



Full Length Article

Herbicidal Potential of *Eichhornia crassipes* Leaf Extract against *Mimosa pigra* and *Vigna radiata*

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Abstract

Despite reports of phytotoxicity of water hyacinth (*Eichhornia crassipes*) extract, little is known about its effects on weed species. Similarly, current understanding of the mode of action of water hyacinth extract as a bioherbicidal agent is limited. In this study, we assessed the effects of water hyacinth leaf extract on the germination, growth and several biochemical parameters of *Mimosa pigra*, an invasive weed. *Vigna radiata*, a crop species, was also tested for comparison. Control studies were conducted to separate the effects of extract pH and osmolarity on the parameters measured. Water hyacinth extract reduced the total percentage and speed of germination of *M. pigra* but not *V. radiata*. Root length and fresh weight were consistently compromised in the non-pregerminated and pregerminated seedlings of *M. pigra* and *V. radiata*. Water hyacinth extract induced similar biochemical responses in the root tissues of non-pregerminated and pregerminated seedlings of both test species. Hydrogen peroxide content and cell wall-bound peroxidase activity in the root tissues were increased, whereas soluble peroxidase activity was inhibited in both test species. Malondialdehyde content decreased in the root tissues of *V. radiata* but showed no significant changes in *M. pigra*. Overall, our study demonstrated the bioherbicidal activity of water hyacinth extract against *M. pigra* and *V. radiata*. In addition, the inhibition of root growth by water hyacinth extract may be mediated by enhanced cell wall-bound peroxidase activity and hydrogen peroxide accumulation in root tissues. © 2013 Friends Science Publishers

Keywords: Allelopathy; Biochemical change; *Eichhornia crassipes*; Germination; Growth; *Mimosa pigra*; *Vigna radiata*

Introduction

Allelopathy is a phenomenon in which secondary metabolites produced by plants, algae and microorganisms stimulate or inhibit the growth and development of biological systems (Rice, 1984; Cheema *et al.*, 2012). Since the introduction of the concept of allelopathy for weed management (Cheema *et al.*, 1988), interest in the use of allelopathic natural products as bioherbicides has continued to grow. Fuelling such growing interest is the recognition that the application of allelopathy for weed control would incur minimal environmental impacts (Khanh *et al.*, 2005; De Albuquerque *et al.*, 2011; Farooq *et al.*, 2011).

Water hyacinth (*Eichhornia crassipes*) is an allelopathic aquatic plant (Gross, 2003; Xie *et al.*, 2010) which has gained notoriety as an invasive weed worldwide (Villamagna and Murphy, 2010). Despite current interest in tapping into this plant as a bioresource with multiple applications (Patel, 2012), there has been little progress in exploiting the plant as a source of bioherbicidal agent.

At present, little is known about the effects of water hyacinth extract on the germination behaviour and early

growth of terrestrial weed species. Current evidence of the phytotoxicity of water hyacinth on terrestrial plants is largely derived from germination bioassays of crop species, such as rice, lentil, chickpea (Paul and Sultana, 2004), pearl millet (Kumar *et al.*, 2010), radish (Ahmed *et al.*, 1982), turnip, and bean (Anaya *et al.*, 1992). In general, these studies found seed germination and post-germination growth of crop species to be inhibited by water hyacinth extract. These investigations, nevertheless, did not carry out control studies to separate potential effects of extract pH and osmolarity from the effects of extract toxicity. Osmotic potential and pH of plant extracts may affect seed germination and seedling growth, hence their effects on the test plants should be evaluated (Sampietro *et al.*, 2009b).

The biochemical basis of the phytotoxicity of water hyacinth on terrestrial plant species is not well-understood. Phytochemical analysis revealed phenolic compounds to be the major group of secondary metabolites in water hyacinth, followed by terpenoids and alkaloids (Shanab *et al.*, 2010). Naringenin, a phytotoxic flavonoid (Bido *et al.*, 2010), is a key phenolic compound in water hyacinth (Chantiratikul *et al.*, 2009). Phenolic compounds are the most common class

of allelochemicals. Their modes of action are diverse, which include disruptions of membrane function, hormonal balance, respiration and photosynthesis, as well as induction of oxidative damage (Einhellig, 2004; Weir *et al.*, 2004; Li *et al.*, 2010; John and Sarada, 2012). Currently, the mechanism of action of the phytotoxicity of water hyacinth is unclear. However, the inhibition of soluble peroxidase in soybean roots by exogenously supplied naringenin (Bido *et al.*, 2010) as well as the ubiquity of oxidative stress induction by allelochemicals (Weir *et al.*, 2004) led us to speculate that the phytotoxicity of water hyacinth may be mediated by cellular oxidative damage.

