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Assessment of allelopathic potential of *Cassia sophera* L. on seedling growth and physiological basis of weed plants

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Allelopathy is described as both beneficial and deleterious biochemical interaction between plant and weeds, and/or plant and microorganisms through the production of chemical compounds that escape into the environment and subsequently influence the growth and development of neighbouring plants. The present laboratory experimental study was conducted to evaluate the allelopathic effect of *Cassia sophera* (L.) on three weed plants (*Chenopodium album* L., *Melilotus alba* Medik and *Nicotiana plumbaginifolia* Viv.). Aqueous extracts of *Cassia* at 0.5, 1.0, 2.0 and 4.0% concentrations were applied to determine their effect on seed germination, seedling growth, dry biomass, leaf area, relative water content, chlorophyll and protein content of test plants under laboratory conditions. The aqueous extracts had a significant retardatory effect on seed germination of test plants which varied among species and also with the different concentrations used. Root length and shoot length of weed species decreased significantly when plants were exposed to increasing aqueous concentration (0.5, 1, 2 and 4%). The noticed reduction in dry biomass, leaf area and relative water content were also significant. Physiological parameters (total chlorophyll content and protein content) in relation to three test species (*M. alba*, *C. album* and *N. plumbaginifolia*) were significantly reduced with the different concentrations of aqueous extract used. From this we can predict that cassia might possess allelochemicals that causes the suppressive ability.

Key words: Allelopathy, aqueous extract, *Melilotus alba*, *Chenopodium alba* and *Nicotiana plumbaginifolia.*

INTRODUCTION

Weeds have been a persistant problem for agriculture systems because it causes economic losses by reduction in crop yield, increase cost of crop production, and often cause total crop failure (Bhuler et al., 1998). Concerns about negative effects of herbicide use, such as environmental contamination, development of herbicide resistant weeds and human health problems, make it necessary to diversify or other weed management options (Holethi et al., 2008). The use of allelopathic behavior is one of the new options for sustainable weed management (Olofsdotter and Navarez, 1996). The invasive nature of weeds is due to the allelopathy which can be defined as the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment. All these compounds usually are called

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Abbreviations: DAS, Day after sowing; **CRD,** completely randomized design; **AOSA,** Association of Official Seed Analysts; **DMSO,** di-methyl sulphoxide; **CSAE,** *C. sophera* aqueous extract; **GP,** germination percentage; **PL,** plumule length; **RL,** radicle length; **DW,** dry weight; **RWC,** relative water content.

secondary plant products or waste products of the main metabolic pathways in plants (Turk et al., 2003; Yokotani et al., 2003; Iqbal et al., 2006). The production of allelochemicals is widely influenced by genetics as well as environmental factors at different growth stages (Yu et al., 2003). They enter the environment through various routes such as leaching, volatilization, root exudation, seed-coat exudation after imbibition, and decomposition of diverse parts of the plant (Rice, 1984; Bertin et al., 2003; Higashinakasu et al., 2004). All plant parts of the weed including leaf, stem, root, and fruit depending upon plant species have allelopathic potential (Mahmood et al., 1999; Alam and Islam, 2002; Tinnin and Muller, 2006). Different groups of plants, crops and weeds have wide known allelopathic interactions (Inderjit and Dakshini, 1994; Ahmad et al., 2007; Uddin et al., 2007). There are several reports that some weed species have allelopathic effects on seed germination and seedlings growth of economically important crop plants (Mulatu et al., 2011; Shibu and Andrew, 1998; Rice, 1984; Delabays et al., 2004; Gulzar and Siddiqui, 2014; Sisodia and Siddiqui, 2012). The multiple effects resulting from allelochemicals include decreases in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure, osmotic potential, dry matter production, leaf area expansion, stomatal aperture size, stomatal diffusive conductance, and photosynthesis (Booker et al., 1992; Chou and Lin, 1976; Einhellig et al., 1970; Einhellig and Kuan, 1971; Einhellig and Rasmussen, 1979; Einhellig et al., 1985; Gerald et al., 1992; Patterson, 1981; Weir et al., 2004).

