest monocotyledonous as well as a cosmopolitan family (Muasya *et al*. 1998) consisting of about 100 genera and 5000 species (FNAEC 2002). *Cyperus difformis* belongs to the Cyperaceae family, is the weed of rice field (Valverde *et al*. 2014) in 46 countries infesting both dry direct and wet-seeded systems (Chauhan and Johnson 2009). Although it has a short life cycle (30 days), but produces large quantities of seed to propagate in a short day. In the field, it forms a mats of vegetation in the young crops and causes about 12-50% yield loss of rice (Kern 1974; Holm *et al*. 1977).

Some researches has been conducted to find out the allelopathic potentiality of different plant species from Cyperaceae family, i.e., *C. esculentus* and *C.*

rotundus (Johnson *et al*. 2007). *C. difformis* has been reported to evolve resistance to propanil and acetolactate synthase-inhibiting herbicides in rice fields of California (Valverde *et al*. 2014). To the best of our knowledge, no research has been taken on *C. difformis* to evaluate its allelopathic potentiality. Therefore, the present study was conducted to evaluate the allelopathic potentiality from the aqueous methanol extracts of *C. difformis* against several weeds and crop species in laboratory environment.

MATERIALS AND METHODS

Collection of plant materials: Whole plant (stems, leaves and roots) of *C. difformis* was collected from the village of Boyra and Sutiakhali under the Sadar Upazilla of Mymensingh district in Bangladesh during the month of July-August, 2014. After collection, the plants were cleaned with water to remove the dirt particles and sun dried. The dried plants were then ground into powder and stored at 2°C in a plastic bags until extraction.

Test plant species: Four dicotyledonous such as cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus* L.) and four monocotyledonous such as Italian ryegrass, (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* L.), timothy (*Phleum pratense* L.) and sand fescue (*Festuca megalura* Nutt.) were selected as receiver test species in this research. Among these, cress, alfalfa, lettuce, rapeseed and timothy were chosen for their known seedling growth characteristics and model plant for laboratory bioassays. On the other hand, Italian ryegrass, barnyard grass and sand fescue were chosen because of their usual distribution in the crop fields around the universe.

Extraction procedure: Dried *C. difformis* (30 g) was extracted with 500 mL of 70% (v/v) aqueous methanol for 48 h. The extract was then filtered through one layer of filter paper (No. 2; 125 mm, Advantec® Toyo Roshi Kaisha, Ltd., Tokyo, Japan) using a vacuum pump. The residue was re-extracted with same amount of cold methanol for 24 h and filtered again. The two filtrates were combined together and evaporated until complete dryness with a rotary evaporator at 40ºC.

Growth bioassay: Crude extracts from *C. difformis* were diluted in 125 mL of methanol to prepare four assay concentrations [0.01, 0.03, 0.1 and 0.3 g dry weight (DW) equivalent extract/mL]. To prepare those concentrations, an aliquot of the methanol extracts (25, 75, 250 and 750 µL, respectively) was added to a sheet of filter paper (No. 2; 28 mm, Toyo.) in 28 mm Petri dish. Methanol was evaporated in a draft chamber. The filter paper in the Petri dishes was then moistened with 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethylene sorbitan monolaurate (Tween 20; Nacalai Tesque, Inc., Kyoto, Japan), which was used as a surfactant and has no any toxic effects on seedlings growth. Ten seeds of cress, lettuce, alfalfa, rapeseed and ten pre-germinated seeds of Italian ryegrass, barnyard grass, timothy, sand fescue (germinated in the darkness at 25ºC for 88, 75, 90 and 48 h, respectively, after overnight soaking in distilled water in each case) were placed on the filter paper (No. 2; 28 mm, Toyo.) in Petri dishes. Control seeds or seedlings were placed on the filter paper moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 without plant extracts. The seedlings lengths of shoot and root of all test species were measured after 48 h of incubation in the darkness at 25ºC.

The percentage length of seedlings was then calculated by the reference to the seedlings length of control. The inhibition percentage was calculated by using the following equation (Islam and Kato-Noguchi 2014).

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{seedlings length with treatment}}{\text{seedlings length of control}}\right) \times 100$$

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.