In this study, we had two hypotheses. First, we hypothesised that water hyacinth leaf extract is phytotoxic to weed species *Mimosa pigra*. Second, we hypothesised that the phytotoxicity of water hyacinth extract is mediated by oxidative stress induction. To test our first hypothesis, we evaluated the effects of aqueous extract of water hyacinth leaves on the germination and growth of *M. pigra*. *M. pigra* is an invasive weed in Australia, Asia and Africa (Paynter and Flanagan, 2004; Shanungu, 2009; Mansor and Crawley, 2011). For comparison, we also assessed the effects of the same extract on *Vigna radiata*, a crop species. To test our second hypothesis, we assessed hydrogen peroxide and malondialdehyde contents as well as peroxidase activities in the roots of extract-treated seedlings. To our knowledge, this is the first study which reports the phytotoxicity of water hyacinth extract on an invasive weed and the biochemical basis of the toxic effects.

Materials and Methods

Plant Materials

Water hyacinth plants were collected from a lake in Temoh, a rural town situated about 8 km south of the university campus. Seeds of *M. pigra* were collected from a natural population on the banks of Kinta River, Tanjung Tualang, Malaysia. Seeds of *V. radiata* were purchased from a local market.

Preparation of Water Hyacinth Leaf Extract

Healthy mature leaves of similar sizes were excised from water hyacinth plants, rinsed, blotted dry and oven-dried at 45°C for 72 h. The dried leaves were then ground into powder. Leaf extract was prepared by soaking 5 g of powder in 100 mL of autoclaved deionised water for 48 h at 25°C in a shaking incubator. The mixture was then filtered through two layers of cheesecloth. The filtrate was clarified by centrifugation at 9000 rpm and 4°C for 15 min. The supernatant, designated as a 5% extract, was kept at -20°C in darkness until used. Extracts of lower concentrations (0.5, 1 and 2.5%) were prepared by diluting the 5% extract with appropriate volumes of autoclaved deionised water.

Preliminary Determination of Extract pH and Osmotic Potential

The pH of leaf extracts of different concentrations was determined using a calibrated pH meter. The osmotic potentials of the extracts were determined based on electrical conductivity (EC) measurements and calculated from the relation of osmotic potential (MPa) = $-0.036 \times \text{EC}$, with EC in dS m^{-1} (Bingham *et al.*, 1987; Raviv and Blom, 2001). EC of the extracts was measured using a calibrated conductivity meter (Oakton Instruments, Vernon Hills, Illinois, USA).

The mean pH values of 0.5, 1, 2.5, and 5% water hyacinth leaf extracts were 5.60, 5.55, 5.50 and 5.00 based on triplicate measurements. The mean osmotic potentials of the same extracts were -0.02, -0.04, -0.08 and -0.16 MPa based on triplicate measurements. Using the information obtained, control studies were conducted to evaluate the influence of extract pH and osmolarity on the germination, growth and biochemical parameters of *M. pigra* and *V. radiata* as described below.

Experiment 1: Seed Germination

The seeds were germinated in Petri dishes, each occupied by either 50 *M. pigra* seeds or 10 *V. radiata* seeds to ensure similar ratios of seed weight to solution volume. Seeds of *M. pigra* were first surface-sterilised with 5% (v/v) Clorox for 30 sec and then rinsed three times with autoclaved distilled water. Next, the seeds were immersed in hot water (80°C) for 3 min to enhance their germinability (Swarbrick and Mercado, 1987; Lonsdale, 1993). The seeds were then transferred to a Petri dish (9 cm diameter) fitted with two layers of filter paper wet with 10 mL of 0.5, 1, 2.5 and 5% extracts. Control seeds were germinated in autoclaved deionised water (0% extract). The Petri dish was sealed with Parafilm and incubated at 25°C in darkness in a thermostatically controlled cabinet. One mL of extract solution of the same concentration was added to the Petri dish every 48 h. Seeds of *V. radiata* were prepared for germination as described above, except that the step of seed immersion in hot water was omitted.