The *Cassia sophera* belongs to the Leguminosae family and the subfamily of Caesalpinioideae, extending from Africa to India and South East Asia. *C. sophera* is believed to be native of South America. The species grows along roadsides and on waste ground and is reported by Mulay and Sharma (2012) to be a common weed in uncultivated lands. Its invasive nature is due to its fast growth rate, high reproductive and vegetative potential, adaptable to changing environmental conditions, wide ecological amplitude and allelopathy. Triterpenes from cassia are known for their allelopathic responses and great ecological significance with respect to invasion (Ghayal et al., 2007). The aqueous extract of the whole plant and leaves produces an inhibitory allelopathic effect on many weeds. The powdered leaves of *C. sophera* also affect the growth and metabolism of associated weeds. Recently, a study showed that the allelopathic effect of *Cassia occidentalis* L. causes the suppression of seed germination and growth in *Parthenium hysterophorus* L., a detrimental weed in India (Knox et al., 2011). Similarly, the allelopathic effect of *Cassia tora* on seed germination and growth of mustard has been elucidated by Sarkar et al. (2012). In ethno botanical literature, it is mentioned to be effective in the treatment of pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes and convulsions of children (Chopra et al., 1956; Agharkar, 1991; Dutt, 1995; Kirtikar and Basu, 2000). For this reason, the plants are not destroyed and instead allowed by the local people to grow near crop fields.

Therefore, in the present work, an attempt has been made to evaluate and compare the allelopathic potentiality of the common invasive weed (*C. sophera*) on the germination, seedling growth, dry biomass, leaf area and physiological parameters of three weed species (*M. alba*, *C. album* and *N. plumbaginifolia*).

MATERIALS AND METHODS

Collection of plant materials

The *C. sophera* was collected from the campus of Aligarh Muslim University, Aligarh (27°, 29 to 28°, 100 N.L and 77°, 29° to 78°, 38° E.L) where it was growing abundantly. The plants were uprooted at maturity. They were washed thoroughly with distilled water and air-dried at room temperature for 96 h. The aerial portions were separated, chopped into 1 cm long pieces, and ground into fine powder with mortar and pestle. Stock aqueous extract was obtained by soaking 4 g powder in 100 mL of cold distilled water (4% w/v) at room temperature (20±2°C) for 24 h with occasional shaking. The mixture was filtered through two layers of cheesecloth and centrifuged for 20 min to remove particulate material and the purified extract was adjusted to pH 6.8 with 1 M HCl. Different concentrations (0.5, 1 and 2%) were prepared from the stock solution in addition to the control (Singh et al., 1989). The aqueous extracts were individually bottled, tagged, and to maintain the efficacy of extracts, it is kept in refrigerator for further use.

Germination in Petri dishes

For growth studies, seeds of *C. album, M. alba* and *N. plumbaginifolia* were procured from National research centre for weed science, Jabalpur (M.P). Seeds of each test plants were first surface sterilized with 2% sodium hypochlorite solution for 2 min and washed thoroughly with distilled water. Next, sets of autoclaved Petri dishes were prepared, each containing a single layer of Whatman No. 1 filter paper and 5 mL of test extract for each concentration (0.5, 1, 2 and 4%) of aerial shoots. The Petri dishes treated with distilled water were taken as a control and considered to be set 0. The treatments were arranged in completely randomized design (CRD) with 10 replicates at room temperature on a laboratory bench with 12 h supply of fluorescent light during the night. The whole experiment was repeated once. The plants were sampled on 15th day after sowing (DAS) to record various observations.

Determination of germination percentage, root length, shoot length, dry biomass, leaf area and relative water content

Germinated seeds were counted daily according to the Association of Official Seed Analysts (AOSA) method (AOSA, 1990). The seeds were considered as germinated when the radical size was 2 mm. Fifteen (15) days after sowing, germination percentage was calculated using the formula: germination percentage = (germinated seed/total seed × 100) for each replication of the treatment followed by seedling root length (cm), shoot length (cm) and dry weight (mg) determination. The root and shoot length were measured by using a meter scale; while, the dry weight was measured with the help of four digital balance of Scientech, Model ZSA 120, Colorado (USA). The leaf area was measured manually by using a graph sheet, where the squares covered by the fresh leaf were counted to calculate the leaf area. Using the equation of Deef and Abd El-Fattah (2008), the relative water content (RWC) was evaluated as RWC% = (FW - DW) / FW × 100.

Protein determination

The method as given by Lowry et al. (1951) was adopted for this purpose.

Chlorophyll determination

The total chlorophyll content from leaves of treated or control plants were extracted in di-methyl sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979). Finely cut uniform discs (100 mg fresh weight) were made from fully expanded leaves of test plants. Dry weight equivalents of each of the treated samples were determined by keeping 100 mg fresh weight discs in an oven. The weighted material (100 mg fresh weight leaf disc) was suspended in 10 ml of di-methyl sulphoxide (DMSO) incubated at 65°C for 1 h (the period of incubation was found sufficient for the complete extraction of chlorophyll). The DMSO was recovered by thorough decantation. The final volume was corrected to 10 ml with fresh DMSO. The extinction of chlorophyll thus, recovered in DMSO was measured at dual wavelength of 645 and 663 nm on spectro-photometer against DMSO as blank. The extinction values were read and the amount of chlorophyll was calculated according to the equation given by Arnon (1949), with modification by Hiscox and Israelstan (1979).

