Sains Malaysiana 51(10)(2022): 3153-3162
http://doi.org/10.17576/jsm-2022-5110-03

Allelopathic Effect of *Eichhornia crassipes* Aqueous Extract against Growth of *Mimosa pudica*

(Kesan Alelopati Ekstrak Akueus *Eichhornia crassipes* terhadap pertumbuhan *Mimosa pudica*)

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Received: 23 November 2021/Accepted: 10 May 2022

ABSTRACT

Allelopathy is a phenomenon in which a plant produces allelochemicals that affect neighboring plants' growth and physiological processes. This study aimed to investigate the allelopathic effect of *Eichhornia crassipes* on the growth of *Mimosa pudica* seedlings. The experiment was conducted in the pot, where *M. pudica* seedlings were irrigated with aqueous leaf extract of *E. crassipes* at 5, 10 and 15% (w/v) concentrations once a week for four consecutive weeks. The experiment was carried out by Completely Randomized Design (CRD) with three replicates. The allelochemical contents of leaf extract were quantified using a spectrophotometric method, and the total phenolic content was 129.54 mg GAE/g DW. The maximum percentage of electrolyte leakage was detected in *M. pudica* seedlings when treated with a higher concentration of *E. crassipes* leaf extract. The allelopathic effect of *E. crassipes* extract on growth, chlorophyll content and lipid peroxidation was also determined. The result showed a significant decrease in length, weight and chlorophyll contents treated with 10 and 15% concentrations of *E. crassipes* leaf extracts. However, malondialdehyde (MDA) in the root of *M. pudica* seedlings treated with aqueous leaf extract of *E. crassipes* at 10% and 15% (w/v) concentrations were found to be remarkably higher compared to control. These results indicated that the *E. crassipes* leaf extract exhibited allelopathic effects on *M. pudica* growth. However, the increase of the allelopathic effect of *E. crassipes* leaf extract was concentration-dependent.

Keywords: Allelopathy; *Eichhornia crassipes*; *Mimosa pudica*

ABSTRAK

Alelopati ialah fenomenon apabila tumbuhan menghasilkan alelokimia yang mempengaruhi pertumbuhan dan proses fisiologi tumbuhan bersebelahan. Kajian ini bertujuan untuk mengkaji kesan alelopati *Eichhornia crassipes* terhadap pertumbuhan anak benih *Mimosa pudica*. Uji kaji dijalankan di dalam pasu dengan anak benih *M. pudica* disiram dengan ekstrak akueus daun *E. crassipes* pada kepekatan 5, 10 dan 15% (w/v) sekali seminggu selama empat minggu berturut-turut. Uji kaji telah dijalankan secara Reka Bentuk Rawak Sepenuhnya (CRD) dengan tiga replikasi. Kandungan alelokimia ekstrak daun dihitung menggunakan kaedah spektrofotometri dan jumlah kandungan fenol ialah 129.54 mg GAE/g DW. Peratusan maksimum kebocoran elektrolit dikesan pada anak benih *M. pudica* apabila dirawat dengan kepekatan ekstrak daun *E. crassipes* yang lebih tinggi. Kesan alelopati ekstrak *E. crassipes* terhadap pertumbuhan, kandungan klorofil dan peroksidasi lipid juga ditentukan. Hasil menunjukkan penurunan ketara pada panjang, berat dan kandungan klorofil yang dirawat dengan kepekatan 10% dan 15% ekstrak daun *E. crassipes*. Walau bagaimanapun, malondialdehid (MDA) dalam akar anak benih *M. pudica* yang dirawat dengan ekstrak akueus daun *E. crassipes* pada kepekatan 10% dan 15% (w/v) didapati lebih tinggi berbanding kawalan. Keputusan ini menunjukkan bahawa ekstrak daun *E. crassipes* menunjukkan kesan alelopati terhadap pertumbuhan *M. pudica*. Walau bagaimanapun, peningkatan kesan alelopati ekstrak daun *E. crassipes* bergantung kepada kepekatan.