Seeds with an emerging radicle of at least 2 mm long were considered to have germinated (Haugland and Brandsaeter, 1996). Total numbers of germinated seeds were recorded at two-day intervals over six days. These data were used to estimate germination rate using a modification of Timson's index of germination velocity as previously described (Khan and Ungar, 1984). After six days, total percentage of seed germination in each Petri dish was determined.

The effects of extract pH on total germination and germination rate were assessed by germinating the seeds in deionised water adjusted to pH 5, 6 and 7 with 0.1 M NaOH and 0.1 M HCl. This pH range spanned the pH of different concentrations of the extract. Osmotic effects of

the extract on the two germination parameters were evaluated by germinating the seeds in mannitol solutions adjusted to -0.02, -0.04, -0.06, -0.08, or -0.16 MPa, spanning the osmotic potential of different concentrations of the extract. Mannitol concentrations corresponding to the aforementioned osmotic potential values were calculated from data published by Sosa *et al.* (2005). Deionised water (osmotic potential taken as 0.0 MPa) was used as control.

Experiment 2: Growth of Non-pregerminated and Pregerminated Seedlings

Two growth parameters were assessed, namely the length and fresh weight (FW) of the whole seedling, shoot and root of *M. pigra* and *V. radiata* seedlings treated with 0, 2.5 and 5% extracts. Non-pregerminated seedlings were prepared as described in Experiment 1. Hence the growth measurements were made on seedlings that were germinated directly in the presence of the extract. On the other hand, pregerminated seedlings were prepared by first germinating the seeds in autoclaved deionised water for two days. Germinated seeds with about 2 mm radicle protrusion were transferred to new Petri dishes containing extracts of different concentrations and grown for six days as in Experiment 1. The length and FW of the whole seedling, shoot and root of the pregerminated seedlings were then determined.

The effects of extract pH on the length and FW of non-pregerminated and pregerminated seedlings were assessed by replacing water hyacinth extract with deionised water adjusted to pH 7, 5.5 and 5. These pH values corresponded to the pH of 0, 2.5 and 5% extract, respectively. The effects of extract osmolarity on the growth parameters were assessed by replacing water hyacinth extract with mannitol solutions adjusted to 0.00, -0.08, or -0.16 MPa. These osmotic potential values corresponded to the osmolarity of 0, 2.5 and 5% extracts, respectively.

Experiment 3: Biochemical Responses of Non-pregerminated and Pregerminated Seedlings

Non-pregerminated and pregerminated seedlings treated with 0, 2.5 and 5% extracts were prepared as described in Experiment 2. At the end of the six-day growth period, root tissues of the treated seedlings were taken for biochemical analyses. Hydrogen peroxide (H_2O_2) content was determined as described in Velikova *et al.* (2000) and expressed as $nmol\ g^{-1}$ FW. Malondialdehyde (MDA) content was estimated as described in Dhindsa *et al.* (1981) and expressed as $nmol\ g^{-1}$ FW. Assays of soluble and cell wall-bound (CW-) peroxidase (POD) activities were performed as previously described (Lin and Kao, 2000). Peroxidase activities were calculated using the extinction coefficient of $26,600\ M^{-1}\ cm^{-1}$ and expressed in unit mg^{-1} protein. One unit of enzyme was defined as the amount of enzyme required to catalyse the formation of $1\ \mu mol$ of tetraguaiacol per min. The protein content of the

enzyme extract was determined according to Bradford (1976).

The effects of extract pH and osmolarity on the biochemical parameters of non-pregerminated and pregerminated seedlings were assessed by exposing the two test species to pH and osmotic treatments as described in Experiment 2.

Statistical Analysis

Data reported are mean \pm standard errors. Three replicates were performed for each treatment, with each Petri dish representing one replicate. Statistical analysis was performed using Microsoft Office Excel 2003. Data were analysed by the ANOVA test and means of significant differences were compared using Student's T-test at the 0.05 level of probability.