Statistical analysis: Each bioassay experiment was conducted with three replications and 10 seedlings using completely randomized design for each treatment and the experiment was repeated two times. Experimental data were analysed using SPSS version 16.0 (IBM Corp. 2007). All measured variables were subjected to analysis of variance (ANOVA) and the significant differences between treatment and control plants were calculated by post-hoc Tukey's test at 5% level of probability for each test species. The I_{50} values of each test species in the assay were analysed by using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, California, USA).

RESULTS

Effects of aqueous methanol extracts of *Cyperus difformis* on the shoot growth of test plant species: The effects of aqueous methanol extracts of *C. difformis* on the shoot growth of eight test plant species are shown in the Figure 2. The extracts showed a significant inhibition on shoot growth of test plants and the inhibition was increased with increasing extracts concentration (Fig. 1). A complete (100%) inhibition of shoot growth of lettuce was found from the extracts obtained from 0.1 g dry weight of the *C. difformis* plant/mL. At the same concentration, the extracts also significantly inhibited the shoot growth of cress, alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and sand fescue to 74.9, 94.4, 89.1, 84.8, 88.9, 61.6 and 83.2%, respectively (Fig. 2). Exposure the test species to the concentration of 0.3 g DW equivalent extract/mL, shoot of cress and lettuce also

exhibited a complete inhibition, whereas alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and sand fescue showed a significant inhibition to 99.5, 95.4, 96.1, 96.3 and 89.8%, respectively (Fig. 2). On the other hand, the shoot growth of all test species were inhibited more than 20% except of Italian ryegrass, timothy and sand fescue where those showed slight stimulation non-significantly by the extracts obtained from 0.01 g dry weight of the *C. difformis*/mL. At the concentration of 0.03 g DW equivalent extract/mL, more than 50% inhibition was found in cress, lettuce, alfalfa, rapeseed, barnyard grass, whereas Italian ryegrass, timothy and sand fescue showed about 25% inhibition (Fig. 2). The I_{50} (concentration required for 50% inhibition) values of the extracts of *C. difformis* for the shoot growth of eight test species ranges from 0.007 to 0.062 g DW equivalent extract/mL (Fig. 4). Considering I_{50} values, rapeseed was the most sensitive to the extract of *C. difformis* whereas timothy was the least sensitive test species (Fig. 4).

Effects of aqueous methanol extracts of *Cyperus difformis* on the root growth of test plant species: Roots of all test plants were inhibited significantly by the extracts and the inhibition was extracts concentration dependent (Fig. 1, Fig. 3). At the concentration of 0.3 g DW equivalent extract/mL, the root growth of cress, lettuce, alfalfa, timothy and sand fescue were inhibited completely (100%) and the root growth of rapeseed, Italian ryegrass and barnyard grass were inhibited by 94.7, 97.3 and 99.3%, respectively (Fig. 3). When the test species were exposed to the concentration of 0.1 g DW equivalent extract/mL, lettuce root growth was arrested completely (100%) whereas cress, alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and sand fescue showed root inhibition to 88.6, 94.5, 87.5, 78.8, 97.8, 93.9 and 72.0%, respectively (Fig. 3). The root growth of all test species also exhibited considerable inhibition at the concentration of 0.01 and 0.03 g DW equivalent extract/mL. Lettuce showed highest inhibition (76.1%) followed by rapeseed (73.1%), sand fescue (72.1%), barnyard grass (64.1%), alfalfa (54.6%), timothy (53.4%), Italian ryegrass (42.8%) and cress (38.6%) at the concentration of 0.03 g DW equivalent extract/mL (Fig. 3). On the other hand, 15.7, 41.4, 30.4, 32.9, 27.1, 32.1, 24.0 and 29.3% inhibition were found from cress, lettuce, alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and sand fescue, respectively at the concentration of 0.01 g DW equivalent extract/mL (Fig. 3). Considering I_{50} values of the extracts of *C. difformis* for the root growth of eight test species ranges from 0.004 to 0.041 g DW equivalent extract/mL (Fig. 4). Rapeseed was the most sensitive whereas barnyard grass was least sensitive by the aqueous methanol extracts of *C. difformis* and also root growth inhibition of test species was found more than shoot growth.

