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Original Article

Characterization of phytochemical profile and phytotoxic activity of *Mimosa pigra* L.

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ABSTRACT

The search for new biological agents produced by plants represents an alternative strategy in sustainable agriculture. The phytochemical profile of *Mimosa pigra* L. was investigated and the phytotoxic potential was assessed. Phytochemical analyses revealed the presence of flavonoids, tannins, phlobatannins, alkaloids and saponins in the methanolic extract of *M. pigra* (mimosa extract). The contents of both total phenolic compounds and flavonoids increased in a similar manner with increases in the mimosa extract concentrations. The phytotoxicity of the extract was evaluated on seedling growth and on some physiological processes in lettuce (*Lactuca sativa* L.) and popping pod (*Ruellia tuberosa* L.). Root and shoot growths were inhibited by mimosa extract in a concentration-dependent manner. Mimosa extract significantly inhibited mitosis resulting in root growth retardation. Moreover, mimosa extract caused cell death and induced lipid peroxidation in both plants. Therefore, the primary action of mimosa extract on seedling growth inhibition was related to the alteration in mitosis, loss in cell viability and trigger lipid peroxidation in affected tissues. Phytotoxicity was due to the presence of several secondary metabolites in the extract. Consequently, the mimosa extract could be considered as a source of metabolites to be used as alternatives for biological weed control.

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Introduction

Due to an increase in the number of herbicide-resistant weeds and environmental concerns in the use of synthetic herbicides, there has been considerable effort to design alternative weed management strategies (Bhowmik and Inderjit, 2003; Dayan et al., 2009). Pesticide intoxication is one of the major public health problems in Thailand and it is caused by intensive use and exposure to pesticides (Panuwet et al., 2012). Herbicides constitute the highest proportion (about 62–79%) of imported pesticides, valued in excess of USD 680 million in 2013 (Tawatsin et al., 2015). Furthermore, herbicides are a major cause of water pollution (Tirado et al., 2008). In order to reduce the intensive use of herbicides, there is an urgent need to search for effective biological agents to replace synthetic herbicides. Phytotoxic natural products have several benefits over synthetic compounds—they have a short half-life, are considered safe for the environment, are full of bioactive materials and compounds with unexploited properties and are present in many secondary products generated by plants (Duke et al., 2002). Plant-derived phytotoxic compounds have been well investigated as sources of new compounds for weed control (Dayan et al., 2012); for example, sorgoleone in *Sorghum* spp. (Dayan et al., 2010) and momilactone B in rice (Kong et al., 2004).

Invasive weed species may produce chemicals for growth inhibition of competing vegetation directly through obstructing native species, or indirectly by altering the habitat or community composition, thereby providing the invader with a competitive advantage (Chengxu et al., 2011). Several studies have indicated the phytotoxic effect of weed extracts including *Centaurea maculosa* Lam. (Bais et al., 2003), *Imperata cylindrica* (L.) Beauv. (Anjum et al., 2005), *Cyperus rotundus* L. (Sharma and Gupta, 2007), *Euphorbia helioscopia* L. (Madany and Saleh, 2015), *Chenopodium murale* L. (Dmitrović et al., 2015) and *Tridax procumbens* L. (Mecina et al., 2016). While many weed species produce phytotoxins to inhibit the growth of other plants, in most cases, the mode of action remains unknown (Xuan et al., 2004; Anjum et al., 2005; Sharma and Gupta, 2007; Mecina et al., 2016). Thus, understanding the target processes of natural plant compounds opens up the possibility to

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take advantage of natural agents for weed management because target sites can serve as the focus of traditional herbicide discovery efforts (Dayan and Duke, 2014).