Results

Effects of Extract on Seed Germination

The total percentage of germination and germination rate of *M. pigra* both decreased in an extract concentration-dependent manner (Fig. 1). Total germination of *M. pigra* decreased from 80% to 61% with increasing concentrations of water hyacinth extract. Germination rate of *M. pigra* decreased by up to 42% over the range of extract concentrations tested. By contrast, water hyacinth extract had no effects on the germination behaviour of *V. radiata*. Control studies showed that pH and osmotic treatments had no effects on the total percentage of germination and germination rate of both test species (data not shown).

Effects of Extract on the Growth of Non-pregerminated and Pregerminated Seedlings

Root and shoot growth of non-pregerminated seedlings of *M. pigra* and *V. radiata* were significantly inhibited by water hyacinth extract (Table 1). In both test species, root length was more severely affected than root FW, shoot length and shoot FW. When treated with 5% extract, the root length of non-pregerminated seedlings of *M. pigra* and *V. radiata* was reduced by 63% and 89%, respectively.

Control studies showed that pH treatments had no effects on either the length or FW of non-pregerminated seedlings. Likewise, osmotic treatments of -0.08 and -0.16 MPa, which simulated the 2.5 and 5% extracts, had no effects on the root and shoot length of non-pregerminated *M. pigra* and *V. radiata* seedlings (data not shown). By contrast, the same osmotic treatments reduced the root and shoot FW of non-pregerminated seedlings (Table 2). Hence, the root and shoot FW of non-pregerminated seedlings treated with 2.5 and 5% extracts were adjusted accordingly to eliminate the osmotic effects of the extracts and presented as adjusted values in Table 1.

Table 1: Effects of water hyacinth leaf extract on the growth parameters of non-pregerminated *Mimosa pigra* and *Vigna radiata* seedlings

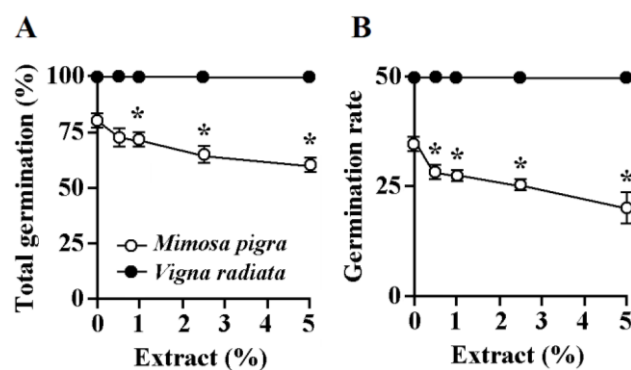
Extract (%)	<i>Mimosa pigra</i>				<i>Vigna radiata</i>			
	Root length (cm)	Shoot length (cm)	Root FW (mg)	Shoot FW (mg)	Root length (cm)	Shoot length (cm)	Root FW (mg)	Shoot FW (mg)
0.0	2.42 ± 0.17	5.99 ± 0.17	16 ± 0	71 ± 1	13.69 ± 0.67	12.05 ± 0.18	100 ± 5	463 ± 8
2.5	1.36 ± 0.08*	3.62 ± 0.20*	13 ± 1*	69 ± 2	3.06 ± 0.30*	9.34 ± 0.43*	83 ± 1*	399 ± 7*
5.0	0.89 ± 0.04*	2.59 ± 0.03*	13 ± 1*	71 ± 0	1.54 ± 0.13*	7.21 ± 0.35*	73 ± 1*	371 ± 13*

Data are means ± standard errors (n=3). Asterisks (*) indicate treatments where the means were significantly different ($P < 0.05$) from the control groups (0% extract) as determined by Student's t-test