Kata kunci: Alelopati; *Eichhornia crassipes*; *Mimosa pudica*

INTRODUCTION

Allelopathy is a biological occurrence in which a plant releases secondary metabolites and translocates them to receiver plants through various processes such as volatilization, leaching and root exudation. They may have either positive or negative impacts on the growth of receiver plants (Ferguson et al. 2009; Rice 1984; Srivasava et al. 2017). These secondary metabolites are

also known as allelochemicals (Bachheti et al. 2019). Li et al. (2010) have characterized these allelochemicals into 10 categories according to their different chemical structures and properties: water-soluble organic acids, simple lactones, long-chain fatty acids and polyacetylenes, quinines, phenolics, cinnamic acid and its derivatives, coumarins, flavonoids, tannins, steroids, and terpenoids. Over the last few years, the application of allelochemicals to weed management has been increasing (Bachheti et al. 2019) as weed infestation exhibits a major impact on the quantity and quality of crop yields (Rao 2000).

Recent studies recorded allelopathic effects from many plant extracts, including ones from water hyacinth, *Eichhornia crassipes* which originated in South America and later spread to other regions of South Africa. Due to its rapid growth rates and extensive dispersal capabilities, water hyacinth is recognized as one of the most invasive aquatic weeds (Gopal & Sharma 1981; Kadono 2004). In addition, water hyacinth can release allelopathic substances that affect other organisms. Previous research has reported the water hyacinth inhibitory effect on the growth of a wide variety of weeds, such as ryegrass (Kato-Noguchi et al. 2014), *Mimosa pigra* (Chai et al. 2013), wild oat and milk thistle (Gul et al. 2016). One of the active allelopathic substances isolated from water hyacinth containing the inhibitory effect on various organisms is loliolide. Kato-Noguchi et al. (2014) extracted the loliolide from water hyacinth with methanol, and this pure extract inhibited the growth of ryegrass. The mixing of water hyacinth powder with potting soil also led to inhibition of seed germination of barnyard grass seedlings.

Mimosa pudica, commonly known as a sensitive plant, is classified as a Mimosaceae family. Due to the lack of persistent weedy stems above ground, *M. pudica* is botanically characterized as herbaceous. The distinguishing features of *M. pudica* are bipinnately compound leaves, slender stems that are often prickly, and small purplish-pink flowers borne in the leaf axils with an average size of 1 cm in diameter. The fruit consists of clusters of flat pods that contain 3-5 seeds inside, and when the seeds are ripened, those pods will dramatically turn brown. *M. pudica* is considered invasive in a wide range of tropical countries in Asia-Pacific, Australia and America (Holm et al. 1977; Rusea & Uleanu 2017; Sankaran & Surat 2013; United States Department of Agriculture 2014). Sankaran and Surat (2013) reported that the rapid growth of *M. pudica* out-competing other plants. Similar cases were noted by Parsons and Cuthbertson (2001), that *M. pudica* is regarded as extremely invasive in plots where various crops are grown. Control methods of *M. pudica* are heavily based on herbicides such as dicamba, picloram and triclopyr (Sankaran & Surat 2013). However, persistent herbicides can remain active in the environment for a long time, potentially causing soil and water contamination. Additionally, herbicide residues may pose adverse effects to humans (Marin-Morales et al. 2013). The studies from Chai et al. (2013), Gul et al. (2016), and Kato-Noguchi et al. (2014), have proved the inhibitory effects of allelopathy on weed control in both greenhouse and field conditions leading to the strike of interest in applying allelopathic plant extract as bioherbicides in agroecosystems. Accordingly, the *E. crassipes* are commonly found and provide allelopathic effects on the plants; it could benefit from utilizing this widespread plant as an allelochemical substance to inhibit other invasive plants. In this case, the *M. pudica* that has not yet been reported for the inhibition effect was selected to determine the inhibitability of *E. crassipes*. Thus, the objective of this study was to investigate the allelopathic effect of *E. crassipes* leaf extract on growth, chlorophyll contents, and lipid peroxidation in *M. pudica*.

Materials and Methods

Plant samples

The complete and intact leaves of approximately equal size (cm) of *E. crassipes* were first sampled and washed with tap water to remove any debris. They were then air-dried and dried at 50 °C for 72 h in a hot air oven. Dried samples were then powdered with an electrical blender and stored in a zip lock bag at 4 °C until use.