Statistical analysis

Using standard procedures of statistical data analysis (including the software BioStat 2009, version 5.7.8.1, and the inbuilt mathematical functions of sigma plot version 10, the effects of different concentrations of *C. sophera* were correlated with the rate of germination, root length, shoot length, leaf area, DW, RWC, chlorophyll content and protein content of test species. Figures 1 to 4 show change in these parameters (the bars represent the standard deviation of measurement)

RESULTS

Germination

The germination percentage (GP) of *M. alba* was significantly affected by the increase in concentration of *C. sophera* aqueous extract (CSAE) (Figure 1). In control and 0.5% CSAE, GP values were 100 and 98%. The percentage was reduced to 57% at 1% and 55.6% at 2% CSAE concentration levels and to 30% at 4% CSAE concentration. Generally, GP of *C. album* seeds varied with CSAE concentrations (Figure 1) and it is supported statistically. In the control series, GP values were 80% but decreased upon applying 0.5 and 1% CSAE concentrations (67 and 70%, respectively). However, the reduction goes to a markedly lower level at 2 and 4% concentrations (47 and 50%, respectively). Figure 1 shows a great variation in the calculated values of GP of *N. plumbaginifolia* seeds. The GP was significantly affected by the increase in CSAE concentrations. In control, 0.5 and 1% CSAE, GP values were 100, 95 and 92%. The percentage was reduced at 2 and 4% CSAE concentration levels.

Seedling growth

The results of plumule length (PL) of *M. alba* imply that allelopathic substances affect negatively the seedling stage (Figure 2). PL was significantly reduced at different concentrations of treatments given. The value of PL was 20.45 cm at control level, but reduced to 18.20 cm at 0.5% CSAE concentration. The retardatory allelopathic action was recorded in 1, 2 and 4% CSAE concentrations, which significantly reduced PL. Allelopathic effect of CSAE concentration on PL of *C. album* is given in Figure 2. The plumule elongation was not completely inhibited by the extract, but the length was reduced at higher concentration levels. Obviously, all allelopathic concentrations have reduced PL. In control series, PL of *C. album* was 19 cm. In 0.5, 1, 2 and 4% concentrations, inhibition was observed and the values were 17.71, 17.77, 15.81 and 15 cm, respectively (Figure 2). PL of *N. plumbaginifolia* was significantly reduced in each treatment (Figure 2). PL was 18.17 cm in control series but was reduced to 17 cm at 0.5% CSAE concentration. The maximum allelopathic action of 1, 2 and 4% CSAE concentrations was observed in the form of reduction as 16, 14.90 and 14 cm, respectively.

A slight difference was observed among *M. alba* RL (Figure 1). The control value was 12.48 cm. Elevated CSAE concentrations had significant retardatory effect on radical growth. At 0.5% CSAE concentration, it was 11.48 cm. Upon applying highest CSAE concentration (4%), it was reduced to 5.99 mm. A gradual decrease in RL of *C. album* was observed with gradual increase in CSAE concentrations. RL was significantly affected by the treatment at 4% concentration. In the control, values of RL were 10 cm, but at higher concentrations of CSAE radicle emergence were clearly affected. At 0.5 and 1% concentrations, RL decreased to 9.50 and 8.31 cm; till it attained a value of ~6.39 and 4 cm at 2 and 4% concentrations. In the control series RL of *N. plumbaginifolia* was 9.00 cm. High CSAE concentrations had significant retardatory effect on radical growth (Figure 1). At 0.5% CSAE concentration it was 8.01 cm. Upon applying the highest CSAE concentration (4%), it was reduced to 3.99 cm and at 1 and 0.5% CSAE, the values were 7.11 and 8.01 cm, respectively.