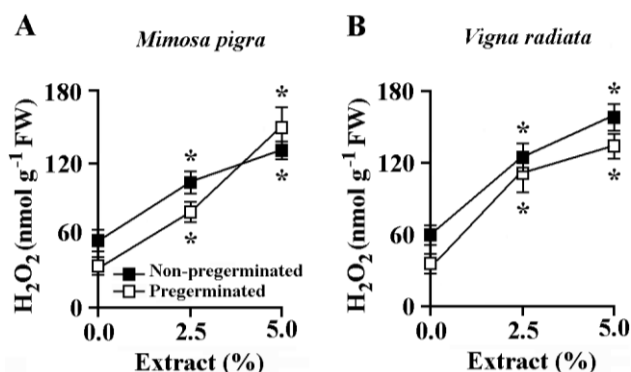
Table 2: Percent inhibition of root and shoot FW of non-pregerminated seedlings of *Mimosa pigra* and *Vigna radiata* subjected to mannitol treatments

Osmotic potential (MPa)	Inhibition in comparison with control value (%)			
	<i>Mimosa pigra</i>		<i>Vigna radiata</i>	
	Root FW	Shoot FW	Root FW	Shoot FW
-0.08	8.7	3.5	9.7	15.6
-0.16	16.7	16.9	20.0	19.4

Data are mean values based on three replicates. Percent inhibition was calculated from the FW of mannitol solution-treated seedlings, which were significantly different ($P < 0.05$) from the FW of water-treated control seedlings as determined by Student's T-test. Mannitol treatments of -0.08 and -0.16 MPa corresponded to the osmotic potential of 2.5 and 5% extracts, respectively

**Fig. 1:** Effects of different concentrations of water hyacinth leaf extract on total germination (A) and germination rate (B) of *Mimosa pigra* and *Vigna radiata*. Data are presented as mean ± standard errors (n = 3). Asterisk (*) indicates significant difference from the control (0% extract) as determined by Student's T-test at $P < 0.05$

Root growth of pregerminated *M. pigra* and *V. radiata* seedlings was significantly inhibited by water hyacinth extract (Table 3). When treated with 5% extract, the root length of *M. pigra* and *V. radiata* was reduced by 50% and 79%, respectively. Meanwhile, the same extract concentration induced a 31% reduction in the root FW of both *M. pigra* and *V. radiata* seedlings. Water hyacinth extract had no statistically significant effects on the shoot growth of *V. radiata*. Conversely, 5% extract induced a 18% reduction in the shoot length of *M. pigra*. Control studies showed that the root and shoot growth of pregerminated seedlings of both test species were unaffected by pH and osmotic treatments (data not shown).

**Fig. 2:** Effects of different concentrations of water hyacinth leaf extract on hydrogen peroxide (H_2O_2) content in the root tissues of non-pregerminated and pregerminated seedlings of *Mimosa pigra* (A) and *Vigna radiata* (B). Data are presented as mean ± standard errors (n = 3). Asterisk (*) indicates significant difference from the control (0% extract) as determined by Student's T-test at $P < 0.05$

Effects of Extract on Biochemical Parameters of Non-Pregerminated and Pregerminated Seedlings

H_2O_2 contents of the root tissues of *M. pigra* and *V. radiata* seedlings significantly increased with increasing concentrations of water hyacinth extract (Fig. 2). Relative to their respective control values, H_2O_2 contents of pregerminated seedlings were enhanced by a greater magnitude compared with non-pregerminated seedlings. Treatment with 5% extract increased the H_2O_2 contents of pregerminated *M. pigra* and *V. radiata* seedlings by 4.3 folds and 3.8 folds, respectively.

MDA contents of the root tissues of *M. pigra* seedlings did not show any statistically significant changes in response to water hyacinth extract (Fig. 3). Conversely, 5% extract reduced the MDA contents of the non-pregerminated and pregerminated seedlings of *V. radiata* by 18% and 44%, respectively.

Soluble POD activities of *M. pigra* and *V. radiata* seedlings showed a declining trend with increasing extract concentrations (Fig. 4). Exposure to 5% extract resulted in 66% and 59% inhibition in the soluble POD activities of non-pregerminated and pregerminated *M. pigra* seedlings, respectively. Less drastic decline in soluble POD activities was detected in the non-pregerminated and pregerminated seedlings of *V. radiata*.