Seeds of *M. pudica* were collected from the Prince of Songkla University Pattani campus. Brownish pods were selected for seed viability tests. Randomly chosen seeds were placed on a filter paper rinsed with distilled water of 5 mL. The germination rate was recorded after leaving those seeds at room temperature for 3 days.

Determination of total phenolic content in *E. crassipes* extract

The *E. crassipes* leaf extract was prepared according to Agaba and Fawole (2014) with slight modifications. The dried leaf powder of *E. crassipes* (500 mg) was dissolved in 25 mL distilled water and incubated at 4 °C for 24 h, followed by filtration. The clear supernatant was collected for analysis using a method described by Chan et al. (2007). The 250 mL of clear supernatant was then mixed with 750 µL of 10% (v/v) Folin and

Ciocalteu's reagent and 1000 µL of 7.5% (w/v). The 1000 µL of 7.5% (w/v) sodium carbonate was then added to the mixture. The reaction was kept in the dark at room temperature for 90 min. After incubation, absorbance was measured at 765 nm wavelength using a UV-visible spectrophotometer. Total phenolic content was calculated as gallic acid equivalents GAE/g of dry plant material based on the standard curve of gallic acid at concentrations of 10-80 µg/mL.

EFFECT OF *E. crassipes* LEACHATE ON ELECTROLYTE LEAKAGE IN *M. pudica* SEEDLINGS

The dried leaf powder of *E. crassipes* (5, 10 and 15 g) was added with distilled water of 100 mL and incubated at 4 °C for 48 h. After incubation, the mixture was then filtered through No. 1 filter paper. Seeds of *M. pudica* were rinsed with distilled water three times before submerging in hot water (80 °C) for 1 min. The seeds were placed on No. 1 filter paper layered in a petri dish, and 5 mL of distilled water was then poured over the seeds. Seed incubation was carried out at room temperature for 72 h. The 10 seedlings were collected and transferred to another petri dish, where each petri dish contained 15 mL of aqueous *E. crassipes* extract at a concentration of 5, 10 and 15% (w/v). The Completely Randomized Design (CRD) with 3 replicates was used in this experiment. The plant extract was replaced with distilled water for the control group. Incubation was carried out at room temperature for 24 h. After incubation, the seedlings were carefully rinsed and stored in a beaker containing distilled water for another 24 h. The initial electrical (EC1) conductivity was then measured. Then, the test tubes containing each sample were autoclaved at 121 °C for 20 min and left to cool at room temperature, followed by the measurement of final electrical conductivity (EC2). The percentage of electrolyte leakage was calculated using a method described by Jaballah et al. (2017):

$$\text{Electrolyte leakage} = (EC1/EC2) * 100$$

EFFECT OF *E. crassipes* LEAF EXTRACT ON GROWTH AND VIGOR OF *M. pudica* SEEDLINGS

Germination of *M. pudica* seeds was carried out in a petri dish (25 × 150 mm) layered with a No. 1 filter paper. Seeds were soaked with 5 mL distilled water and incubated at room temperature for 24 h. The 10 germinated seeds were transplanted into pots containing 30 g of potting soil. This experiment was carried out using a Completely Randomized Design (CRD) with 3 replicates. For this purpose, seedlings were divided into 4 distinct groups with different treatments: control (distilled water), aqueous *E. crassipes* leaf extract of 5%, 10% and 15% (w/v) concentrations, respectively. For the first week of transplantation, seedlings were watered with distilled water, and replaced with aqueous *E. crassipes* leaf extract for each treatment group, except for the control group for the next three weeks. Seedlings were harvested at the end of the fourth week after transplantation. Seedlings' growth parameters (shoot and root lengths, fresh weight and dry weight) were then measured and recorded. The seedling vigor index (SVI) was also calculated as described by Papenfus et al. (2013).

EFFECT OF *E. crassipes* LEAF EXTRACT ON CHLOROPHYLL CONTENT OF *M. pudica* SEEDLINGS

The determination of leaf pigment was conducted using a method described by Inskeep and Bloom (1985). Seedlings of *M. pudica* were collected individually from each treatment group and dried over 48 h at 80 °C. The 50 mg of dried seedling sample was ground using 20 mL of 80% (v/v) acetone, then incubated at 4 °C for 48 h, followed by centrifugation at 8000 rpm for 15 min. The clear supernatant was then analyzed using a UV-visible spectrophotometer with absorbance at 645 and 663 nm. Results were obtained in the form of chlorophyll a and b content (mg/g dry weight).