Dry biomass, leaf area and relative water content

As per the dry biomass of test species affected by CSAE at 4% concentration, the percentage reduction observed was 34, 40 and 44% in *M. alba*, *C. album* and *Nicotiana plumbaginifolia* over the control (Figure 2). In contrast to

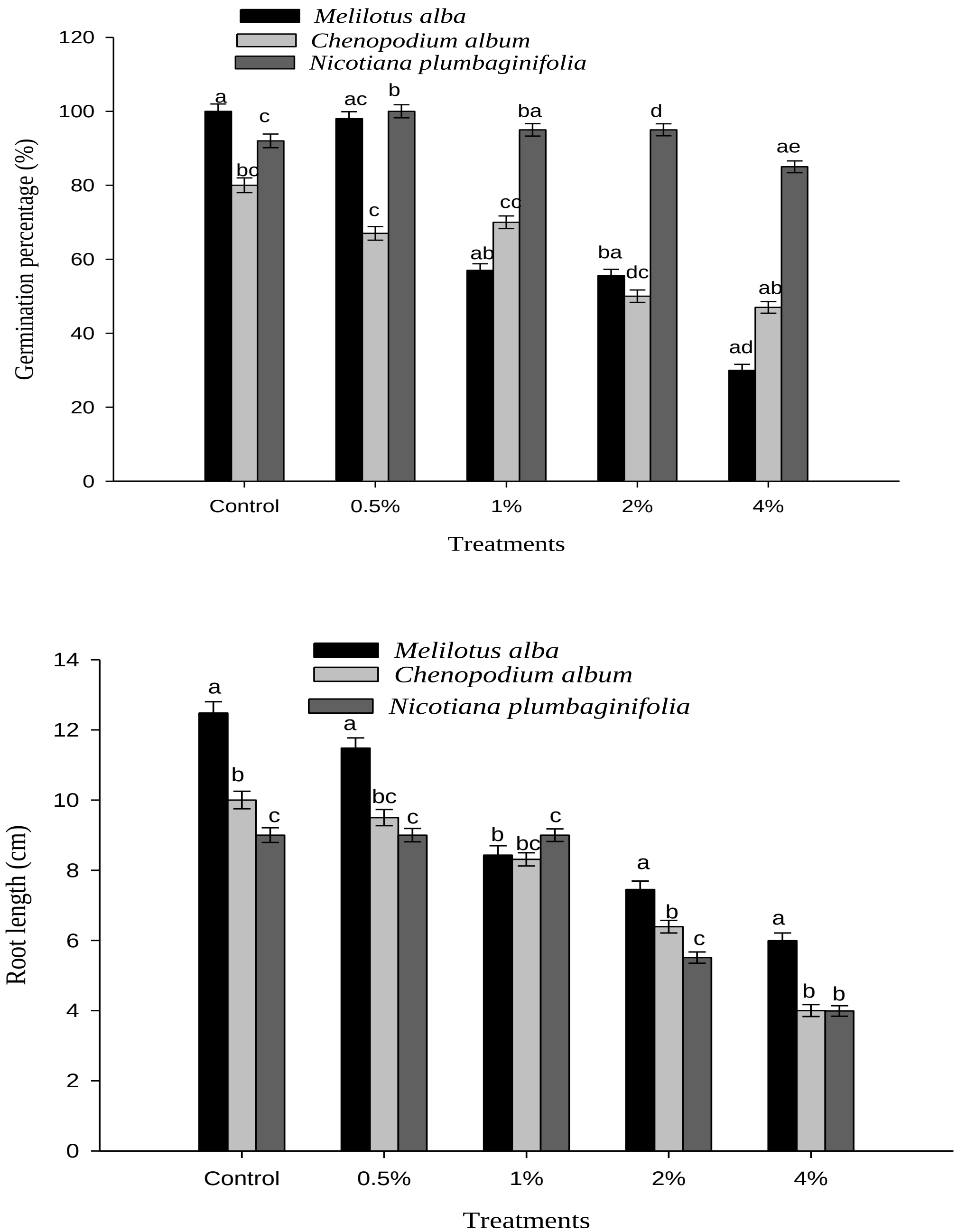


Figure 1. The effects of increasing concentrations of *Cassia sophera* on germination percentage (%) and root length (cm) of test species (n = 10).The bars indicate standard deviation. Different superscript symbols within each column represent significant difference among themselves at P<0.05 applying DMRT.

control, leaf area shows decrease with increase in extract concentration, reduced by 16, 10 and 19.65% in *C. album*, *M. alba* and *N. plumbiginifolia* at 4% CSAE concentration (Figure 3). Leaf relative water content (RWC) of the stressed plants showed significantly lower values at higher CSAE concentration than their control plants and that were 26.6% in *N. plumbiginifolia* and 14.28% in *M. alba* (Figure 3).