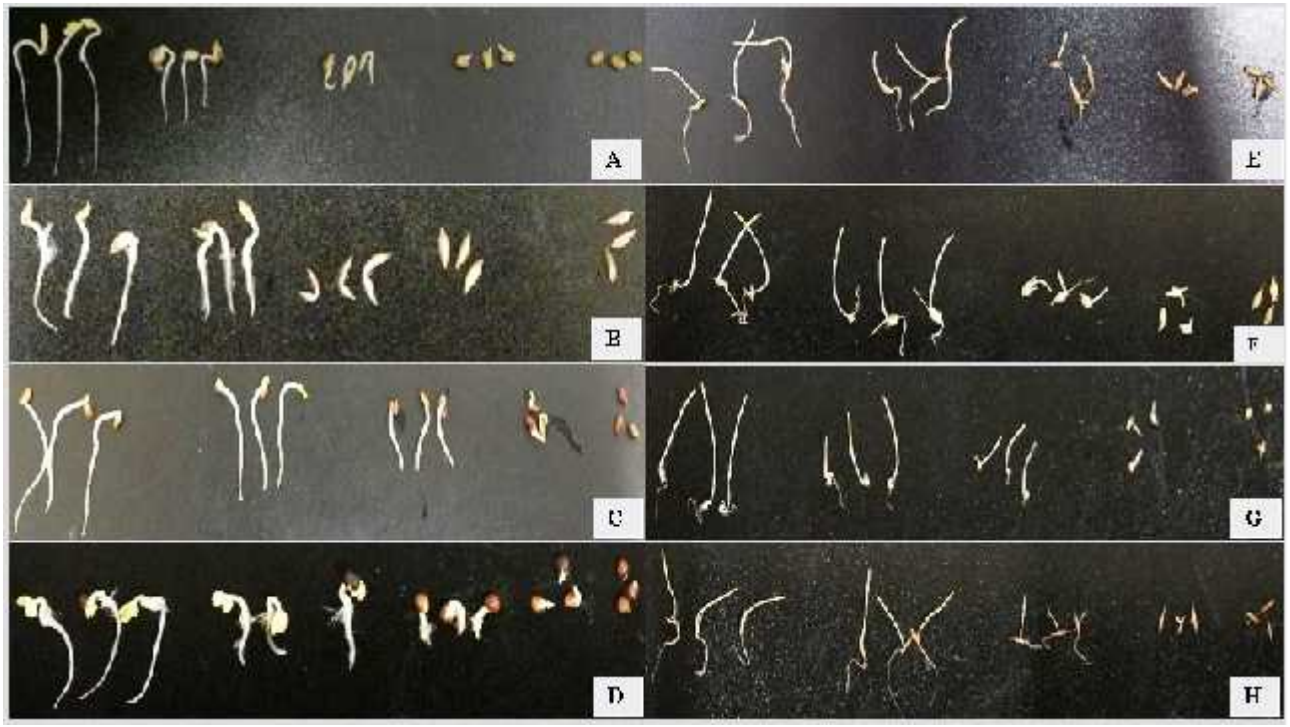

Fig. 1. Effects of aqueous methanol extracts of *Cyperus difformis* on the seedlings growth of cress (A), lettuce (B), alfalfa (C), rape seed (D), Italian ryegrass (E), barnyard grass (F), timothy (G) and sand fescue (H). Treatment concentrations (from left to right in each test species): control, 0.01, 0.03, 0.1, 0.3 g dry weight equivalent extract of *Cyperus difformis*/mL