EFFECT OF *E. crassipes* LEAF EXTRACT ON THE INDUCTION OF LIPID PEROXIDATION

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) accumulated in seedling roots using a method described by Devasagayam et al. (2003). The plant root sample of 500 mg was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 8000 rpm for 15 min. The clear supernatant 500 µL was then mixed with 4 mL of 20% TCA containing 0.5% thiobarbituric acid. The mixture was heated at 95 °C for 30 min and then cooled in an ice bath for 5 min. The absorbance of the supernatant was taken at 532 and 600 nm. The concentration of MDA was calculated using the extinction coefficient of 155 mM cm^{-1}.

DATA ANALYSIS

Statistical comparison of means was carried out using a one-way analysis of variance (ANOVA). Multiple comparisons were conducted using Duncan's multiple

range test, and differences were considered significant at $P < 0.05$.

Results and Discussion

The total phenolic content in *E. crassipes* aqueous extract was recorded at 129.54 mg gallic acid equivalent (GAE)/g dry weight. Based on previous studies, variations in phenolic content yielded from *E. crassipes* are attributed to different polarities of solvent and phytochemical compounds present in *E. crassipes* (Tyagi & Agarwal 2017). Additionally, phenolics have been widely regarded as the most common allelochemicals (Bachheti et al. 2019; Li et al. 2010). Many studies in recent years have reported that various phenolic compounds can act as allelochemicals that interfere with physiological processes involved with the germination, growth and development of the seeds (Li et al. 2010).

The electrolyte leakage in *M. pudica* seedlings after treatment with *E. crassipes* leaf extract at various concentrations was determined. The electrolyte leakage observed in the treatment group correlated with increased concentrations of aqueous *E. crassipes* extract: 55.56%, 57.62% and 62.88% for 5%, 10% and 15% concentrations, respectively. In addition, the level of electrolyte leakage of *M. pudica* seedlings found in the treatment groups was significantly greater than the control group (29.07%), as shown in Figure 1. Evidently, the 15% concentration of *E. crassipes* extracts induced the highest electrolyte leakage level. This finding aligned with previous studies that observed allelopathic effects from many plant species on electrolyte leakage. Jaballah et al. (2017) reported the disruption of cell membrane integrity of two lentil varieties caused by significant stimulatory effects of the aqueous extracts of chickpea. Ladhari et al. (2014) reported that the allelochemical in the extract of the Capparidaceae species triggered lipid peroxidation in lettuce, as evidenced by the disruption in the membrane permeability caused by strong electrolyte leakage.