Mimosa pigra L. (Family Leguminosae), a woody neotropical legume, is native to Central America, and is regarded as one of the worst weeds because of its invasiveness, potential for spread, and economic and environmental impacts (Paynter, 2005; Global Invasive Species Database, 2016). Rosado-Vallado et al. (2000) reported that *M. pigra* has antimicrobial activity, and phytochemical analysis of this species indicated the presence of flavonoids, quinones, saponins, sterols, and tannins. Some isolated flavonoids, such as quercetin, have shown antioxidant and anti-inflammatory properties (Rakotomalala et al., 2013). However, a few studies have made reference to the phytochemical analysis of *M. pigra* (Rosado-Vallado et al., 2000), and there was no report on the relative amounts of compounds in this plant. Furthermore, no experiments have been conducted to assess the phytotoxic action of *M. pigra* for plant suppression. Therefore, this study was conducted to examine the phytochemical profile and also investigated the phytotoxic action of *M. pigra* on plant growth.

Materials and methods

Plant materials

Matured leaf samples of *M. pigra* during its vegetative stage were collected from its natural habitats in the field in Kochan district, Chonburi province, Thailand. The leaves were air-dried then ground to a fine uniform texture and stored in a poly bag until used. Leaves were used in this study because of their proportionately greater biomass. Seeds of lettuce (*Lactuca sativa* L.) were purchased from Chia Tai Group, Thailand and seeds of popping pod (*Ruellia tuberosa* L.) were manually collected from the Kasetsart University, Kamphaeng Saen campus, Thailand and used throughout the experiments.

Preparation of M. pigra extract

A stock of methanolic extract was prepared by soaking the leaf powder in methanol at 20 g g dry weight per liter (DW/L)7 and placing on an orbital shaker at 50 rpm in dark conditions for 48 h. The solution was filtered through Whatman no. 1 filter paper, and kept at 4 °C until used. The filtrate was used in the experiments as the source of *M. pigra* crude extracts (mimosa extract). Previous research conducted by Koodkaew (2015) reported that *M. pigra* crude extract at concentrations of 1 and 10 g DW/L had an effect on plant growth. Therefore, different concentrations of mimosa extract at 2.5, 5.0, 7.5 and 10 g DW/L were prepared from the stock solution.

Phytochemical screening

M. pigra leaf samples were extracted with methanol at room temperature for 24 h and the solvent was rotary evaporated to yield a dried sample of *M. pigra*. Then the dried sample was subjected to phytochemical screening using standard procedures to identify the constituents as described by Harborne (1973) and Trease and Evans (1989).

Total phenolic compound content assay

The total phenolic compound content (TPC) was determined using the Folin Cioculteu (FC) method with some modifications according to Singleton et al. (1999). Fifty microliters of each extract concentration were mixed with 250 μL of FC reagent and kept at room temperature for 8 min, before adding 750 μL of 20% (w/v) Na_2CO_3 and 950 μL of distilled water. The mixture was vortexed and incubated at 25 °C for 30 min. After incubation, the solutions were then centrifuged at 3000 rpm for 15 min and the supernatants were collected. Thereafter, the absorbance was measured using the UV–Vis spectrophotometer (model S-20; BOECO; Hamburg, Germany) at 765 nm. Gallic acid was used as the standard phenolic compound. Experiments were done in three replicates.

Total flavonoid content assay

A total flavonoid content (TFC) assay was performed using the $AlCl_3$ colorimetric method (Tunna et al., 2015). Five hundred μL of each extract concentration were mixed with 2 mL of distilled water and 15 μL of 5% (w/v) $NaNO_3$ and incubated at room temperature for 6 min. Then, 150 μL of 10% (w/v) $AlCl_3$, 2 mL of 2 M NaOH and 200 μL of distilled water were added. The mixture was vortexed and incubated at 25 °C for 30 min. After incubation, the supernatants were collected and the absorbance was taken using the UV–Vis spectrophotometer at 415 nm. Quercetin was used as the standard flavonoid compound. Experiments were done in three replicates.