Table 3: Effects of water hyacinth leaf extract on the growth parameters of pregerminated *Mimosa pigra* and *Vigna radiata* seedlings

Extract (%)	<i>Mimosa pigra</i>				<i>Vigna radiata</i>			
	Root length (cm)	Shoot length (cm)	Root FW (mg)	Shoot FW (mg)	Root length (cm)	Shoot length (cm)	Root FW (mg)	Shoot FW (mg)
0.0	2.03 ± 0.08	7.20 ± 0.08	13 ± 1	82 ± 1	12.59 ± 0.68	12.68 ± 0.15	102 ± 2	443 ± 9
2.5	1.54 ± 0.12*	6.90 ± 0.07*	10 ± 0*	80 ± 2	4.79 ± 0.62*	10.85 ± 1.46	86 ± 5*	440 ± 40
5.0	1.02 ± 0.05*	5.92 ± 0.25*	9 ± 1*	78 ± 2	2.64 ± 0.55*	11.94 ± 0.34	70 ± 3*	409 ± 22

Data are means ± standard errors (n=3). Asterisks (*) indicate treatments where the means were significantly different (P<0.05) from the control groups (0% extract) as determined by Student's t-test

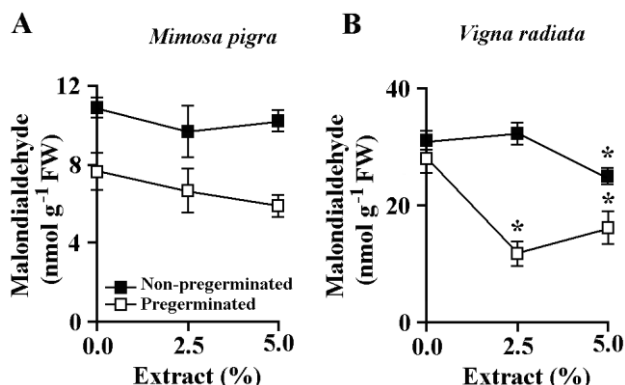


Fig. 3: Effects of different concentrations of water hyacinth leaf extract on malondialdehyde content in the root tissues of non-pregerminated and pregerminated seedlings of *Mimosa pigra* (A) and *Vigna radiata* (B). Data are presented as mean ± standard errors (n = 3). Asterisk (*) indicates significant difference from the control (0% extract) as determined by Student's T-test at P<0.05

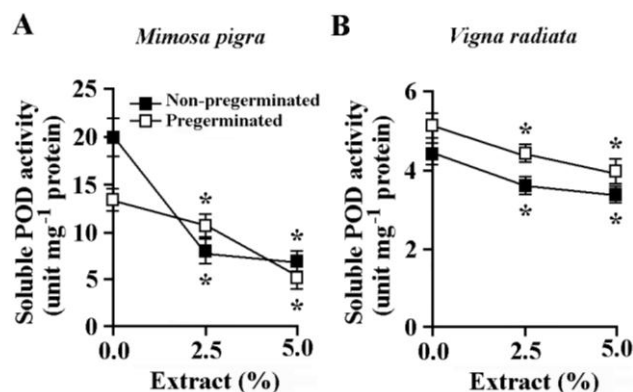


Fig. 4: Effects of different concentrations of water hyacinth leaf extract on the soluble peroxidase (POD) activities in the root tissues of non-pregerminated and pregerminated seedlings of *Mimosa pigra* (A) and *Vigna radiata* (B). Data are presented as mean ± standard errors (n = 3). Asterisk (*) indicates significant difference from the control (0% extract) as determined by Student's T-test at P<0.05

CW-POD activities of the root tissues of *M. pigra* and *V. radiata* seedlings were induced to varying degrees by water hyacinth extract (Fig. 5). In comparison with non-pregerminated seedlings, the pregerminated