Protein and chlorophyll content

The protein and chlorophyll content of test species were affected significantly when treated with the different concentrations of CSAE. Although, treatment with 0.5% CSAE had very little impact on the protein and chlorophyll content of test seedlings, higher concentrations (4%) reduced the protein contents by 41.3, 20 and 33% in *M.*

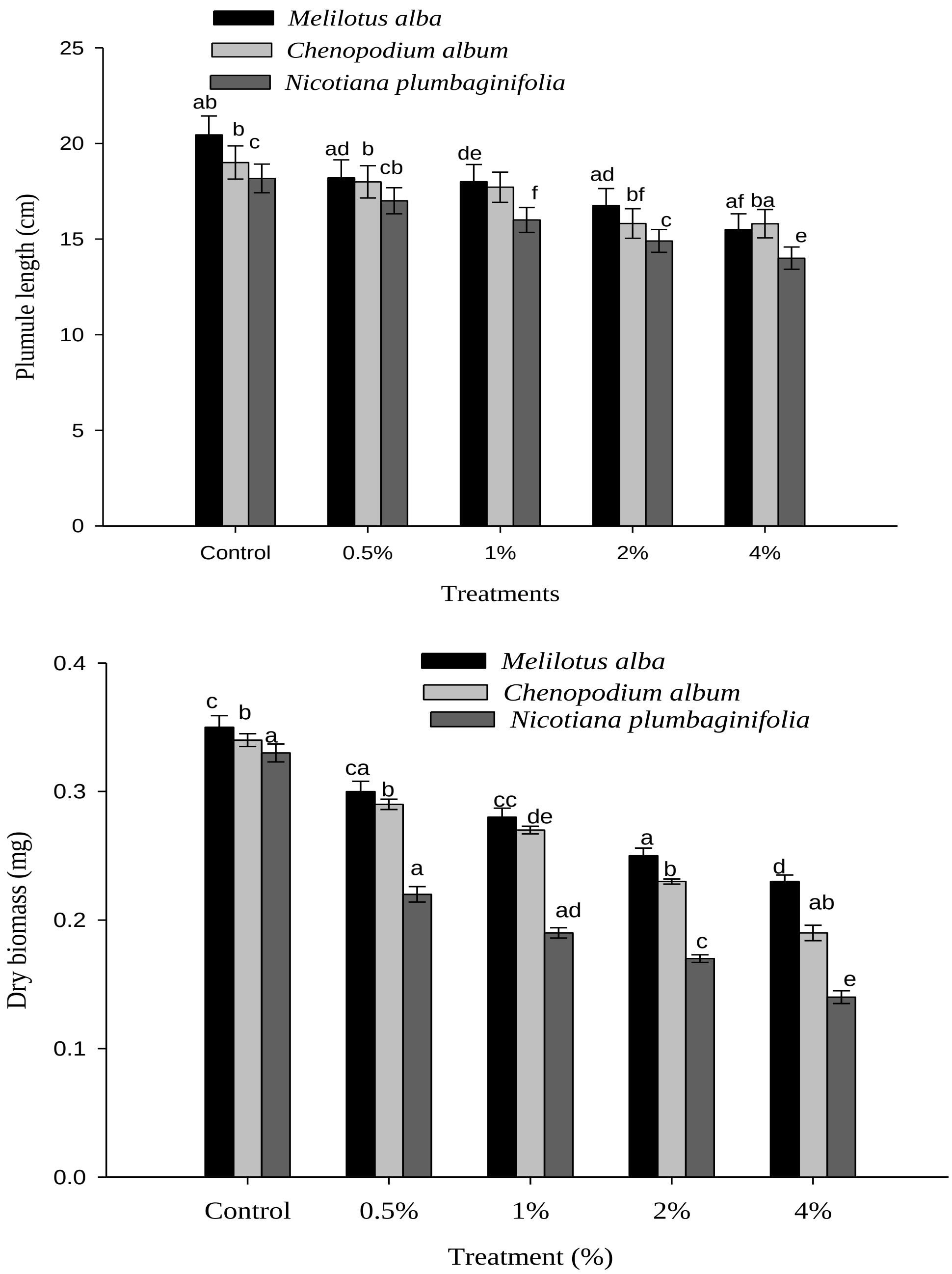
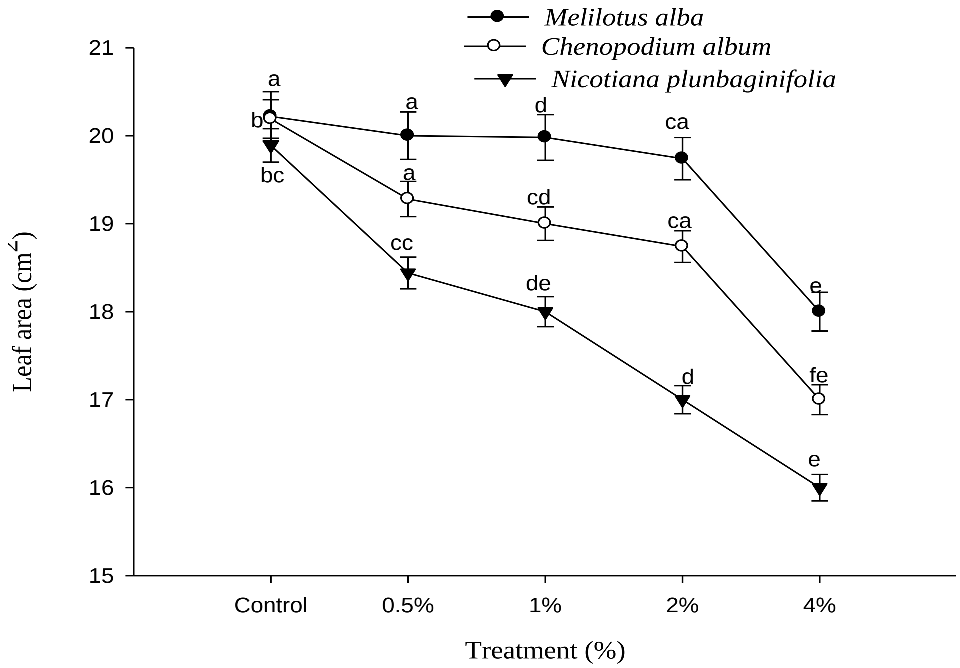