Early seedling growth experiment

Seeds of both lettuce and popping pod were pre-germinated in distilled water for 48 and 72 h, respectively; seedlings with an average radicle length of 2 mm were used in this experiment. Two milliliters of each concentration of the extract were added to filter paper in Petri dishes (5 cm in diameter) and evaporated to dryness in a fume hood for 20 min. For the control experiment, methanol was used and evaporated to dryness. Then, 2 mL of distilled water were added to each Petri dish and five germinated seeds were placed onto filter paper in each dish and kept at ambient temperature. Seven days after treatment (DAT), the root length and shoot length, as well as the fresh weight and dry weight were recorded. The root and shoot lengths were calculated as the inhibition rate percentage.

Determination of mitosis

The mitotic index (MI) was determined using the method of Armbruster et al. (1991) modified as follows. After 3 DAT of mimosa extract, root segments approximately 1 cm from the tip were cut off and fixed in ethanol:acetic acid (3:1 vol per volume) for 24 h before transferring to 70% (v/v) ethanol for storage. Roots were washed with distilled water for 15 min and the 2 mm region of the tip was excised. Then, the roots were hydrolyzed with 5 N HCl for 15 min and later stained with 2% (w/v) acetic orcein (Sigma–Aldrich; St. Louis, MO, USA) solution for 10 min. Thereafter, the roots were placed on a microscope slide, heated for a few minutes and fragmented into separated cells using the squash technique. Mitotic figures were evaluated randomly under a light microscope using at least 1000 cells for each replication. Each mitotic phase was counted, and the MI was calculated using the equation MI (%) = (Number of cells in mitotic phase/Total number of cells) × 100.

Determination of cell viability in root tips

The root cell viability was estimated using the Evans blue staining method (Sunohara and Matsumoto, 2008). After 3 d, the treated root segments (approximately 1 cm length from the tip) were excised and washed with distilled water. Root segments were stained with 0.25% (w/v) aqueous Evans blue solution (Sigma–Aldrich; St. Louis, MO, USA) for 1 h at room temperature. After washing the roots with distilled water for 30 min, the dye was

extracted using 500 μL of *N*,*N*-dimethylformamide without grinding for 24 h at room temperature in the dark. The absorbance of released Evans blue was measured at 600 nm using the UV–Vis spectrophotometer.

Determination of lipid peroxidation

The level of lipid peroxidation product (malondialdehyde, MDA) was estimated using the thiobarbituric acid reactive substance method following Velikova et al. (2000) with some modifications. After 7 d treatment with mimosa extract, 0.2 g each of fresh root and shoot samples were ground in liquid N_2 and homogenized in 2 mL of 10% (w/v) trichloroacetic acid (TCA) solution for 10 min. The homogenate was centrifuged at 10,000 rpm for 20 min, and then 1 mL of supernatant was mixed with 2 mL of 0.5% (w/v) thiobarbituric acid in 20% (w/v) TCA. The mixture was incubated at 95 °C for 30 min, and then the reaction was stopped by placing in an ice bath for 5 min. After centrifugation at 5000 rpm for 5 min, the absorbance of the supernatant was measured at 532 and 600 nm. The concentration of MDA was quantified using a molar extinction coefficient of 155 m/M/cm.

Statistical analyses

Data were expressed as the mean ± SE. One-way analysis of variance was used and the mean differences among groups were analyzed using Tukey's test in the SPSS software version 11 (SPSS Inc.; Chicago, IL, USA). Significance was tested at $p < 0.05$. Correlation between the phytotoxic parameters was established using Pearson's correlation coefficient.

Results and discussion

Chemical class identification and quantitative determination

Secondary metabolites may present in different plants parts including the leaves, bark, flowers or roots (Weir et al., 2004). Foliar leachates have been regarded to be most phytotoxic in nature, probably owing to the production of more metabolites (Xuan et al., 2004). The extract from leaves of *M. pigra* was tested for the presence of different major classes of organic compounds such as flavonoids, tannins, phlobatannins, alkaloids, terpenoids, cardiac glycosides and saponins. The results showed that flavonoids, tannins, phlobatannins, alkaloids and saponins were present in the methanolic extract of *M. pigra* (Table 1). This was in agreement with data presented by Rosado-Vallado et al. (2000) and Rakotomalala et al. (2013) who found that leaves of *M. pigra* contain flavonoids, quinones, saponins, sterols and tannins.