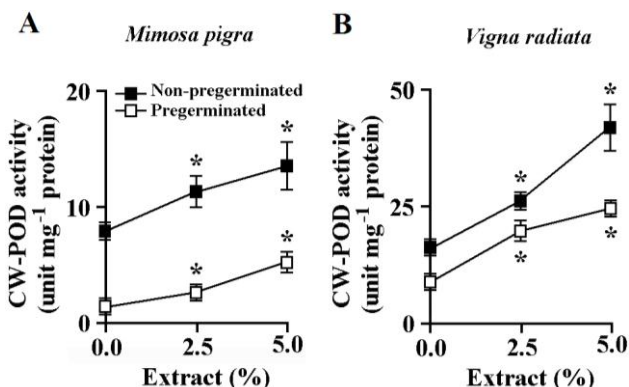


Fig. 5: Effects of different concentrations of water hyacinth leaf extract on the cell wall-bound peroxidase (CW-POD) activities in the root tissues of non-pregerminated and pregerminated seedlings of *Mimosa pigra* (A) and *Vigna radiata* (B). Data are presented as mean ± standard errors (n = 3). Asterisk (*) indicates significant difference from the control (0% extract) as determined by Student's T-test at P<0.05

seedlings of both test species exhibited a greater extent of increase in CW-POD activities relative to the controls. Treatment with 5% extract resulted in 1.7-fold and 4-fold increase in the CW-POD activities of non-pregerminated and pregerminated *M. pigra* seedlings, respectively. The same extract concentration enhanced CW-POD activities of non-pregerminated and pregerminated *V. radiata* seedlings by 2.6 folds and 2.8 folds, respectively. The absolute levels of CW-POD activities of non-pregerminated seedlings, however, were higher compared with those of the pregerminated seedlings.

Our control studies found that pH and osmotic treatments had no statistically significant effects on the H₂O₂ content, soluble POD activity and CW-POD activity of *M. pigra* and *V. radiata*. Similarly, pH and osmotic treatments had no effects on the MDA contents of non-pregerminated seedlings. MDA contents of pregerminated seedlings were largely unaffected by the pH and osmotic treatments. However, -0.16 MPa treatment, which simulated the 5% extract, induced a 60% increase in MDA content in the root tissues of pregerminated *M. pigra* seedlings (data not shown).

Discussion

Compromised root growth of *M. pigra* and *V. radiata* as well as compromised germination of *M. pigra*, collectively, demonstrated the phytotoxicity of water hyacinth. Notably, this is the first report of the susceptibility of invasive weed *M. pigra* to water hyacinth extract.

Water hyacinth extract disrupted the germination behaviour of *M. pigra* but not that of *V. radiata*. This implies that the inhibition of seed germination by water hyacinth extract is target species-dependent. In support of this suggestion is the observation that water hyacinth leaf extract inhibited the germination of rice and lentil, but not that of wheat (Paul and Sultana, 2004). The mechanism of inhibition of *M. pigra* germination by water hyacinth extract is unclear. However, previous studies suggest that inhibition of seed germination by allelopathic extracts and/or allelochemicals may result from disruptions in mitochondrial respiration and/or mobilisation of seed reserves for development (Weir *et al.*, 2004; Gniazdowska and Bogatek, 2005).

Osmotic potential and pH are two factors which may affect germination and seedling growth (Sampietro *et al.*, 2009b). Hence, we carried out control studies to evaluate the osmotic and pH effects of the extract on all parameters measured. Osmotic treatments were applied using mannitol solutions adjusted to the osmotic potentials of the extract as previously recommended (Wardle *et al.*, 1992; Sampietro *et al.*, 2009a). Overall, extract osmolarity and pH had no statistically significant effects on germination, root elongation and biochemical parameters of the test species. Thus, the inhibitory effects of the extract on these parameters are very likely due to the chemical constituents of the extract. Furthermore, FW reduction due to osmotic treatments was observed in the non-pregerminated seedlings of *M. pigra* and *V. radiata*. This suggests that control osmotic treatments are necessary when analysing the effects of plant extracts on seedling growth, particularly FW changes.

Concerns were raised that apparent growth reduction in non-pregerminated seedlings in response to toxic extracts could be due to delayed germination, rather than due to direct effects of the extracts on seedling growth (Hoagland and Williams, 2004; Sampietro *et al.*, 2009a). Hence, in this study, both non-pregerminated and pregerminated seedlings were analysed. This should allow a more reliable assessment of the effects of water hyacinth extract on post-germination growth.

Our growth data show that root growth was more sensitive to the effects of water hyacinth extract than was shoot growth in both non-pregerminated and pregerminated seedlings. More specifically, root length was more sensitive than root FW to the extract. Also, despite unperturbed seed germination, reduced root growth was detected in *V. radiata*. Our observations therefore agree with the proposal that root length is a sensitive indicator of phytotoxicity in

germination bioassay (Haugland and Brandsaeter, 1996; Colvin and Gliessman, 2012).