Figure 2. The effects of increasing concentrations of *Cassia sophera* on plumule length (cm) and dry biomass (mg) of test species (n = 10). The bars indicate standard deviation. Different superscript symbols within each column represent significant difference among themselves at P<0.05 applying DMRT.

alba, *C. album* and *N. plumbiginifolia* as compared to the control (Figure 4). The chlorophyll content was similarly affected resulting to more reduction at higher concentration (4%) in comparison to control (Figure 4).

DISCUSSION

Treatment by aqueous extract resulted in delayed germination and low germination rate of the weeds. Through delayed germination, lowered seed germination rate and reduced seedling growth, reduced root-shoot ratio, the treatments lowered dry matter. The inhibitory effects were increased with increasing concentrations. This study shows that different concentrations of leachate showed distinct allelopathic inhibitory effects on the weed species; lower concentrations (1 and 0.5%) showed weak inhibitory or even positive effects, whereas higher



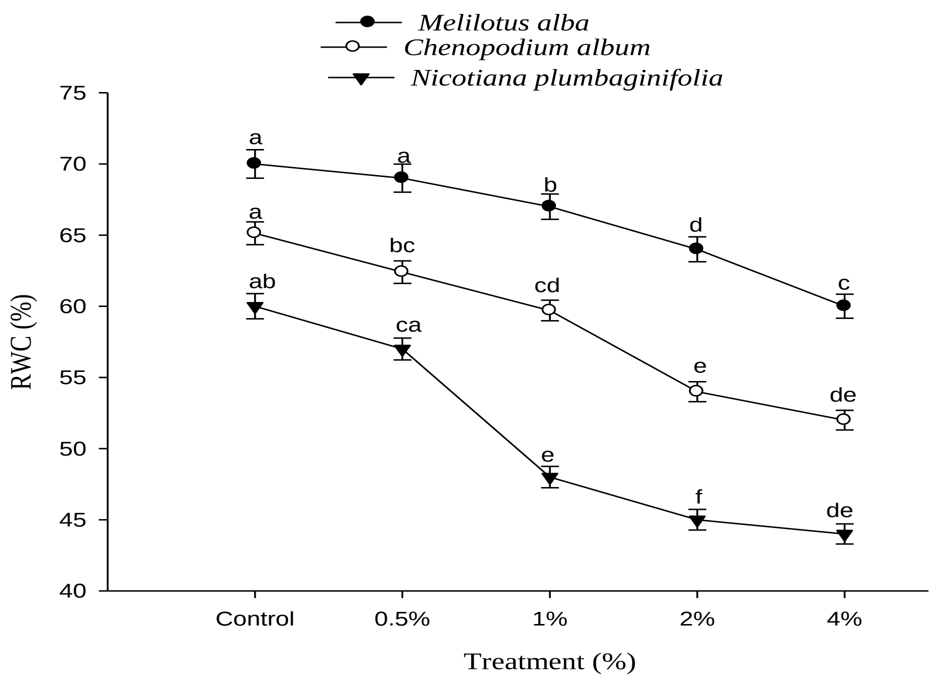


Figure 3. The effects of increasing concentrations of *Cassia sophera* on leaf area (cm^2) and Relative water content (%) of test species (n = 10). The bars indicate standard deviation. Different superscript symbols along each represent significant difference among themselves at P<0.05 applying DMRT.

concentrations (4 and 2%) showed stronger inhibitory effects. Therefore, considering that the plant allelochemicals could have beneficial or inhibitory effect, in order to control.

