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Phytotoxic effects of *Echinochloa colona* (L.) Link. (Poaceae) extracts on the germination and seedling growth of weeds

N. H. Gomaa* and H. R. AbdElgawad

Department of Botany, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

Abstract

This paper aims to study the phytotoxic potential of *Echinochloa colona* in order to explore its potential as bioherbicide. In this way, the phytotoxic effects of the most active fraction and pure compound from methanol (MeOH) extract of *E. colona* shoots on germination and seedling growth of ten weed species and on *E. colona* itself were evaluated. A lettuce seed bioassay indicated that dichloromethane (CH_2Cl_2) soluble fraction of MeOH extract had the most phytotoxic activity. The most phytotoxic active compound of CH_2Cl_2 fraction was isolated and identified as tricin. Different concentrations of CH_2Cl_2 fraction (100, 500, 1000 mg L^{-1}) and tricin (50, 100, 200 µmol L^{-1}) were used to assess their phytotoxicity on germination and seedling growth of *Portulaca oleracea*, *Corchorus olitorius*, *Brachiaria reptans*, *Euphorbia heterophylla, Dinebra retroflexa, Hibiscus trionum, Amaranthus graecizans, Amaranthus hybridus, Convolvulus arvensis, Setaria pumila* and *E. colona*. Low concentration of CH_2Cl_2 fraction (100 mg L^{-1}) significantly stimulated germination and seedling growth of some test species. There was no significant effect of the low concentration of tricin (50 µmol L^{-1}) on germination and elongation of roots and shoots of species. Higher concentrations of CH_2Cl_2 fraction (500, 1000 mg L^{-1}) and tricin (100, 200 µmol L^{-1}) inhibited germination and seedling growth of most species. The results suggest that CH_2Cl_2 fraction and tricin might be potentially useful as bioherbicide for weed control in agriculture.

Additional key words: allelochemicals; bioherbicide; tricin; weed control.

Resumen

Efectos fitotóxicos de extractos de *Echinochloa colona* (L.) Link. (Poaceae) sobre la germinación y el crecimiento de plántulas de malas hierbas

En este trabajo se estudia el efecto fitotóxico de la planta *Echinochloa colona* para explorar su potencial como herbicida natural. Se evaluaron los efectos fitotóxicos de la fracción más activa y del compuesto más puro del extracto de metanol de tallos de *E. colona* sobre la germinación de semillas y el crecimiento de plántulas de diez malas hierbas incluyendo *E. colona*. El ensayo biológico en semillas de lechuga indicó que la fracción soluble del diclorometano (CH_2Cl_2) mostró la actividad fitotóxica más importante. Se aisló e identificó el componente activo más fitotóxico del CH_2Cl_2, que fue la tricina. La fitotoxicidad de estos compuestos se analizó con tres concentraciones distintas de CH_2Cl_2 (100, 500 y 1000 mg L^{-1}) y tres de tricina (50, 100, 200 µmol L^{-1}) sobre la germinación de semillas y el crecimiento de plántulas de las especies *Portulaca oleracea*, *Corchorus olitorius*, *Brachiaria reptans*, *Euphorbia heterophylla, Dinebra retroflexa, Hibiscus trionum, Amaranthus graecizans, Amaranthus hybridus, Convolvulus arvensis, Setaria pumila*, y también *E. colona*. La concentración 100 mg L^{-1} de CH_2Cl_2 estimuló significativamente la germinación de semillas y el crecimiento de plántulas de algunas de las especies de estudio. No hubo ningún efecto significativo de la concentración 50 µmol L^{-1} de tricina sobre la germinación de semillas o la elongación de raíces y tallos de las especies. Concentraciones más altas de CH_2Cl_2 (500, 1000 mg L^{-1}) y tricina (100, 200 µmol L^{-1}) inhibieron la germinación de semillas y el crecimiento de plántulas de la mayoría de las especies. Los resultados sugieren que la fracción soluble del CH_2Cl_2 y la tricina podrían ser potencialmente empleadas como herbicidas para el control de malas hierbas en las prácticas agrícolas.

Palabras clave adicionales: compuestos aleloquímicos; control de malas hierbas; herbicidas biológicos; tricina.

*Corresponding author: nhgomaa@yahoo.com
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Abbreviations used: HPLC (high performance liquid chromatography); NMR (nuclear magnetic resonance); ODS (octadecylsilane); RGR (relative growth rate); TLC (thin layer chromatography).