Analysis of pregerminated seedlings of *M. pigra* and *V. radiata* revealed that water hyacinth extract compromised post-germination root growth. Hence, the inhibited root growth of non-pregerminated *M. pigra* seedlings may be ascribed to both delayed germination and direct effects of the extract on emerged roots. On the other hand, inhibited root growth of non-pregerminated *V. radiata* seedlings was possibly due to direct effects of the extract on developing roots.

H₂O₂ content in root tissues was inversely related to root elongation in non-pregerminated and pregerminated seedlings of both test species. Hence, water hyacinth extract very likely inhibited root growth by promoting H₂O₂ production or accumulation. In support of this suggestion is the earlier finding that root elongation of *Arabidopsis* seedlings was inhibited by exogenous application of H₂O₂ and umbelliferone, a coumarin that enhances H₂O₂ formation *in vivo* and *in vitro* (Dunand *et al.*, 2007). Furthermore, Dunand *et al.* (2007) found that exogenous application of potassium iodide, an H₂O₂ scavenger, promoted root elongation.

Elevated H₂O₂ content in the roots of extract-treated seedlings correlated with decline in soluble POD activities. This observation indicates that the extract may have attenuated H₂O₂ detoxification in the root cells by inhibiting soluble POD, an H₂O₂-metabolising enzyme. Inhibition of soluble POD activity by allelopathic extracts and/or allelochemicals was previously reported (Sánchez-Moreiras and Reigosa, 2005; Li and Jin, 2010). Notably, naringenin, a key phenolic compound identified in water hyacinth (Chantiratikul *et al.*, 2009), inhibited soluble POD in soybean roots (Bido *et al.*, 2010).

The toxicity of some allelopathic extracts and/or allelochemicals is mediated by oxidative stress (Weir *et al.*, 2004; Gniazdowska and Bogatek, 2005). Inhibited soluble POD activity under allelochemical stress was also associated with elevated levels of lipid peroxidation (Sánchez-Moreiras and Reigosa, 2005; Li and Jin, 2010). Surprisingly, MDA content, an indicator of oxidative stress, did not increase despite elevated H₂O₂ levels in the root tissues of extract-treated seedlings. There was also no clear inverse relationship between MDA content and the root growth or germination of *M. pigra* and *V. radiata*. Thus, our findings did not support the hypothesis that the toxicity of water hyacinth leaf extract was mediated by oxidative stress.

Premature or enhanced lignification may have restricted root elongation of the extract-treated seedlings. In this study, an inverse relationship between root growth and CW-POD activity was observed. CW-POD catalyses the last step of the phenylpropanoid pathway, which involves H₂O₂-dependent polymerisation of monolignol to form lignin (Boerjan *et al.*, 2003). In our experiments, elevated H₂O₂ levels coincided with enhanced CW-POD activity in *M. pigra* and *V. radiata*, which may have contributed to the

activity of CW-POD, hence to lignin biosynthesis. The association between reduced root growth and concurrent increase in both CW-POD activity and lignification was previously observed in seedlings treated with allelochemicals (Dos Santos *et al.*, 2004; Zanardo *et al.*, 2009; Bido *et al.*, 2010). Hence, the possibility of cell wall being the key target site of water hyacinth toxicity, particularly through the induction of premature lignification should be investigated further.

In conclusion, water hyacinth leaf extract was toxic to both *M. pigra* and *V. radiata*, by inhibiting the germination of both the species. Inhibition of root growth by water hyacinth extract may be attributed to enhanced H₂O₂ levels, inhibited soluble POD activity and enhanced CW-POD activity. Notably, reduced or unaltered levels of MDA in the extract-treated seedlings did not support the hypothesis that phytotoxicity of water hyacinth was mediated by oxidative stress. The use of water hyacinth as a bioherbicidal agent may be a beneficial approach which allows concurrent management of weeds and identification of allelochemicals responsible for the phytotoxicity especially to *M. pigra* needs further studies.

Acknowledgments

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