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Effects of *Mimosa pigra* L. leaf extract on growth behavior of *Ruellia tuberosa* L. and *Echinochloa crus-galli* (L.) P. Beauv.

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Abstract

Mimosa pigra L. is an invasive species which could produce some biological compounds to control other plant species. This study investigated the potential role of leaf extracts of *M. pigra* L. in plant interference. Aqueous (water), 80% aqueous methanolic (80% MeOH) and methanolic (MeOH) extracts at different concentrations of 0, 1, 10 and 100 grams dry weight per liter were prepared and used as treatment solutions to assess their effects on the plant growth and the chlorophyll content of two target weed species, *Ruellia tuberosa* L. and *Echinochloa crus-galli* (L.) P. Beauv. The experiment was arranged as a 3 × 4 factorial in a completely randomized design and was carried out under greenhouse conditions. The results revealed that the three different solvent extracts inhibited the growth and decreased the chlorophyll accumulation in both tested weed species. The inhibition percentage in most cases for each growth parameter increased with increasing concentration of each different solvent extract. Among the three extracted solvents, the 80% MeOH and MeOH extracts showed greater efficiency than the water extract. Consequently, the *M. pigra* extracts had phytotoxic potential to control weed growth.

Keywords: *Mimosa pigra* L., *crude extract, phytotoxic activity*

1. Introduction

Weeds are the most severe and widespread biological constraint to crop production and cause invisible damage till the crop is harvested. Interference by weeds with agricultural crops causes huge economic losses to farmers in two ways; firstly, weeds reduce crop quality and quantity and secondly, they increase the cost of labor and herbicides used for weed control [1]. Furthermore, the use of herbicides is a problem with regard to health through malformation induced by genetic effects and to the environment through pollution [2]. Therefore, the biological control of weeds is a topic that has recently garnered much interest.

Plants offer a rich source of biologically active substances [3] and one group of these phytochemicals is the allelochemicals. Allelochemicals, secondary metabolites produced naturally by plants, serve a crucial role in the allelopathy phenomenon—any beneficial or deleterious effects of one plant on the growth or development of another [4]. Several researchers have suggested the use of extracted allelochemicals for weed control, used either directly as a crude preparation or as pure compounds, in suppression trials undertaken in the laboratory and also under field conditions [1, 5, 6, 7]. Natural compounds offer a possible alternative weed control that may contribute to reduce use of commercial herbicides [8]. In addition, natural products are considered to be more environmentally friendly than most synthetic compounds and are easily biodegradable [8, 9].

Recently, researchers have focused on invasive species to identify and isolate phytochemicals for their potential use as herbicides [10, 11, 12]. Allelochemicals released by nonlocal species were thought to be the primary reason that some species can invade new habitats and out-compete native species [13]. Consequently, allelochemicals from invasive species have the potential to be explored as a form of natural weed control.

Mimosa pigra L. is a giant sensitive plant belonging to the family Leguminosae and while native to Central America, it has invaded ecosystems worldwide, especially in parts of South East Asia and Australia and has been named as one of the top 100 world's worst invasive species [14]. Data from previous work indicated that *M. pigra* leaf methanolic extract inhibited the early seedling growth of popping pod under laboratory conditions [15].

Therefore, this study aimed to elucidate the inhibitory effects of different solvents extracts and different concentrations of *M. pigra* leaf extract on plant growth at maturation stage under greenhouse conditions. Such information should be beneficial for the application of *M. pigra* extract for weed control in agro-systems.

2. Materials and Methods

2.1 Plant materials

Fully grown, healthy leaves of *Mimosa pigra* L. at the vegetative stage were harvested in areas of Kochan district, Chonburi province, Thailand. The *M. pigra* leaf samples were air-dried in the shade with a well-ventilated place at an average temperature of 33 ± 3˚C for 14 d and then ground to a fine, uniform texture and kept in a desiccator until used. Two weed species—*Ruellia tuberosa* L. (popping pod) and *Echinochloa crus-galli* (L.) P. Beauv. (barnyard grass)—were chosen as dicotyledonous and monocotyledonous weed species, respectively. These two species are common weeds in Thailand. Seeds of *R. tuberosa* and *E. crus-galli* were collected from a natural population in Nakhon Pathom province, Thailand, from healthy and vigorous plants with good fruit production.