Introduction

Allelopathy is any direct or indirect, harmful or beneficial effect that a plant or microorganism exerts on another plant by the release of chemical compounds into the environment (Rice, 1984). These allelochemicals are secondary metabolism products produced by plants. Such natural compounds have the potential to be exploited as antibacterial agents, fungicides, insecticides and herbicides or as leads for discovery of new derivatives (Duke *et al.,* 2000; Copping & Duke, 2007).

Weeds affect crops by competing with them for different environmental resources, as well as by the release of allelochemicals. When these effects occur concomitantly, the harm caused by weeds becomes even greater. Losses in crop yield and production caused by weeds are well documented in many studies (*e.g.,* Akobundu, 1987; Swanton *et al.*, 1993). The application of synthetic herbicides reduces the amount of human labor necessary for hand weeding and controls weeds not economically or feasibly controlled by other methods. Moreover, they allow greater flexibility in the choice of management systems. Despite the benefits of commercial herbicides, the negative impacts of their use in relation to environmental contamination make it necessary to diversify the weed management options (Putnam, 1988; Weston, 1996). A large number of allelochemicals including phenolics, sequiterpenes, flavonoids, terpenes, alkaloids that exhibit strong phytotoxic activity (Qasem & Foy, 2001) have been identified in various weeds. Some allelochemicals from weeds have been extracted, purified and applied as bioherbicides, such as parthenin from *Parthenium hysterophorus* L. (Batish *et al.*, 1997) and artemisinin from *Artemisia* sp. (Dayan *et al.*, 1999; Duke *et al.*, 2000). Studies on weeds are important in order to explore weed species with phytotoxic potential. These weeds could play effective roles for weed control instead of chemical herbicides application (Fujii, 2001), thereby reducing the risk of environmental toxicity due to application of chemical herbicides.

Echinochloa colona (L.) Link. is an annual weed belonging to the family Poaceae. It has become one of the world's most serious grass weeds (Holm *et al.*, 1991; Chauhan & Johnson, 2009). It is a major weed in many crops, including rice, corn, sorghum, sugarcane, cotton, peanut, and cassava (Holm *et al.*, 1991). Losses in crop yield production due to *E. colona* have been reported in several studies (Chander *et al.*, 2008). This weed is also an alternate host of diseases, insects, and nematodes (Holm *et al.*, 1991). *E. colona* is widespread throughout different habitat types and is a dominant species of weed communities of summer crops and orchards in Egypt (Shaltout *et al.*, 1992; Hegazy *et al.*, 2004). It is characterized by a high relative growth rate (RGR) together with a high dry matter investment into leaves, during seedling and juvenile stages. This promotes the competitive ability of the species and may ensure a resource turnover from vegetative to reproductive structures later in the plant life cycle (Hegazy *et al.*, 2005). *E. colona* begins to produce flower buds early in its lifespan, a behavior that ensures some seed production even in years with a short growing season (Hegazy *et al.*, 2005). Though *E. colona* is one of the most serious grass weeds, its phytotoxic potential has received little attention. Swain *et al.* (2008) evaluated the allelopathic potential of *E. colona* leachates on rice. They pointed out that rice root growth was completely inhibited with 10% w/v leachates of 60 days old plant, and that the decomposing and decomposed leachates reduced rice shoot growth by 57% and 84%, respectively.

The objectives of our study were: i) to determine the most phytotoxic active organic solvent fraction of MeOH extract of *E. colona* shoots; ii) to isolate and identify the most phytotoxic active compound in that fraction; and iii) to analyze the phytotoxic effects of the most active fraction and the isolated compound on the germination and seedling growth of ten weed species and of *E. colona* itself.

Material and methods

Vegetation composition of *E. colona* communities

The weed vegetation was sampled in some 20 stands representing maize (*Zea mays* L.) and cotton (*Gossypium barbadense* L.) fields where *E. colona* grew during August 2009 in Minya governorate, Egypt. Minya governorate (27°30' N–28°36' N, 30°23' E–31°09' E; 47 m a.s.l) lies about 245 km south of Cairo. It is an important agricultural region in Egypt. The soil of the cultivated land is fine-grained and very fertile, because it is composed of natural minerals and organic material. The governorate is characterized by dry climate. The average annual rainfall is 5.3 mm.