Increasing amounts of phenolic compounds and flavonoids were observed with increasing concentrations of methanolic mimosa extract as shown in Table 2. Total phenolic compounds ranged from 21.95 ± 1.52 μg/mL to 42.25 ± 1.43 μg/mL. Total flavonoids ranged from 13.84 ± 0.66 μg/mL to 33.36 ± 0.63 μg/mL at different extract concentrations. Yusuf et al. (2003) and Rakotomalala et al. (2013) reported that flavonoids in *M. pigra* consisted of quercetin, quercitrin, kaempferol, luteolin and myricitrin. In addition, triterpenoid saponins have been reported from the plant (Englert et al., 1995), and recently, two new acylated flavonol glycosides were isolated and identified by Okonkwo et al. (2016). Moreover, Wink (2013) proposed that the legumes (mostly members of the Papilionoideae and Mimosoideae) can produce more nitrogen-containing secondary metabolites (especially glucosinolates, amines and alkaloids) because they contain symbiotic bacteria within their root system which can fix atmospheric nitrogen into cells.

Effect on seedling growth

The experiments tested the effects of mimosa extract on lettuce and popping pod seedling growth using a Petri dish bioassay. Lettuce and popping pod were chosen as the target species in the phytotoxic assays because lettuce was more sensitive to phytotoxin than another relevant species (preliminary study) and because popping pod is an abundant weed species on the Kasetsart University, Kamphaeng Saen campus. The results showed that mimosa extract had an inhibitory effect on lettuce and popping pod growth. The degree of inhibition increased with the concentration of the extract. The greatest inhibitory activity was found at the highest concentration (10 g DW/L). For lettuce, the root and shoot lengths were inhibited by 69.78 ± 0.2% and 41.44 ± 0.6%, respectively (Table 3). For popping pod, the root and shoot lengths were inhibited by 59.58 ± 0.5% and 42.86 ± 2.4%, respectively (Table 4). A

Table 1
Phytochemical screening for chemical class identification of *M. pigra*.

Chemical class	Test/procedure[a]	Positive test	Observation[b]
Flavonoid	Shinoda's test	Pink or red coloration	+
Tannin	Ferric chloride test	Blue-black coloration	+
Phlobatannin	Hydrochloric test	Red precipitate	+
Alkaloid	Draggendorff's reagent	Reddish brown precipitate	+
Terpenoid	Salkowski's test	Reddish brown coloration of the inter face	−
Cardiac glycoside	Glacial acetic + sulfuric test	Blue or blue green coloration	−
Saponin	Frothing test	Formation of froth	+

[a] Test details are available in Harborne (1973) and Trease and Evans (1989).
[b] +, presence; −, absence.

Table 2
Total phenolic compounds (TPC) and total flavonoid contents (TFC) in different concentrations of *M. pigra* extract.

Concentration (g DW/L[†])	TPC (μg/mL)	TFC (μg/mL)
0	0.00 ± 0.00^{d,‡}	0.00 ± 0.00^{e}
2.5	21.95 ± 1.52^{c}	13.84 ± 0.66^{d}
5.0	29.42 ± 1.66^{b}	16.70 ± 0.86^{c}
7.5	34.87 ± 2.78^{b}	22.89 ± 0.36^{b}
10.0	42.25 ± 1.43^{a}	33.36 ± 0.63^{a}

[†] g DW/L = gram dry weight per liter.
[‡] Values represent mean ± SE of three replications and same lowercase superscript letters in the same column are not significantly ($p \geq 0.05$) different using Tukey's test.

Table 3
Effect of *M. pigra* extract on lettuce seedling growth.