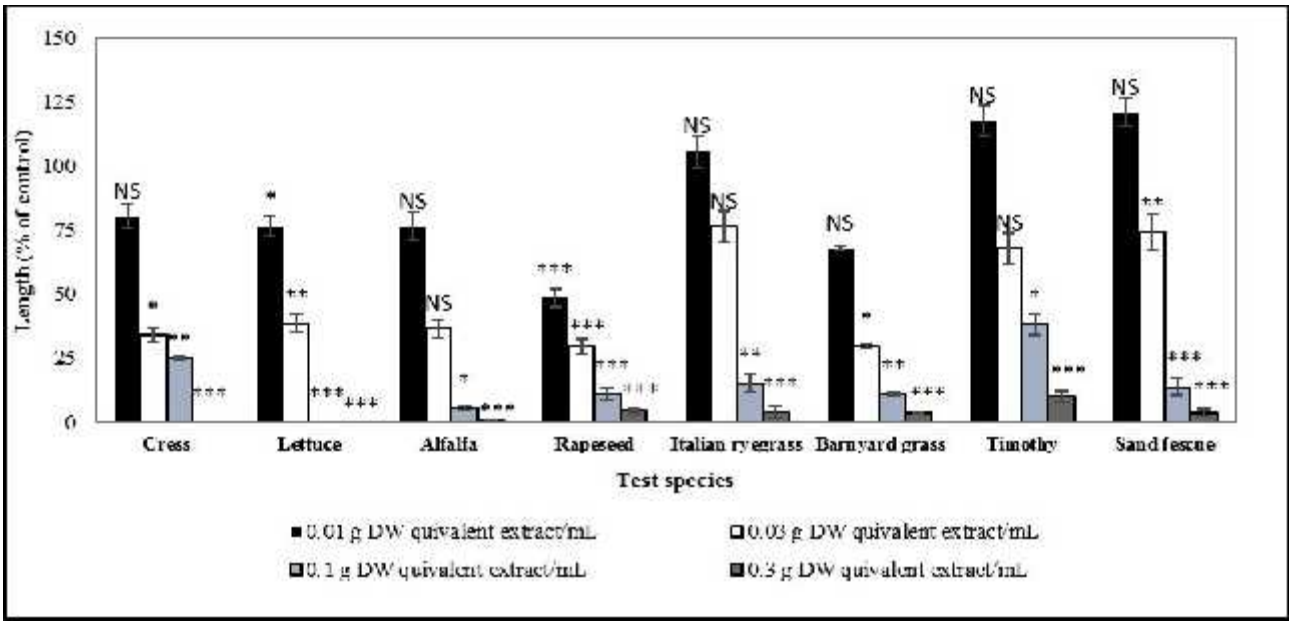

Fig. 2. Allelopathic effects of aqueous methanol extracts of *Cyperus difformis* on the shoot growth of cress, lettuce, alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and sand fescue. All the test plants species were exposed to the concentrations equivalent to the extracts obtained from 0.01, 0.03, 0.1 and 0.3 g dry weight of *C. difformis*/mL. Mean ± SE from two independent experiment with 3 replicates for each treatment are presented (seedlings per treatment=10, n=60). Each vertical bar represents standard error of the mean. Significant differences between treatments and control are denoted by asterisks. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$