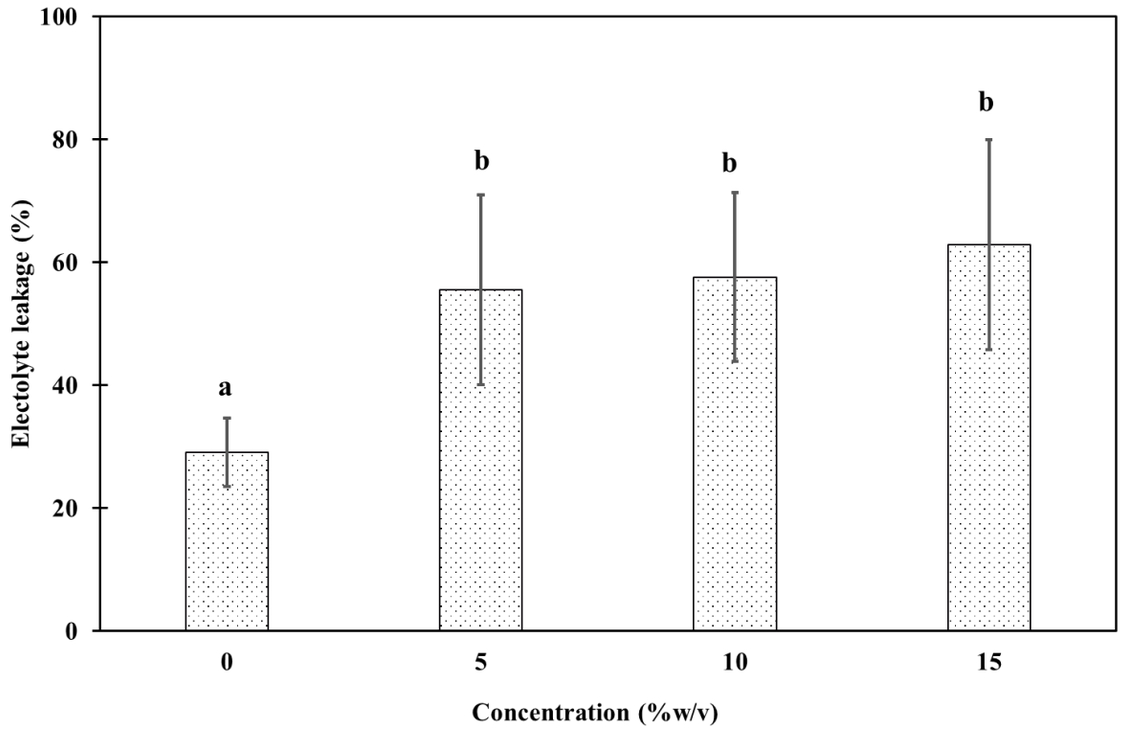

FIGURE 1. Effect of *E. crassipes* leachate on electrolyte leakage in *M. pudica* seedlings. Every column in each graph represents the mean ±SD of three replicates. The different letter indicates significant differences at the 0.05 level compared to control

Compared to control, the seedling growth of *M. pudica* in the treatment groups was markedly stunted, especially in the growth inhibition (Table 1). Results also showed the inhibitory effect of different *E. crassipes* extract concentrations on seedling growth in terms of shoot and root lengths. It was observed that the shoot length of seedlings treated with *E. crassipes* leaf extract at 10% and 15% concentration significantly decreased by 37%-45%. While similar results were observed in seedlings' root length, the decrease was not significant. In addition, the longest *M. pudica* seedling length was exhibited in the control treatment, while the shortest seedling length was observed in *M. pudica* treated with 15% of *E. crassipes* leaf extract. Furthermore, the shoot and roots fresh weight and dry weight were reduced significantly under the influence of *E. crassipes* leaf extract at 10% and 15% concentration compared to control. The lowest fresh weight of shoot and root (44.93% and 38.00%, respectively) was recorded in seedlings treated with *E. crassipes* leaf extract at 15% concentration. The lowest dry weight of shoot and root also occurred through the influence of the *E. crassipes* leaf extract at 15% concentration (43.47% and 26.00%, respectively). Additionally, Figure 2 showed that the seedling vigor index of *M. pudica* treated *with E. crassipes* leaf extract at 10% and 15% concentration was significantly reduced compared to control (1.76% and 1.49%, respectively).

The similarity was reported by Mendez and Miranda (2015) when investigating the inhibitory effect of water hyacinth extract on various growth parameters of chickpea seedlings. Chai et al. (2013) also noted that the water hyacinth extract at 2.5%-5.0% concentration suppressed the length and fresh weight of *M. pigra* seedling root, but the shoot length did not show any suppression compared to the control. In contrast to the findings reported by Chai et al. (2013) and Gul et al. (2016), the influence of water hyacinth on stunted shoot length of both wild oat and milk thistle was observed. The results indicated that the growth inhibition (length and fresh weight) of *M. pudica* seedlings was influenced by the allelopathic effect of *E. crassipes* in correlation with concentration. The higher concentration at 10%-15% (w/v) provides a more inhibitory effect than the 5% concentration and control treatments, maybe because of the different amount of phenolic content. This is also supported by previous studies that suggested the allelopathic activity of extract attributed to allelochemicals constituent and their contents (Al-Hawas & Azooz 2018; Radwan et al. 2019).