The area of the stand was 20 × 20 m. In each stand, the present species were recorded and their cover was

evaluated visually as percentage of the ground surface in 10 randomly sampled quadrats (1 × 1 m each). Species identification and nomenclature followed Boulos & El-Hadidi (1994) and Boulos (2009). The species composition of *E. colona* communities are presented in Table 1. Weed communities in most of the surveyed stands were dominated by *E. colona*. Codominant species were *Portulaca oleracea, Corchorus olitorius* and *Brachiaria reptans*. The common associated species were represented by *Euphorbia heterophylla, Dinebra retroflexa, Hibiscus trionum, Amaranthus lividus, Amaranthus hybridus, Sida alba,* and *Convolvulus arvensis.*

Collection of plant material

Shoots of *E. colona* were collected at the flowering stage from a maize field, dried at room temperature for two weeks and then ground to fine powder (2 mm size) using a grinder. Seeds of ten weed species (*P. oleracea*, *C. olitorius*, *B.reptans*, *E. heterophylla, D. retroflexa, H. trionum, Amaranthus graecizans, A. hybridus, C. arvensis,* and *Setaria pumila*) representing the different abundance categories in *E. colona* communities as well as seeds of *E. colona* were collected from cotton and maize fields in Minya governorate during August and September 2009. The collected seeds were air-dried at room temperature for one week. These weed species were used as test species to determine the phytotoxic potential of *E. colona*.

Extraction

Dried shoots of *E. colona* (2 kg) were extracted by soaking with 10 L of a high polar solvent (methanol) for recovering most of plant secondary metabolites like polyphenols and antioxidants (Peschel *et al.*, 2006; Bushra *et al.*, 2009). Methanol (MeOH) extract showed the highest activity compared to other used solvents (ethanol and ethyl acetate) according to our preliminary test (data not shown). MeOH extract was then dried using rotary evaporator (SENCO, Shanghai Senco Technology Company, Shanghai, China) at 45°C and reduced pressure. Using soxhlet apparatus (Linuo Group Co., Ltd., No 38, Shandong, China), the dried extract (117.4 g) was separated into four groups using gradual solvent extraction method (from low to high

Table 1. Mean cover values of the species constituting *E. colona* communities in 20 surveyed stands

Species	Family	Cover (%)
Amaranthus graecizans L.	Amaranthaceae	0.07
Amaranthus hybridus L.	Amaranthaceae	0.80
Amaranthus lividus L.	Amaranthaceae	0.80
Brachiaria eruciformis (Sm.) Griseb.	Poaceae	0.07
Brachiaria reptans (L.) C.A. Gardner & C.E.Hubb.	Poaceae	4.60
Cenchrus biflorus Roxb.	Poaceae	0.01
Convolvulus arvensis L.	Convolvulaceae	0.60
Corchorus olitorius L.	Tiliaceae	3.70
Cynodon dactylon (L.) Pers.	Poaceae	1.70
Cyperus rotundus L.	Cyperaceae	0.08
Dactyloctenium aegyptium (L.) Willd.	Poaceae	0.01
Digitaria sanguinalis (L.) Scop.	Poaceae	0.01
Dinebra retroflexa (Vahl.) Panz.	Poaceae	0.09
Echinochloa colona (L.) Link.	Poaceae	10.70
Eragrostis cilianensis (All.) F. T. Hubb.	Poaceae	0.01
Euphorbia heterophylla L.	Euphorbiaceae	1.80
Euphorbia prostrata Aiton	Euphorbiaceae	0.05
Hibiscus trionum L.	Malvaceae	0.50
Panicum repens L.	Poaceae	0.03
Paspalum distichum L.	Poaceae	0.01
Portulaca oleracea L.	Portulacaceae	7.30
Sesbania sesban (L.) Merr.	Fabaceae	0.02
Setaria pumila (Poir.) Roem. & Schult.	Poaceae	0.02
Setaria verticillata (L.) P. Beauv.	Poaceae	0.01
Sida alba L.	Malvaceae	0.50

polarity): 1 L of heptane (F1, 21.2 g, 1.06% of dry weight), dichloromethane (CH_2Cl_2) (F2, 47.5 g, 2.37% of dry weight), n-butanol (n-BuOH) (F3, 18.9 g, 0.95% of dry weight) and 70% ethanol (F4, 28.6 g, 1.43% of dry weight).