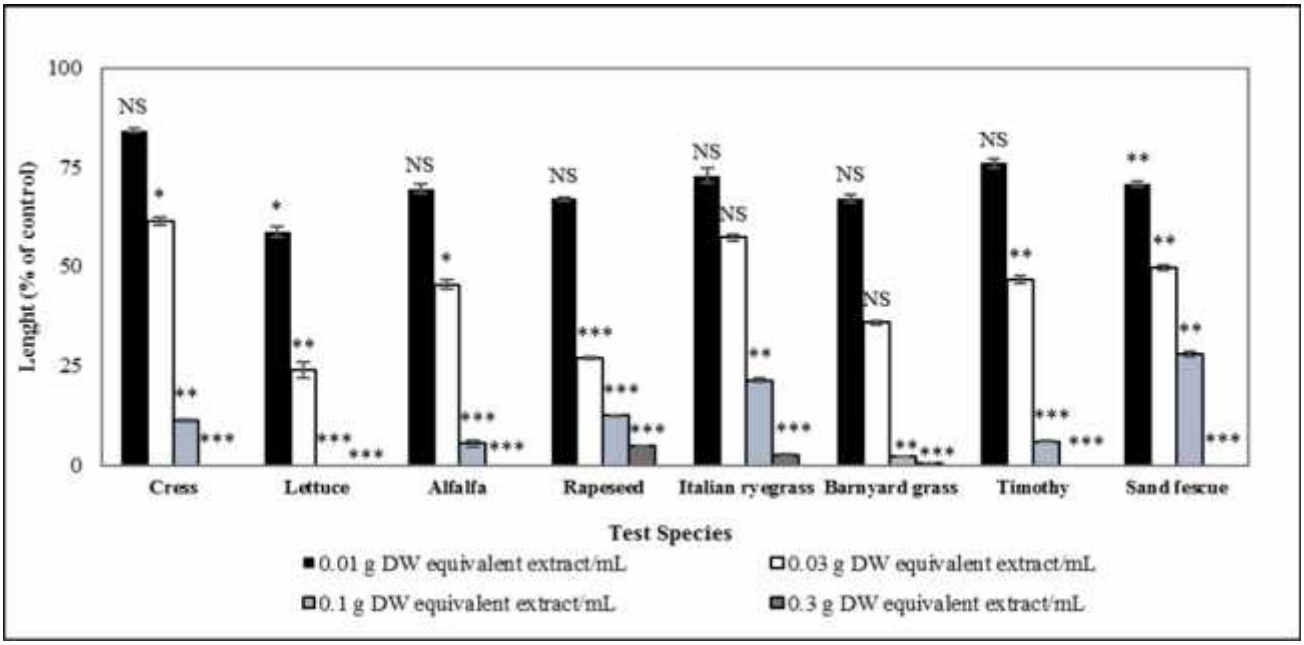

Fig. 3. Allelopathic effects of aqueous methanol extracts of *Cyperus difformis* on the root growth of cress, lettuce, alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and sand fescue. All the test plants species were exposed to the concentrations equivalent to the extracts obtained from 0.01, 0.03, 0.1 and 0.3 g dry weight of *C. difformis*/mL. Mean ± SE from two independent experiment with 3 replicates for each treatment are presented (seedlings per treatment=10, n=60). Each vertical bar represents standard error of the mean. Significant differences between treatments and control are denoted by asterisks. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$