As observed in many studies (Hong et al., 2003; 2004; Mulatu et al., 2011; Lungu et al., 2011; Shapla et al., 2011), allelochemicals extracted from Melia (extracted with ethanol and water) inhibited germination and growth of receiver plants are in line with our studies. Similar observation was also concluded by Bogatek and Gniazdowska (2007) and Javed (2011) in radish germination inhibition due to the result of induction of

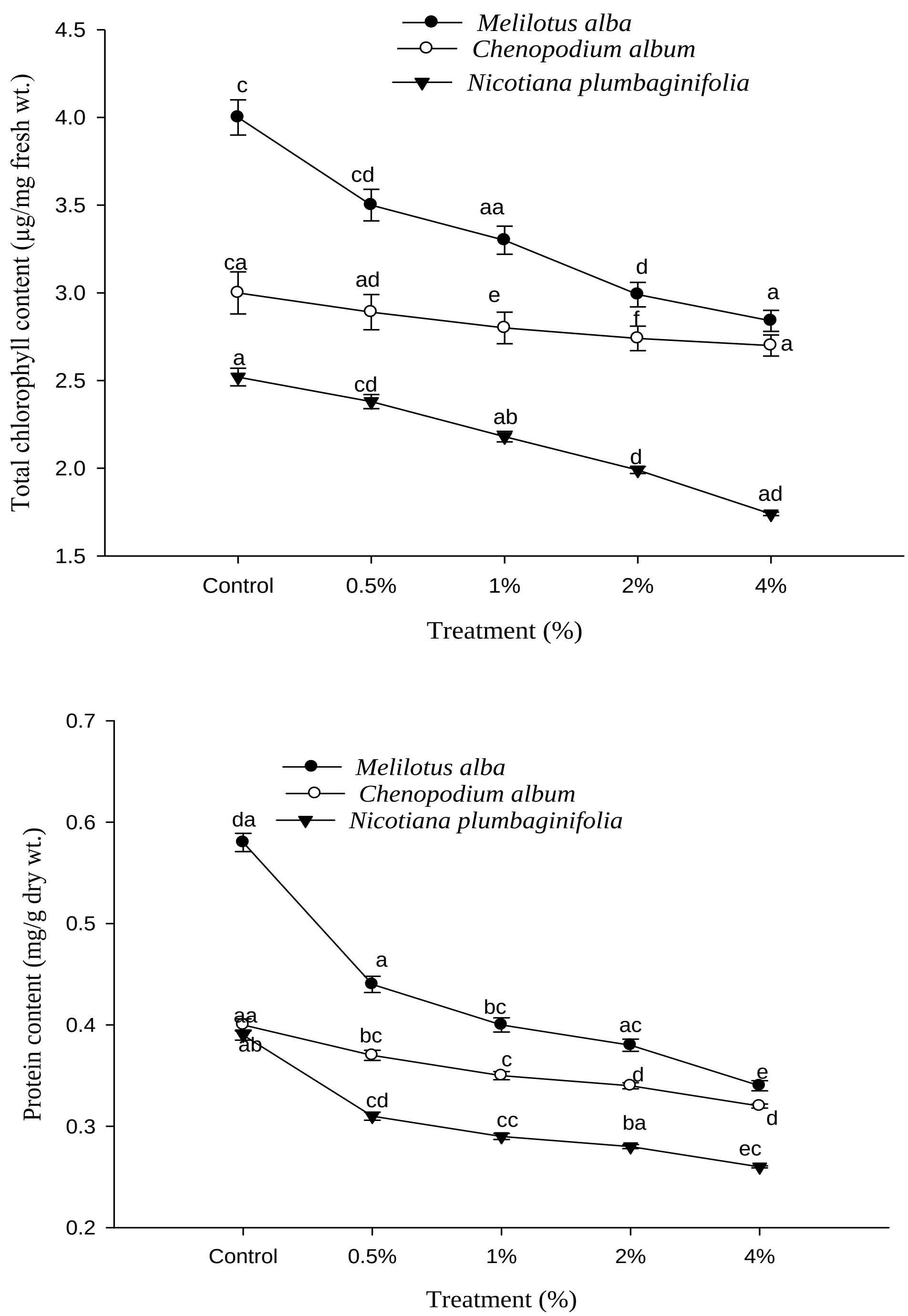


Figure 4. The effects of increasing concentrations of *Cassia sophera* on chlorophyll content (mg/g fr. Wt.) and protein content (mg/g dry weight) of test species (n = 10).The bars indicate standard deviation. Different superscript symbols along each curve represent significant difference among themselves at P<0.05 applying DMRT.