Lettuce seed bioassay

Lettuce (*Lactuca sativa* L.) seeds were used to test germination response to different concentrations of fractions and sub-fractions collected during column chromatography. A very little amount of dimethyl sulfoxide (DMSO) was used to redissolve the dry fractions and sub-fractions then diluted by distilled water to five concentrations as 25, 50, 100, 200 and 400 mg L^{-1}. Seeds were surface sterilized with 0.1% mercuric chloride for 5 min, then washed three times in distilled water. Three replicates, each of 50 seeds, were prepared for each treatment using sterile Petri dishes (4 cm) lined with one sterile filter paper (Whatman, No. 1). Five milliliters of test solutions were added to each Petri dish. The control groups were treated with 5 mL of 1% DMSO. Petri dishes were then placed in an unilluminated incubator adjusted at 25°C (NO: G150, Biotech Co. for Medical & Laboratory Equipments, Cairo, Egypt). After five days, the seedling length was measured and the sensitivity of the lettuce seeds test indicated which of the fractions and sub-fractions were active.

Isolation, purification and identification of the most active pure compound

Results of lettuce bioassay showed that the CH_2Cl_2 fraction (F2) which got most of MeOH extract compounds exhibited the highest phytotoxic activity and it was selected for further analysis. Further separation of fraction on a silica gel column chromatography (Merck KGaA, Darmstadt, Germany) was carried out using a gradient of acetone: ethanol (30:1 to 5:1) as a mobile phase depending upon thin layer chromatography (TLC) analysis (Merck KGaA, Darmstadt, Germany) to obtain 12 sub-fractions. Actually we got most of compounds in F2 within the early sub-fractions (F2-1 to F2-5) as we started with high polar solvent mixture and the rest sub-fractions were of low weight. The sub-fractions were subjected to another bioassay for lettuce seeds germination to determine the most active sub-fraction (F2-9, 2.57 g, 0.128% of dry weight) which was then concentrated under pressure using rotary evaporator. The concentrated sub-fraction (F2-9) was eluted on Sephadex LH-20 chromatography (Merck KGaA, Darmstadt, Germany) by using CH_2Cl_2: chloroform (20:1 to 10:1), and then the elute was divided into other four sub-fractions (F2-9-1 to F2-9-4) that represented 0.017, 0.021, 0.027, 0.062 % of dry weight, respectively. The sub-fraction F2-9-4 was the most active in comparison with the rest sub-fractions which then concentrated under reduced pressure and purified by high performance liquid chromatography (HPLC) with octadecylsilane (ODS) column to yield a pure compound. The isolated pure compound was identified based on spectroscopic analyses (^{1}H and ^{13}C nuclear magnetic resonance, NMR) and comparison of obtained data with previous literature values of Watanabe (1999), Stochmal *et al.* (2001) and Kuwabara *et al.* (2003).

HPLC analysis of free phenolic compounds in CH_2Cl_2 fraction

After drying, the residue of most active fraction was dissolved in HPLC grade MeOH to give 1000 mg L^{-1} and then 20 µL of methanol-dissolved sample was injected into HPLC system (Shimadzu class, Shimadzu Corporation, Kyoto, Japan). HPLC system consisted of diode-array detector and column Lichrosorb Si-60, 7 µm, 3 × 150 mm. Mobile phase consisted of water/acetonitrile, Linear Gradient from 5 to 100% in 40 min. The analysis was based on the comparison of the retention time of 25 standard pure phenolic compounds (Sigma-Aldrich Laborchemikalien, Seelze, Germany) with those in the plant samples detected at 254 nm. Eight compounds were identified by peak area measurement relative to the standard peak area (coumarin, resorcinol, apigenin and cinnamic, syringic, chlorogenic, ferulic, protocatechuic acids).

Effects of the most active fraction and pure compound on the seed germination and the seedling growth of test species

The most active fraction against lettuce was dissolved in a very little amount of DMSO and then diluted with distilled water to prepare the test solutions as 100, 500 and 1000 mg L^{-1}. A 200 µmol L^{-1} solution of the pure compound was made up in 1% DMSO and serially diluted with distilled water to obtain 100 and 50 µmol L^{-1}

solutions. Distilled water containing 1% DMSO was used as the control. Before the experiment was carried out, the seeds of the test weeds were put into a solution of H_2SO_4 (90%) for 2 min to loosen the seed coat. The seeds were then cleaned several times with distilled water and then air-dried. Twenty five seeds of each test species were placed in separate Petri dishes that contained filter paper (Whatman, number 1) wetted with 10 mL of CH_2Cl_2 fraction or the pure compound. The Petri dishes were placed in an unilluminated incubator at 28°C (NO: G150, Biotech Co. for Medical & Laboratory Equipments, Cairo, Egypt). The percentage of germination and the length of seedling root and shoot of the test species were measured after ten days.