2.2 Preparation of extract

Stock extract solutions were prepared by soaking 50 g of leaf powder with 500 mL of three different solvents—distilled water (water), 80% (v/v) methanol (80% MeOH) and 100% (v/v) methanol (MeOH)—and placed on an orbital shaker at 50 rpm at room temperature under dark conditions for 48 h. Then, the three stock solutions were filtered through Whatman no. 1 filter paper and stock solutions of 100 gram dry weight per liter (g L^{-1}) of the three different solvents were obtained. For the methanolic extracts (80% MeOH and MeOH), the methanol was evaporated in a vacuum and the extract was then re-dissolved in distilled water. From the stock solutions, each different extract was diluted with distilled water to make a total of three concentrations, 1, 10 and 100 g L^{-1}, and distilled water was used as the control. The extracts were stored at 4 ˚C when not in use.

2.3 Effect of M. pigra extract on weed growth

Weeds were planted in plastic pots (8 cm deep, 10 cm wide) containing approximately 500 g of 50% soil mixed with 50% commercial growing medium. Twenty seeds of each of the two weed species were sown at a depth of 1 cm. Before sowing, the seeds were incubated at room temperature for 1-2 d until the radicle had protruded from the seed coat. After 10 d, when complete germination had been achieved, the number of seedlings per pot was reduced by careful manual thinning to 10 equally healthy seedlings. Five replications were prepared for each treatment along with the control. The pots were arranged in a 3 × 4 factorial design, where the first factor was the different solvent extract—water, 80% MeOH or MeOH—and the second factor was the four different concentrations—0, 1, 10 or 100 g L^{-1}—in a completely randomized design. The pots were placed in a greenhouse under natural solar radiation with an average temperature of 35 ± 5 ˚C and were irrigated daily with sufficient tap water. The extracts were applied as a foliar spray and Tween-20 was added as a spreading agent for each tested treatment. Plants were first sprayed 4 weeks after germination and four successive. Applications of each extract were carried out at 3-day intervals. The control plants were similarly sprayed with distilled water. After 2 d from the last application, the plants were carefully uprooted and washed with water and their root and shoot lengths, fresh and dry weights, as well as the chlorophyll content were measured.

2.4 Chlorophyll determination

One gram of leaf materials from the tested species of each treatment was extracted using 25 mL of 80% (v/v) acetone and filtered through Whatman no.1 filter paper. The filtrate was measured at 645 and 663 nm using a spectrophotometer (model S-20, BOECO, Germany) using 80% acetone as the blank. The chlorophyll content was calculated according to Arnon (16) as follows:

$$\text{Total chlorophyll} = 20.2A_{645} + 8.02A_{663} \times (V/1000 \times W)$$

where A_{645} and A_{663} are the absorbance at wavelengths of 645 and 663 nm, respectively, V is the final volume and W is the weight of the leaf sample.

2.5 Statistical analysis

All results were represented as a mean ± SE, with the inhibition percentage (%) calculated as 100 - [(treatment × 100)/ control]. Data were subjected to analysis of variance using the SPSS (version 15, SPSS Inc, Chicago, IL, USA) statistical program. Tukey's test was used to determine significant differences between treatment groups when the *F* test indicated a significant effect and was considered to be significant at the $P < 0.05$ level.

3. Results

The effects of different extracts from *M. pigra* at different concentrations on the growth and chlorophyll content of *Ruellia tuberosa* and *Echinochloa crus-galli* are shown in Table 1 and Table 2, respectively. The results showed that growth of both *R. tuberosa* and *E. crus-galli* was reduced and the chlorophyll contents were decreased after treatment with *M. pigra* crude extract. The inhibition effect was found to increase with increasing concentration of the different solvent extracts. With *R. tuberosa*, the highest inhibition occurred at 100 g L^{-1} of aqueous methanolic and methanolic extracts for all growth parameters (Table 1). With *E. crus-galli*, the highest inhibitory effects on the shoot length, fresh weight, dry weight and chlorophyll content were recorded for 100 g L^{-1} of methanolic extract (Table 2). Furthermore, the leaves of plants exposed to the extracts, especially at the highest concentration of methanolic extract, exhibited varying degrees of necrosis—the death of the cell in a plant tissue—and chlorosis—abnormal reduction of normal green coloration of leaves (Figure 1 and 2).

Among the solvents, 80% aqueous methanolic extract and the methanolic extract significantly inhibited *R. tuberosa* growth more than the aqueous extract but there was no significant difference in the chlorophyll content between the two methanolic extracts (Table 3). With *E. crus-galli*, the 80% aqueous methanolic and methanolic extracts significantly inhibited shoot length but only the methanolic extract significantly decreased the chlorophyll content whereas the aqueous extract decreased the dry weight more than other the two extracts did (Table 3).