oxidative stress. Suggested mechanism for the inhibition of seed germination is the disruption of ‘dark’ or mitochondrial respiration and by this, a possible disruption of the activity of metabolic enzymes involved in glycolysis and oxidative pentose phosphate pathway (OPPP), which takes substrates from glycolysis and feeds its products back into glycolysis as observed in *Pinus laricio* seeds germination grown in the soils around *P. laricio* and *Fagus sylvatica* trees (Muscolo et al., 2001). In general, the negative effect increased with the concentration of the extract and biological activities of receiver plants to allelochemicals which are known to be concentration dependent as noticed by Zhang et al. (2007), Singh et al. (2006) and Peng et al. (2004), which also is in line with our study.

The root length decreased as the concentration of extract increased and the greatest inhibition was observed at 4% concentration. As root membranes are a primary site of action for phenolics. The contact of phenolic acids with the root cell membrane leads to depolarization, an efflux of ions, and a reduction of hydrolic conductivity, water uptake and net nutrient uptake (Baziramakenga et al., 1995; Lehman and Blum, 1999). Root growth is characterized by high metabolic rates and, for this reason, roots are highly susceptible to environmental stresses such as allelochemicals in soils (Cruz-Ortega et al., 1998). Similarly, Baerson et al. (2005) reported that BOA impaired root system development, resulting in reduced root lengths and a complete absence of lateral root formation in 10-day-old *A. thaliana* seedlings by 50% (I_{50}) and 80% (I_{80}) at 540 µM and µM. In the present study, aqueous extract lowered relative water contents in leaves of all target species. Apparently, the three target species responded differently to the phytotoxic compounds, thereby contributing to species selectivity. Some other phenolic acids such as p-coumaric, caffeic, ferulic, and salicylic acids also cause water stress in plants (Einhellig, 1995; Barkosky and Einhellig, 2003). Sanchez-Moreiras and Reigosa (2005) reported that a 10% decrease in relative water content was correlated with a decline in leaf water potential in BOA-exposed lettuce plants concentration, respectively. From our results, it is clear that decreases in the relative water content of leaves in these plant species initially induce stomatal closure, imposing a decrease in the supply of CO_2 to the mesophyll cells and, consequently, photosynthesis could be lowered resulting in the decrease in chlorophyll content (Hussain and Reigosa, 2011). The significant reduction of chlorophyll content seen with all concentrations may be due to the inhibition of chlorophyll biosynthesis, the stimulation of chlorophyll-degrading substances, or both (Yang et al., 2007; Patterson, 1981). One of the best-characterized phytotoxic mechanisms induced by allelochemicals is the inhibition of photosynthesis and oxygen evolution through interactions with components of photosystem II (PSII) (Einhelling, 1995). Higher concentrations of extracts were found to cause mosaic chlorosis, resulting in the yellowing of leaves and thereby causing the reduction in the chlorophyll content. A significant downgrade in leaf protein contents in all three species due to aqueous extract of different concentration is supported by findings of Baziramakenga et al. (1997); Mersie and Singh (1993); Hussain et al. (2010) that many phenolic acids reduced the incorporation of certain amino acid into proteins and thus reduced the rate of protein synthesis.

The dry mass reduction is supported by previous study of Terzi and Kocacaliskan (2010), where the elongation and DW of barley and wheat seedlings were reported to be reduced by the walnut allelochemical juglone (5-hydroxy-1,4-naphthoquinone) in a similar pattern. Macro- and micronutrient absorption and IAA oxidase in plant root cells is inhibited by various allelochemicals (Yang et al., 2004), which may lead to the observed reductions in DW and RWC of germinating mustard seedling.

Conclusion

The allelopathic effect from aqueous extracts *C. sophera* showed a significant retardatory effect on all the above mentioned parameters of *M. alba, C. album, N. plumbaginifolia*. Hence, the allelochemicals extracted from aqueous extract of *Cassia* can be employed for the natural control of the tested weeds, thus achieving the aim of environmental safety. There is need for further study to be carried out on identifying the inhibiting allelochemical in the parts investigated. By delaying germination, lowering the germination rate of the weeds and inhibiting seedling growth, leachate from *C. sophera* could provide an effective way of controlling the weeds.

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