Statistical analysis

Germination and seedling growth bioassays were conducted with three replications. The data were subjected to one-way analysis of variance, and treatment means were compared to the control at $p < 0.05$ by Tukey's post-hoc test. Statistical analysis was done with SPSS 11.1 for Windows statistical software package (SPSS, Chicago, IL, USA).

Results

Identification of the most active isolated pure compound and HPLC analysis for free phenolic compounds in CH_2Cl_2 fraction

Lettuce seed preliminary bioassay results (data not shown) indicated that CH_2Cl_2 fraction and its two sub-fractions F2-9 and F2-9-4 had the highest phytotoxic activity. The sub-fraction F2-9-4 was purified using HPLC with ODS column to yield the pure compound (95.3 mg, 0.005% of dry weight).

According to the spectroscopic analyses of the pure compound [^{1}H NMR (DMSO-d6) δ; 3.88 (6H, s, CH3O- × 2), 6.20 (1H, d, J = 2.0 Hz, H-6), 6.55 (1 H, d, J = 2.0 Hz, H-8), 6.93 (1H, s, H-3), 7.31 (2H, s, H-2' and 6') and ^{13}C NMR (DMSO-d6) δ; 56.4 (6H, s, CH3O- × 2), 94.1 (C-8), 98.3 (C-6), 103.9 (C-3), 105.6 (C-10), 104.6 (2C, C-2' and 6'), 120.2 (C-1'), 139.9 (C-4'), 148.1 (2C, C-3' and 5'), 156.9 (C-9), 157.7 (C-5), 163.6 (C-2), 164.2 (C-7), 182.1 (C-4)] and by comparing spectrometry analyses results with previous literature values of Watanabe (1999), Stochmal *et al.* (2001) and Kuwabara *et al.* (2003), the compound is tricin (5,7,4'-trihydroxy-3',5'-dimethoxyflavone).

Eight phenolic compounds were identified in the CH_2Cl_2 fraction. Cinnamic acid was recorded as the major component followed by coumarin and resorcinol (32.1, 19.3 and 17.3% of the total concentration of the determined free phenols, respectively). Moreover, chlorogenic acid, apigenin, syringic acid, ferulic acid and protocatechuic acid were identified in smaller amounts (Table 2).

Effects of the CH_2Cl_2 fraction and tricin on the seed germination and the seedling growth of the test species

The effects of CH_2Cl_2 fraction on the germination and seedling growth of the test weed species are shown in Fig. 1. The lowest concentration of CH_2Cl_2 fraction (100 mg L^{-1}) significantly ($p < 0.05$) stimulated germi-

Table 2. Quantitative determination of phenolic compounds present in CH_2Cl_2 fraction of *E. colona* MeOH extract using HPLC analysis

Standard phenolic compounds	Retention time (min)		Concentration (µg g^{-1} dry weight)
	Standard	Sample	
Protocatechuic acid	12.736	12.577	8.063
Resorcinol	13.739	13.923	33.828
Chlorogenic acid	16.331	16.523	17.243
Syringic acid	18.379	18.263	12.530
Coumarin	22.208	21.322	37.650
Ferulic acid	24.853	24.732	9.645
Cinnamic acid	36.149	36.214	62.632
Apigenin	37.984	37.951	13.730
Total			195.258