Concentration (g DW/L[†])	Inhibition rate (%)		Fresh weight (mg)	Dry weight (mg)
	Root length	Shoot length		
0	0.00 ± 0.0^{a,‡}	0.00 ± 0.0^{a}	193.90 ± 13.1^{a}	5.97 ± 0.1^{ns§}
2.5	61.34 ± 0.3^{c}	24.14 ± 0.5^{b}	209.37 ± 9.4^{a,b}	5.67 ± 0.2
5.0	59.67 ± 0.3^{b}	21.80 ± 1.1^{b}	247.03 ± 12.1^{b}	6.13 ± 0.1
7.5	67.14 ± 0.2^{d}	34.05 ± 0.6^{c}	218.00 ± 3.3^{a,b}	5.60 ± 0.2
10.0	69.78 ± 0.2^{e}	41.44 ± 0.6^{d}	213.27 ± 7.1^{a,b}	4.26 ± 1.8

[†] g DW/L = gram dry weight per liter.
[‡] Values represent mean ± SE of three replications and same lowercase superscript letters in the same column are not significantly ($p \geq 0.05$) different using Tukey's test.
[§] ns = no significant difference.

Table 4
Effect of *M. pigra* extract on popping pod seedling growth.

Concentration (g DW/L[†])	Inhibition rate (%)		Fresh weight (mg)	Dry weight (mg)
	Root length	Shoot length		
0	0.00 ± 0.0[a,‡]	0.00 ± 0.0[a]	118.03 ± 6.4[a]	8.03 ± 0.1 [ns§]
2.5	28.90 ± 0.5[b]	28.57 ± 0.9[b]	235.53 ± 5.5[b]	8.30 ± 0.5
5.0	46.07 ± 0.4[c]	27.68 ± 1.5[b]	219.63 ± 3.6[b]	9.07 ± 0.2
7.5	51.10 ± 0.4[d]	41.07 ± 1.5[c]	221.50 ± 10.3[b]	7.73 ± 0.4
10.0	59.58 ± 0.5[e]	42.86 ± 2.4[c]	226.50 ± 20.0[b]	8.43 ± 0.4

[†] g DW/L = gram dry weight per liter.
[‡] Values represent mean ± SE of three replications and same lowercase superscript letters in the same column are not significantly ($p \geq 0.05$) different using Tukey's test.
[§] ns = no significant difference.

marked reduction in the root length was noticed in the seedlings of both plants species compared to the shoot length, suggesting that the mimosa extract was more toxic on roots than shoots. Dmitrović et al. (2015) also demonstrated that root growth of *Arabidopsis* was inhibited (67% inhibition) more than shoot growth (53% inhibition) by *C. murale*. Beside inhibiting root and shoot elongation, other morphological abnormalities occurred in the treated seedlings. The roots were a brownish color compared to the control (data not shown). The mimosa extract modified lettuce and popping pod growth which was exhibited as a reduction in root and shoot lengths. These observations are in line with those made regarding mustard in the presence of leaf extracts of sunflower (Bogatex et al., 2006) and with radish after treatments with leaf extracts of *Ageratum conyzoides* L. (Xuan et al., 2004). Mimosa extract influenced the fresh weight of the lettuce and popping pod seedlings but had no effect on the dry weight of these two plants. The fresh weight of treated seedlings was higher than the control (Tables 3 and 4). This may have been due to the ability of the plants to increase the size of sap vacuoles for the collection of a lot of water, and this in turn dissolved the accumulated phytotoxins, leading to the subsequent increase in the fresh weight, as was reported for a glyphosate resistance mechanism (Ge et al., 2010). The results may be directly related to the phytotoxic action of the mimosa extract on the development of the tested plants. These findings correlated with the reports of Uddin et al. (2007) and Pérez et al. (2015) who found that leaf extracts of *Albizia lebbeck* (L.) Benth. and *Trifolium argutum* Sol., respectively, both within the Family Leguminosae, contain secondary plant products that have phytotoxic potential by causing seedling lost ability to develop normally as a result of reduced radicle elongation and root necrosis.