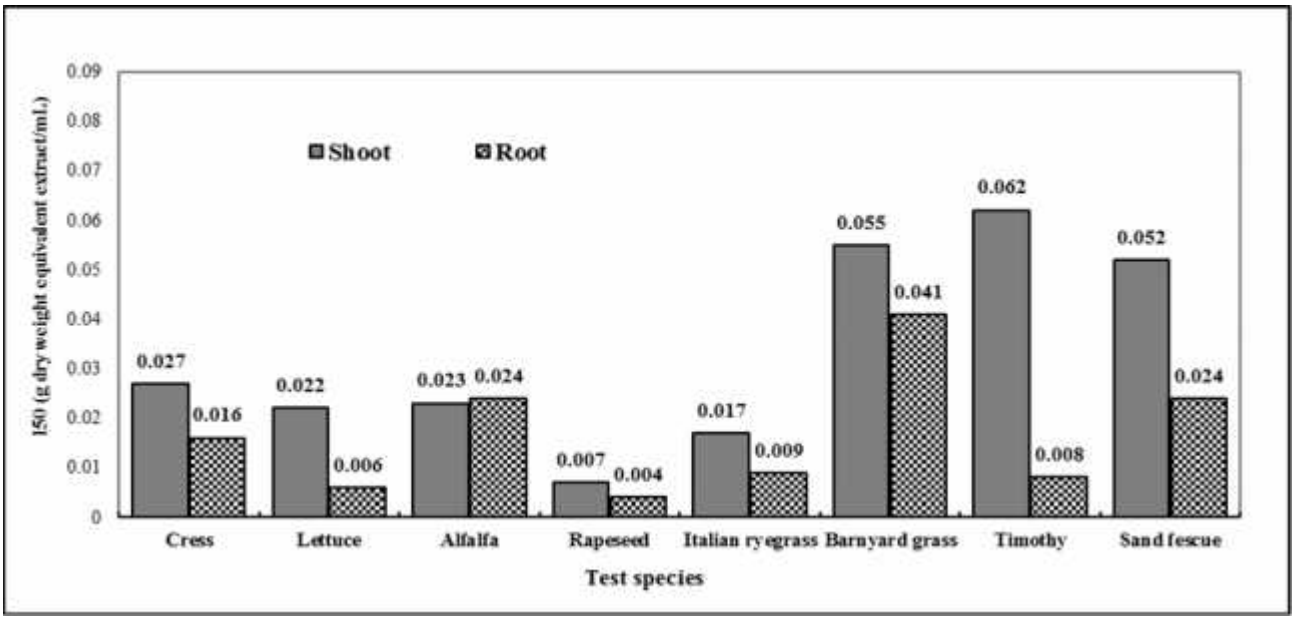

Fig. 4. I_{50} values of the aqueous methanol extracts of *Cyperus difformis* for shoot and root growth of cress, lettuce, alfalfa, rapeseed, Italian ryegrass, barnyard grass, timothy and sand fescue.

DISCUSSION

Aqueous methanol extracts of *C. difformis* had inhibitory effects on both dicotyledonous (cress, lettuce, alfalfa, rapeseed) and monocotyledonous (Italian ryegrass, barnyard grass, timothy, sand fescue) test species. This inhibition was proportional to the extracts concentrations and stronger inhibitory effects were found at higher extracts concentrations, suggesting that inhibitory effects were species specific and concentration dependent (Fig. 1). Such inhibition by the plants extracts was reported by the McEwan *et al.* (2010), where the inhibitory effects of *Lonicera maackii* extracts on *Impatiens wallerana* increased with increasing its extracts concentrations. Similar results are also in agreement with others studies (Batish *et al*. 2002; Kato-Noguchi *et al*. 2014; Duke 2015). Hassan *et al.* (2012) reported that, uptake of allelochemicals by the plants may be influenced by the different seed size, shape as well as different seed coat permeability, which may exposure a plants inhibition to different concentration dependable. In addition, sometimes low concentration although enhance stimulation in plants (Fig. 2) but with the increase of same concentration the plants experienced strong inhibitions (Duke *et al*. 2006; Calabrese 2008; Liu and Chen 2011; Islam and Kato-Noguchi 2012).

The shoot and root growth inhibition on test plants by the extracts of *C. difformis* may be due to the allelochemicals (Mubeen *et al*. 2012) rather than intra species competition for light, space, nutrient and water. Seeds or seedlings of test species used in the experiment were placed to grow in Petri dishes without any supporting nutrient media. In addition, as light is inessential during growing period (Fuerst and Putnam 1983; Ashrafi *et al*. 2008) of seedlings, so the shoot and root inhibition of the test species may be caused due to the effect of allelochemicals. The growth inhibition in the presence of allelochemicals could be for the reason of reduced cell division of the seedlings, altering the ultrastructure of the cells (Li *et al*. 2010). During seedling growth, the reduction of protein and nucleic acids, as well as the alteration of the ion uptake, water and phytohormone balance, photosynthesis, respiration, inactiveness of several enzymes, generation and accumulation of reactive oxygen may be also the possible reasons for such inhibition by the extracts (Fahmy *et al*. 2012; El-Shora and Abd El-Gawad 2014). Root growth of the test species was found more sensitive by the extracts than their shoot growth (Fig. 2 and 3). Parallel results of root sensitivity by the extracts were also reported by Pukclai *et al*. (2010); Netsere and Mendesil (2012). The sensitivity of root to the extracts could be attributed for the direct contact of radicle (younger stage of root) to the allelochemicals (Salam and Kato-Noguchi 2010) as well as higher permeable capacity of allelochemicals to root tissue than shoot tissue (Nishida *et al*. 2005; Yoshimura *et al*. 2011). Furthermore, based on the I_{50} values of all the test species, the experimental results showed a variation in sensitivity of the test species to the extracts of *C. difformis*. These results indicate that, the specificity of test species to different allelochemicals (Khanh *et al*. 2006).

Conclusion: Aqueous methanol extracts of *Cyperus difformis* exhibited inhibitory activities on the growth of both crops and weeds species. Inhibitory activities of *C.*

difformis extracts may be caused for its phytotoxicity and may convey allelochemicals. Therefore, *C. difformis* could be a candidate for further isolation and identification of allelopathic compounds and may lead to develop an environment friendly biological control of weed.

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