TABLE 1. Effect of leaf extract on growth parameters of *Mimosa pudica* after treated with *E. crassipes* leaf extract

Parameters	Concentration (% w/v)			
	0	5	10	15
Shoot length (mm)	94.69 ± 11.82a	61.62 ± 10.95a	59.46 ± 9.86bc	52.16 ± 8.53c
Root length (mm)	33.58 ± 2.90a	31.03 ± 7.88a	28.55 ± 5.81a	26.24 ± 8.50a
Seedling length (mm)	128.54 ± 12.65a	92.64 ± 14.12ab	88.01 ± 11.40b	78.40 ± 10.27b
Shoot fresh weight (mg)	40.77 ± 3.37a	34.42 ± 7.33a	26.87 ± 3.99b	22.45 ± 5.33b
Root fresh weight (mg)	2.63 ± 0.58a	2.15 ± 0.60a	1.85 ± 0.51b	1.63 ± 0.33b
Seedling fresh weight (mg)	43.10 ± 2.98a	36.57 ± 6.88a	28.72 ± 3.62b	24.08 ± 5.11b
Shoot dry weight (mg)	5.52 ± 0.75a	4.88 ± 1.07a	3.55 ± 0.48b	3.12 ± 0.98b
Root dry weight (mg)	0.92 ± 0.12a	0.80 ± 0.60a	0.70 ± 0.13b	0.68 ± 0.13b
Seedling dry weight (mg)	6.43 ± 0.74a	5.68 ± 1.13a	4.25 ± 0.48b	3.80 ± 1.00b

The data represents the mean ±SD of three replicates. The different letter in same row indicates significant differences at the 0.05 level compared to control.

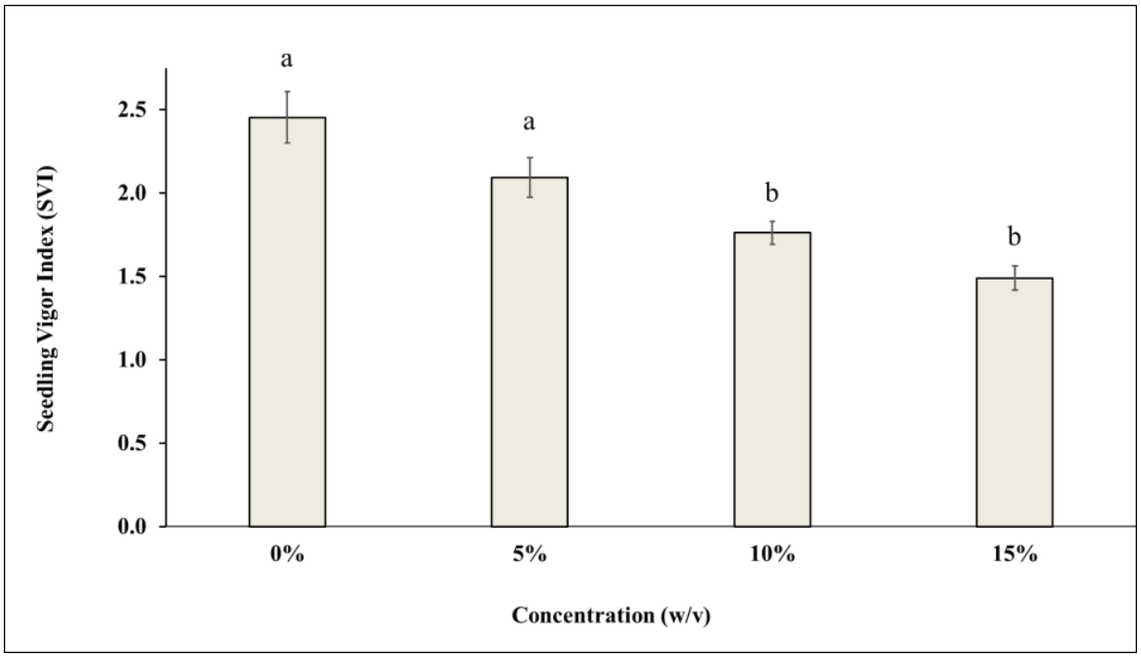

FIGURE 2. Effect of water hyacinth extract at various concentrations on seedling vigor index of *Mimosa pudica*. Every column in each graph represents the mean ±SD of three replicate. The different letter indicates significant differences at the 0.05 level compared to control