Effect on mitosis

Mimosa extract affected the mitotic division of lettuce and popping pod in the same way—the inhibitory effects increased with increases in the extract concentrations. The percentage of dividing cells in mitosis decreased gradually in the root tip treated with mimosa extract. The extract also caused a substantial decrease in the MI; the strongest effect was observed in root cultured in 10 g DW/L (Tables 5 and 6). These observations were in agreement with data obtained on allelochemicals from *Vicia villosa* Roth, where cyanamide had a phytotoxic effect on onion root growth by disturbance of cell division and by inhibition of the proliferation of meristematic cells and the cell cycle (Soltys et al., 2011). The current results revealed that mitotic interference by the extract may have been one factor causing root and shoot growth retardation because root and shoot elongation depends on normal cell division of the meristematic cells. Using flow cytometry, quantification of gene expression and other methods, it has been well established that seedling growth, especially root growth, may be inhibited by different phytotoxic plant extracts through the disturbance of cell division (Soltys et al., 2011; Dmitrović et al., 2015). Cell division is in turn a prerequisite for root growth, as newly formed cells are the only means of organ elongation via an increase in dimensions, mainly in line with long axis of the root (Soltys et al., 2011).

Effect on cell viability

Loss of cell viability is an indicator for the evaluation of toxic substance-induced cell death (Baker and Mock, 1994). Evans blue uptake was determined to measure the level of appearance of dead

Table 5
Effect of *M. pigra* extract on mitosis division in lettuce root tip.

Concentration (g DW/L[†])	Mitotic division (%)				Mitotic index (%)
	Prophase	Metaphase	Anaphase	Telophase	
0	4.32 ± 0.11[a,‡]	1.47 ± 0.27[ns§]	0.97 ± 0.31[a]	0.86 ± 0.10[ns]	7.08 ± 0.24[a]
2.5	3.25 ± 0.36[a,b]	1.39 ± 0.24	0.51 ± 0.08[a,b]	0.69 ± 0.10	6.09 ± 0.29[a,b]
5.0	3.07 ± 0.58[a,b]	1.00 ± 0.00	0.47 ± 0.03[a,b]	0.63 ± 0.19	4.97 ± 0.78[a,b,c]
7.5	2.15 ± 0.62[b]	1.13 ± 0.13	0.14 ± 0.08[b]	0.61 ± 0.22	3.40 ± 1.00[b,c]
10.0	1.59 ± 0.11[b]	0.99 ± 0.01	0.06 ± 0.06[b]	0.56 ± 0.24	2.99 ± 0.28[c]

[†] g DW/L = gram dry weight per liter.
[‡] Values represent mean ± SE of three replications and same lowercase superscript letters in the same column are not significantly ($p \geq 0.05$) different using Tukey's test.
[§]ns = no significant difference.

Table 6
Effect of *M. pigra* extract on mitosis division in popping pod root tip.

Concentration (g DW/L[†])	Mitotic division (%)				Mitotic index (%)
	Prophase	Metaphase	Anaphase	Telophase	
0	3.55 ± 0.58[a,‡]	0.53 ± 0.03[b,c]	0.52 ± 0.02[a]	0.52 ± 0.02[a]	6.02 ± 0.52[a]
2.5	2.56 ± 0.29[a,b]	1.13 ± 0.13[a]	0.54 ± 0.21[a]	0.68 ± 0.03[a]	4.28 ± 0.15[b]
5.0	1.17 ± 0.06[b]	0.82 ± 0.12[a,b]	0.25 ± 0.07[a,b]	0.42 ± 0.08[a,b]	2.67 ± 0.28[c]
7.5	1.12 ± 0.56[b]	0.23 ± 0.01[c]	0.00 ± 0.00[b]	0.19 ± 0.06[b]	1.47 ± 0.38[c,d]
10.0	0.81 ± 0.35[b]	0.16 ± 0.02[c]	0.00 ± 0.00[b]	0.15 ± 0.01[b]	0.96 ± 0.27[d]

[†] g DW/L = gram dry weight per liter.
[‡] Values represent mean ± SE of three replications and same lowercase superscript letters in the same column are not significantly ($p \geq 0.05$) different using Tukey's test.

cells in the mimosa extract-treated roots. The effects of mimosa extract on the loss of cell viability in both tested plants resulted in the same trend, as the relative uptake of Evans blue into lettuce and popping pod roots was significantly increased after treatment with the extract (Table 7). Increased Evan blue staining reflected a loss in membrane integrity. This phenomenon may indicate inhibition in the seedling growth of the two plant species used due to enhanced membrane deterioration. It was speculated that the observed enhancement of cell death in treated roots may also be a response to the elevated lipid peroxidation by reactive oxygen species (ROS) in the affected tissue.