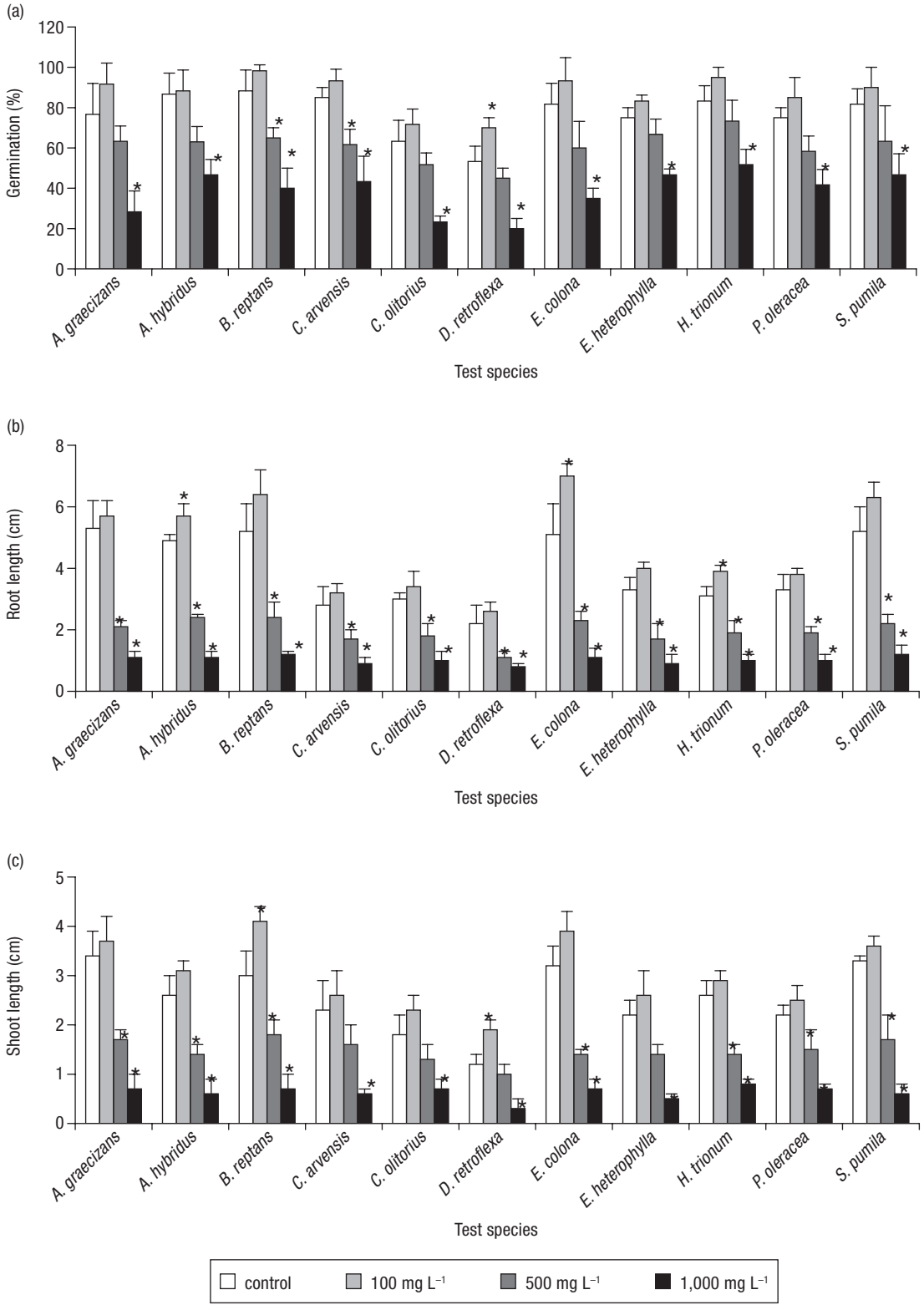

Figure 1. Effects of the CH_2Cl_2 fraction of *E. colona* MeOH extract on (a) germination, (b) root length and (c) shoot length of weed species. Vertical bars are standard deviations from the mean. *: significant differences at $p < 0.05$.

nation of *D. retroflexa*, root elongation of *A. hybridus*, *E. colona* and *H. trionum* and shoot length of *B. reptans* and *D. retroflexa* (31.3, 16.3, 37.3, 25.8, 36.6 and 58.3% stimulation compared with control, respectively). The germination of *B. reptans* and *C. arvensis* was significantly ($p < 0.05$) reduced by 26.4 and 27.4%, respectively in response to the application of 500 mg L^{-1} of CH_2Cl_2 fraction. This concentration also significantly inhibited ($p < 0.05$) the root length of all species and caused significant inhibition ($p < 0.05$) to shoot length of seven of the test species (*A. graecizans*, *A. hybridus*, *B. reptans*, *E. colona*, *H. trionum*, *P. oleracea*, and *S. pumila*). The highest applied concentration of fraction (1000 mg L^{-1}) significantly reduced ($p < 0.05$) germination, root and shoot length of all species. The highest degrees of inhibition were reported for germination of *C. olitorius* (63.2%), *A. graecizans* (63.1%) and *D. retroflexa* (62.5%), root length of *A. graecizans* (79.2%) and *E. colona* (78.4%) and shoot length of *S. pumila* (81.8%) and *A. graecizans* (79.4%) in comparison with control.