Additionally, the abundance of *M. pudica* lateral roots decreased in response to higher *E. crassipes* leaf extract (10% and 15% concentration) compared to control (Figure 3). Based on these findings, it was suggested that stunted growth of root and shoot was the consequence of the inhibition of cell division of *M. pudica*. This was also supported by Huang et al. (2020), which reported that the exogenous application of allelochemical p-hydroxybenzoic acid suppressed the growth of cucumber root by reducing root tip reactive oxygen species (ROS) level, decreasing root meristem activity and reducing root cell length. Additionally, Gatti et al. (2010) studied the allelopathic effects of aqueous extracts of *Aristolochia esperanzae* on germination, root growth and xylem cell development of sesame seedlings. They found that the extract of *A. esperanzae* caused a reduction in the size of root xylem cells and morphological changes in the primary root and the abundance of secondary roots.

The maximum chlorophyll a (4.04 ± 0.06 mg/g DW) and chlorophyll b (1.80 ± 0.121 mg/g DW) were observed in control *M. pudica* seedlings. In contrast, the lowest content of chlorophyll a and b (1.99 ± 0.044 mg/g DW and 0.40 ± 0.21 mg/g DW, respectively) were recorded in seedlings treated with *E. crassipes* leaf extract of 15% concentration (Figure 4A). Additionally, this result demonstrated that both chlorophyll a and b were significantly reduced in seedlings with the highest concentration of the extract. In comparison with control, total chlorophyll a and b content in *M. pudica* treated with the extract at a concentration of 15% was slightly reduced by 30-38%. Previous studies reported that the allelopathic extract could cause a reduction in chlorophyll content. These results were in line with other reports (Elisante et al. 2013; Mandez & Miranda 2015; Oyerinde et al. 2007; Skrzypek et al. 2015). Yang et al. (2004) studied the effect of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings. They reported that allelopathic phenolics affected protoporphyrin IX activity, a well-known precursor for chlorophyll biosynthesis (Granick 1948). According to da Silva and Vieira (2019), allelochemicals affected the synthesis and/or degradation of chlorophylls and enhanced H_2O_2 production may have interrupted the electron transport chain in the photosystems. It is possible that the *E.*

FIGURE 3. Allelopathic effect of water hyacinth aqueous extract on growth of *Mimosa pudica*. A: control B: 5% C: 10 % D: 15 % aqueous extract

crassipes leaf extract exhibited an allelopathic effect *M. pudica* growth by decreasing the chlorophyll content.

MDA is widely regarded as a secondary product of lipid peroxidation, which can be used as a marker of cell membrane damage. Increasing MDA content could indicate the occurrence of lipid peroxidation and cell membrane disruption (Farhoudi & Lee 2013; Repetto et al. 2012). The result obtained from this study showed that the MDA content measured in *M. pudica* seedlings was significantly greater than control (Figure 4(B)). The *E. crassipes* leaf extract at 15% concentration caused the highest MDA content (2.84 ± 0.11 nmole/g FW), while the lowest MDA content (1.44 ± 0.06 nmole/g FW) was observed in control. Additionally, the MDA content in *M. pudica* seedling root was enhanced according to the increasing treatment with 5%, 10% and 15% of *E. crassipes* leaf extract compared with control. Thus, this result indicated that *E. crassipes* leaf extract could trigger oxidative stress in *M. pudica* through increased ROS. Chai et al. (2013) suggested that the lipid peroxidation in seedlings of *M. pigra* was induced by the water hyacinth extract. Furthermore, these results agree with Farhoudi and Lee (2013), who studied the allelopathic effects of barley extract foliar application on lipid peroxidation in *Hordeum spontoneum* and *Avena ludoviciana* seedlings, and found that 30% barley extract increased lipid peroxidation in those seedlings. Al-Hawas and Azooz (2018) reported that *Artemisia monosperma* and *Thymus vulgaris* leaf extracts increased lipid peroxidation and H_2O_2 content in pea seedlings. In addition, Ding et al. (2007) mentioned the response of cucumber roots to cinnamic acid via ROS accumulation, which increased the membrane peroxidation and decreased the H+-ATPase activity, causing root cell death.