Effect on lipid peroxidation

Lipid peroxidation is the metabolic process in which ROS attack lipids containing a carbon-carbon double bond that results in the oxidative deterioration of lipids (Ayala et al., 2014). To determine whether the mimosa extract imposed oxidative stress in the tested plants, the occurrence of MDA (a secondary end product of the oxidation of polyunsaturated fatty acids) has been considered as a useful index of general lipid peroxidation (Hodges et al., 1999). Mimosa extract caused an increase in the MDA content in both the lettuce and popping pod seedlings. The extract at 10 g DW/L significantly increased the MDA content in roots and shoots of lettuce and in the roots of popping pod but had no effect on popping pod shoots (Table 8). It could be assumed that mimosa extract induced the accumulation of ROS, which played a key role in lipid peroxidation in the plants. Therefore, this supports the hypothesis that decreased seedling growth may be associated with membrane lipid peroxidation as a response to the elevated ROS in the affected tissue.

Table 7
Effect of *M. pigra* extract on cell viability in lettuce and popping pod root using Evans blue uptake method.

Concentration (g DW/L[†])	Relative Evans blue uptake (%)	
	Lettuce	Popping pod
0	100.00 ± 13.7[a,‡]	100.00 ± 5.8[a]
2.5	411.73 ± 55.6[b]	161.58 ± 14.8[b]
5.0	424.69 ± 42.0[b]	170.88 ± 14.0[b]
7.5	433.95 ± 13.8[b]	178.25 ± 6.9[b]
10.0	453.09 ± 19.2[b]	182.98 ± 2.0[b]

[†] g DW/L = gram dry weight per liter.
[‡] Values represent mean ± SE of three replications and same lowercase superscript letters in the same column are not significantly ($p \geq 0.05$) different using Tukey's test.

Table 8
Effect of *M. pigra* extract on MDA content in lettuce and popping pod.

Concentration (g DW/L[†])	MDA content in lettuce (mol/g)		MDA content in popping pod (mol/g)	
	Root	Shoot	Root	Shoot
0	5.93 ± 0.4[a,‡]	4.83 ± 1.2[a]	5.43 ± 0.8[a]	8.13 ± 1.0 [ns§]
2.5	7.63 ± 1.6[a]	5.93 ± 0.6[a]	5.53 ± 0.4[a]	7.23 ± 2.4
5.0	4.77 ± 0.9[a]	6.83 ± 1.0[a]	5.83 ± 1.2[a]	8.23 ± 0.3
7.5	7.23 ± 0.3[a]	7.47 ± 1.7[a]	5.57 ± 0.8[a]	9.94 ± 1.7
10.0	12.90 ± 4.0[b]	10.30 ± 3.5[b]	7.87 ± 2.8[b]	9.21 ± 1.2

[†] g DW/L = gram dry weight per liter.
[‡] Values represent mean ± SE of three replications and same lowercase superscript letters in the same column are not significantly ($p \geq 0.05$) different using Tukey's test.
[§]ns = no significant difference.

Phytotoxic effect of mimosa extract

The effects of phytotoxins on plants are diverse. The phytotoxic activity of allelochemicals usually involves alteration in one of the crucial physiological processes such as photosynthesis, respiration, induction of ROS and cell division, which all lead to cell death (Bais et al., 2003; Weir et al., 2004; Soltys et al., 2011). Inhibition of any of these processes results in disturbances in plant growth and development. The current experiment has demonstrated for the first time the effect of mimosa extract toxicity during early seedling growth of lettuce and popping pod. The inhibitory effect of mimosa extract on these two plants was dose dependent (Tables 3 and 4). Therefore, the latter three experiments were conducted to elucidate whether this growth retardation was related to cell division, cell death and oxidative stress.