There was no significant effect of tricin at 50 µmol L^{-1} on germination and the elongation of roots and shoots of test species. Application of 100 µmol L^{-1} significantly ($p < 0.05$) reduced germination of *A. graecizans* (6.5% compared to control). The same concentration significantly ($p < 0.05$) inhibited root elongation of all test species except for *D. retroflexa*. The shoot length of four species (*A. graecizans*, *E. colona*, *H. trionum* and *S. pumila*) of the 11 test weeds was significantly ($p < 0.05$) reduced (44.1, 43.7, 42.3 and 39.4%, respectively) when treated with 100 µmol L^{-1} tricin. Except for *C. arvensis* and *P. oleracea*, germination of all species was significantly ($p < 0.05$) reduced at 200 µmol L^{-1} tricin and the greatest suppressions were reported for *A. graecizans* (52.2%) and *D. retroflexa* (49.9%). The root and shoot elongation of all species was significantly ($p < 0.05$) inhibited in response to treatment with 200 µmol L^{-1} tricin and the highest root inhibition values were recorded for *S. pumila* (76.9%) and *A. hybridus* (71.4%), while the greatest shoot inhibitions were exhibited by *S. pumila* (81.8%) and *E. heterophylla* (72.7%) (Fig. 2).

Discussion

The results indicated that the phytotoxic effects of *E. colona* on the test species are concentration–dependent. Our results agree with the findings of other studies which indicated that lower concentrations of some phytotoxic compounds can stimulate plant growth, while higher concentrations cause inhibition (Ghareib *et al.*, 2010; Ma *et al.*, 2011). This can be attributed to the fact that low dose of phenolic compounds stimulate protein synthesis and activation of antioxidant enzymes (Baziramakenga *et al.*, 1995) which are effective in plant protection (Kleiner *et al.*, 1999), while high levels of phenolic application result in plant damage (Politycka *et al.*, 2004). Under field conditions *E. colona* may stimulate or inhibit other plants depending on its density and cover. The phytotoxic effect of both CH_2Cl_2 fraction and tricin is also weed species-dependent, *i.e.,* the test species showed different responses to both CH_2Cl_2 fraction and tricin indicating different tolerance of the weed species. Several researchers have also reported differences between weeds in their tolerance to phytotoxic effects (*e.g.,* Batish *et al.*, 2004). Root growth is the most sensitive variable followed by shoot growth then seed germination. These results are supported by the findings of Meksawat & Pornprom (2010) who have reported that root length was the most reliable response variable because it had a high sensitivity to allelochemicals.

Tricin is a flavonoid compound that occurs in its glycosidic form in rice bran and other grass species such as wheat, barley and maize (Cai *et al.*, 2005). The findings of the present study prove the phytotoxic activity of tricin on the test weed species. Flavonoids could be associated with plant resistance to weed, where previous studies revealed the higher flavoniods contents of weed resistant varieties of rice than those of sensitive variety (Grayer *et al.*, 1994; Stevenson *et al.*, 1996). Kong *et al.* (2004) reported that tricin isolated from leaves of allelopathic rice accession PI 312777 significantly inhibited the growth of weeds like *Echinochloa crus-galli*, *Cyperus difformis* and *Cyperus iris*, and the spore germination of fungal pathogens such as *Pyricularia oryzae* and *Rhizoctonia solani*.

The phytotoxic effects of the CH_2Cl_2 fraction were stronger than those of tricin. This can be related to the fact that the fraction contains phenolic compounds (as indicated by the HPLC analysis) in addition to tricin. These phenolics may contribute to the phytotoxic activity of the fraction. Phenolic compounds have been identified as phytotoxic agents (Reigosa *et al.*, 1999; Ghareib *et al.*, 2010). The findings of the present study suggest the potential of *E. colona* as bioherbicide. Several studies have documented the potentiality of plant products as bioherbicides. Macías *et al.* (2000)