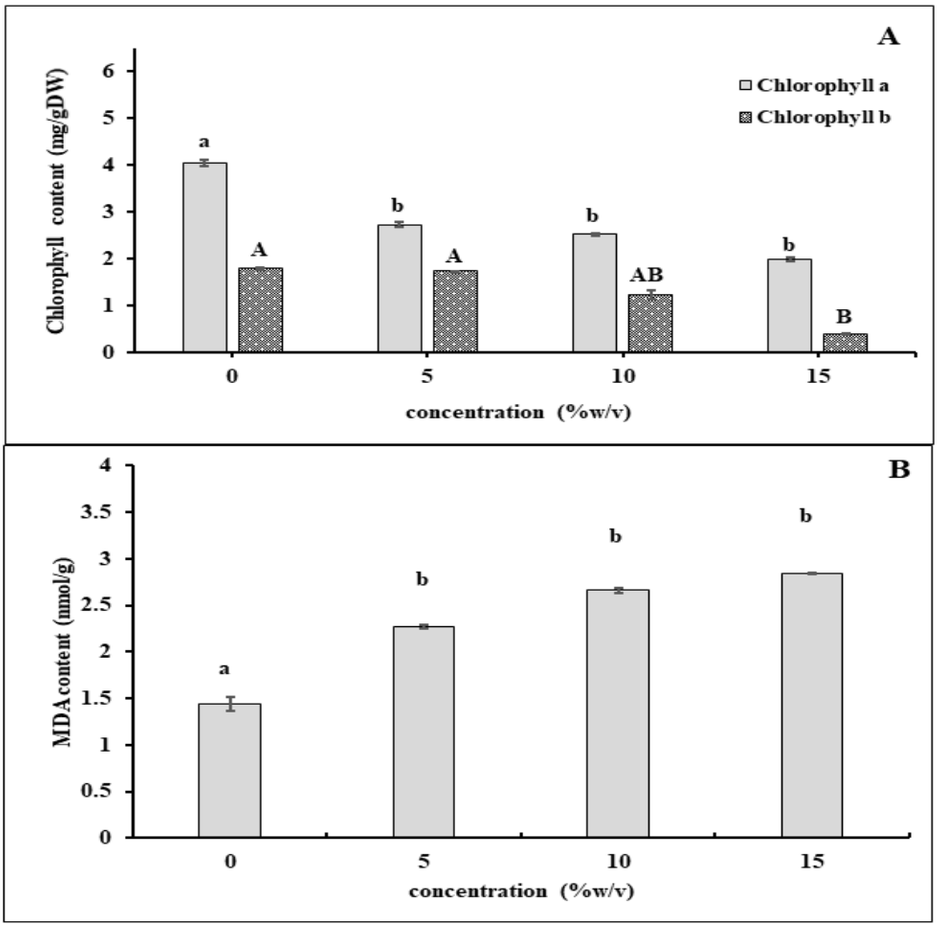

FIGURE 4. Effect of water hyacinth extract at various concentrations on chlorophyll (A) and MDA content (B) in *Mimosa pudica.* Every column in each graph represents the mean ±SD of three replicates. The different letter indicates significant differences at the 0.05 level compared to control

CONCLUSION

Based on the results of this study, the *E. crassipes* leaf extract inhibited the growth of *M. pudica* by reducing biomass, length, seedling vigor and chlorophyll content. It also induced lipid peroxidation in seedling roots. Hence, the highest concentrations of *E. crassipes* leaf extract (15% w/v) had a promised inhibitory effect on *M. pudica* growth. Further investigation is recommended to evaluate the effect of water hyacinth aqueous extract on biochemical responses in *M. pudica*, such as the activity of defense-related enzymes and lipid peroxidation. This could help in understanding the biochemical mechanisms of *E. crassipes* leaf extract against the growth of *M. pudica*. Furthermore, the field experiment needs to be considered for the agricultural application as the bioherbicide compared with the herbicide.

ACKNOWLEDGEMENTS

We are immensely grateful to the Department of Science, Faculty of Science and Technology, Prince of Songkla University Pattani campus for facilities support and the Language Center, Prince of Songkla University for proofreading this article.

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