The results showed that the mimosa extract had effects on the retardation of cell division, induction of cell death and of oxidative stress (Tables 5–8). Correlation analysis revealed the percentage of root inhibition was highly significant ($p < 0.01$) and negatively correlated with MI (lettuce, −0.688; popping pod, −0.949). A strong correlation ($p < 0.01$) between the percentage of root inhibition and a loss in cell viability (lettuce, 0.935; popping pod, 0.880) was also observed. However, there were no significant correlations between the root inhibition and the MDA content in both lettuce and popping pod seedlings (Table 9). These results indicated that mimosa extract disturbed mitosis and induced cell death leading to root growth retardation. On the other hand, mimosa extract may have triggered a wave of ROS-initiated lipid peroxidation, which then rapidly depolarized the cell membrane increasing the membrane permeability, causing generalized cellular disruption that ultimately lead to cell death. The extract was also associated with the inhibition of cell entry to the cell cycle resulting in decreased root growth.

Similarly, other authors have reported a connection between the inhibition of root growth and the disturbance of cell division, induction of cell death or oxidative stress. Hairy root exudates of the allelopathic weed *C. murale* L. showed down-regulation of cell cycle regulators and generation of oxidative stress in the affected plants

Table 9
Correlation coefficients between responses studied.

	Lettuce				Popping pod			
	IR[†] of root	Mitotic index	Cell death	MDA[‡] content	IR of root	Mitotic index	Cell death	MDA content
IR of root	1	−0.688**	0.935**	0.414	1	−0.949**	0.880**	0.261
Mitotic index	–	1	−0.557*	−0.454	–	1	−0.698**	−0.198
Cell death	–	–	1	0.456	–	–	1	0.205
MDA content	–	–	–	1	–	–	–	1

[†] IR = inhibition rate.
[‡] MDA = malondialdehyde.
*, ** = correlation significant at 0.05 and 0.01 level, respectively.

(Dmitrović et al., 2015). The phytotoxin, catechin, from spotted knapweed (*C. maculosa*) showed inhibition of native species growth and germination and triggered ROS-initiation at the root meristem, which led to the death of the root system (Bais et al., 2003). Likewise, a primary action of phytotoxin from the cyanobacteria *Hapalosiphon* sp. suppressed lettuce growth by ROS overproduction, which induced major oxidative damage to membrane lipids, resulting in cell death and growth inhibition (Koodkaew et al., 2012).

Analyses of possible compounds involved in the phytotoxicity of mimosa extract were undertaken in the present study. The phytotoxic potential of many compounds, including the phenolic compounds, is recognized because they can lead to increase cell membrane permeability, lipid peroxidation and cell death and also can inhibit cell division and change the cell ultra-structure, which subsequently interferes with the normal growth and development of the whole plant (Li et al., 2010). It could be suggested that the phytotoxicity of mimosa methanolic extract on lettuce and popping pod may have been due to the activity of these secondary metabolites. This was supported by data presented by Silva et al. (2013) who suggested that high levels of polyphenols and alkaloids in coffee fruit extracts can be included in the mechanism of plant growth inhibition. Due to mimosa extract presenting several phytochemical classes, it could be suggested that perhaps more than one compound is responsible for the effect.

The results of the phytochemical characteristics and phytotoxic action mechanism of the mimosa extract support the conclusion that this species contains phytotoxic compounds capable of directly interfering with seedling growth. The primary action mechanism of mimosa extract on the retardation of seedling growth may be related to an alteration in mitosis, loss in cell viability and triggering lipid peroxidation in affected tissues. Further application of mimosa extract as a potential bioherbicide for weed control should be tested on different weeds and crop species.

Conflict of interest

The authors declare no conflict of interest.

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