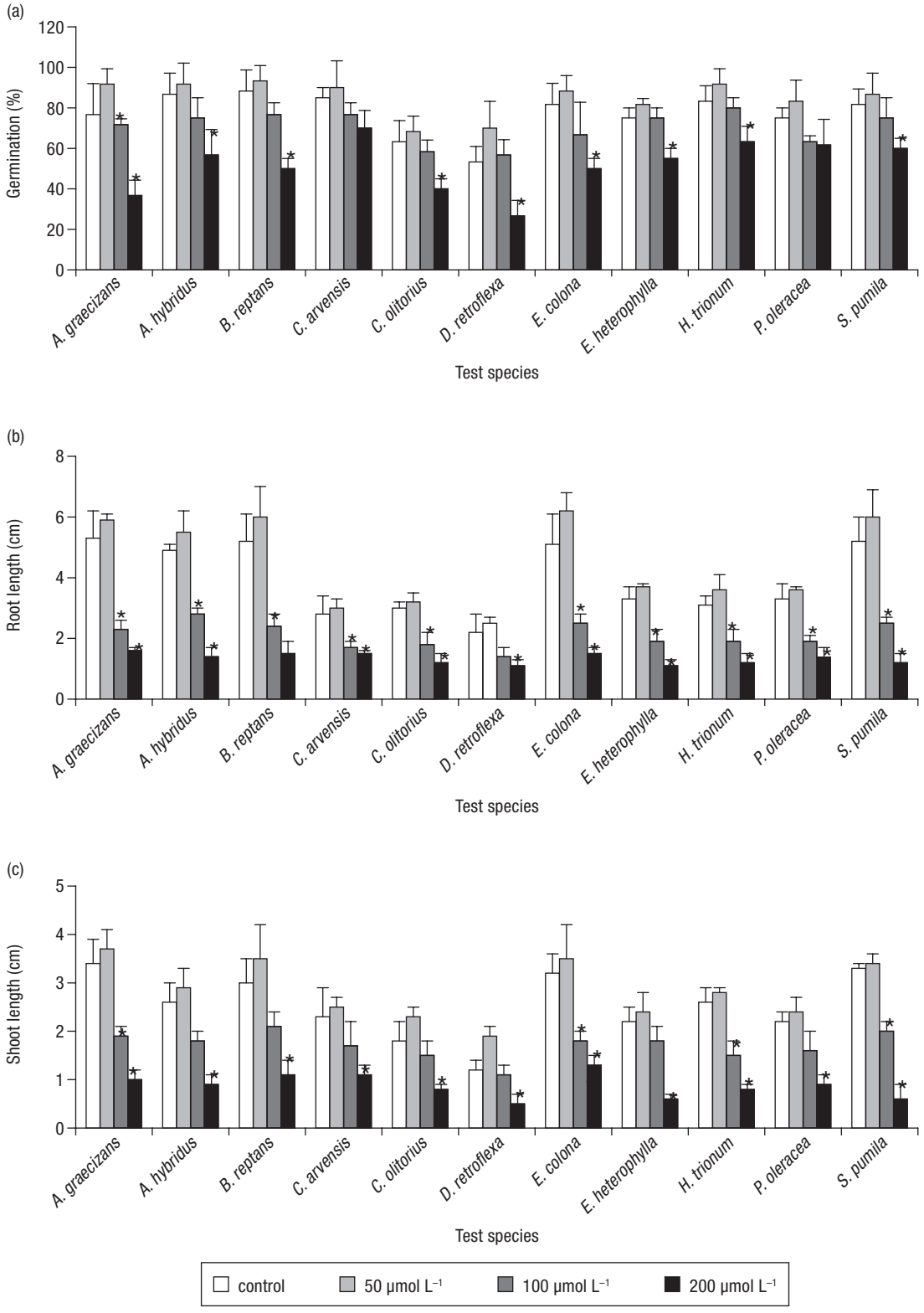

Figure 2. Effects of tricin on (a) germination, (b) root length and (c) shoot length of weed species. Vertical bars are standard deviations from the mean. *: significant difference at $p < 0.05$.

suggest that the natural product dehydrozaluzanin C obtained from different weeds of the Compositae family exhibits phytotoxic activity levels which are comparable (or even higher) with those of the commercial herbicide Logran®. In the same context, confertiorin isolated from *Ambrosia confertifora* has been reported to inhibit the germination of *Amaranthus palmeri* and *Amaranthus retrofexus* (Fischer & Mabry, 1985). Moreover, Khanh *et al.* (2006) proved that *Stylosanthes guianensis* showed significant herbicidal effects against *Echinochloa crus-galli* and *Monochoria vaginalis*. They attributed its phytotoxicity to the presence of several allelochemicals in *S. guianensis* including phenolic acids, coumarin and long-chain fatty acids.

The wide distribution and the dominancy of *E. colona* over other weed species which reported in other studies (Shaltout *et al.*, 1992; Hegazy *et al.*, 2004) and also in this study may be attributed to its phytotoxic stress on other weed species which documented in the present study. El-Khatib (2000) pointed out that allelopathic plants tend to form pure dense patches with relatively few other species growing within their vicinity.

Conclusively, the CH_2Cl_2 fraction of *E. colona* MeOH extract and the isolated compound tricin have significant phytotoxic effects on the germination and seedling growth of weeds. So, they might be potentially useful as bioherbicide for weed control in agriculture which should be investigated further in the field for their practical application.

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