4. Discussion

The *M. pigra* extract showed similar responses against the tested broad and narrow-leaved weeds. Crude preparations of *M. pigra* in different solvent extracts and at different concentrations inhibited the growth and reduced the chlorophyll content in both *R. tuberosa* and *E. crus-galli*. The overall inhibition percentages of the plant growth indices increased in almost all the treatments compared to the control. The reduction in biomass was correlated with the seedling height growth which may have been due to stunt and reduce plant growth. Moreover, once the chlorophyll content decreased, plant growth was also suppressed because chlorophyll is a photosynthetic pigment which is necessary for photosynthesis, an essential process of plant growth and development [17]. The inhibition of the tested weed plants indicated the accumulation of toxic substances in the extract which provided the phytotoxic activity of *M. pigra*.

The three extracts showed varying degrees of efficacy in each parameter. Methanolic extracts—80% aqueous methanol and methanol extracts—imposed stronger effects than the aqueous extract. This finding corroborated Harborne [18] who stated that alcohol is a good, all-purpose solvent for preliminary extraction due to its ability to extract all the low molecular weight compounds from plant tissue. Additionally, alcohol acts more efficiently on cell walls and causes polyphenols to be released from the cells [19]. Thus, it was inferred that methanol could more easily penetrate the cellular membrane to extract the bioactive compounds from the *M. pigra* leaf material than water.

Several studies have shown phytotoxic activity of invasive plants in germination and seedling development assay [7, 11, 20]. The current study is the first report of applying *M. pigra* extract in the maturation stage of tested species. This information should provide valuable knowledge for large-scale application under field conditions.

Previous studies reported the presence of flavonoid, quinone, saponin, sterol, tannin and phenolic compounds classes in *M. pigra* extract [21, 22]. Therefore, the phytotoxic property of the *M. pigra* extract might be due to the synergistic activity of the bioactive ingredients present in the extract. However, further isolation of the individual bioactive substances in *M. pigra* that are responsible for the phytotoxic effects of weeds should be clarified.

Table 1. Effects of *M. pigra* extract on *R. tuberosa* growth and the chlorophyll content.

Solvent	Concentration ($g\ L^{-1}$)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Chlorophyll content ($mg\ g^{-1}$)
Control		6.40 ± 0.19a[1]	14.00 ± 0.73a	10.07 ± 0.29a	2.33 ± 0.07a	0.66 ± 0.07a
Water	1	5.60 ± 0.32ab (12.50)[2]	12.96 ± 0.56ab (7.43)	8.58 ± 0.32b (14.78)	1.83 ± 0.08b (21.29)	0.51 ± 0.04ab (22.42)
	10	5.40 ± 0.18b (15.63)	12.80 ± 0.18ab (8.57)	7.50 ± 0.13bc (25.52)	1.45 ± 0.04c (37.68)	0.49 ± 0.04ab (25.76)
	100	4.40 ± 0.15bc (31.25)	12.10 ± 0.19abc (13.57)	6.08 ± 0.29d (39.66)	1.11 ± 0.10cde (52.27)	0.39 ± 0.04b (40.91)
80% MeOH	1	4.40 ± 0.18cd (31.25)	10.98 ± 0.44bc (21.57)	6.28 ± 0.33cd (37.62)	1.19 ± 0.08cd (48.76)	0.44 ± 0.03ab (33.03)
	10	4.44 ± 0.15cd (30.63)	11.04 ± 0.32bc (21.14)	4.20 ± 0.32e (58.29)	0.87 ± 0.13def (62.66)	0.40 ± 0.03b (39.09)
	100	3.80 ± 0.04cd (40.63)	11.10 ± 0.25bc (20.71)	3.60 ± 0.09e (64.27)	0.54 ± 0.04f (76.74)	0.34 ± 0.03b (49.09)
MeOH	1	4.28 ± 0.13cd (33.13)	11.72 ± 0.43abc (16.29)	4.72 ± 0.27e (53.18)	0.77 ± 0.10ef (66.87)	0.47 ± 0.03ab (29.09)
	10	3.74 ± 0.15d (41.56)	10.82 ± 0.62bc (22.71)	4.32 ± 0.18e (57.06)	0.79 ± 0.05ef (66.09)	0.38 ± 0.03b (41.82)
	100	3.54 ± 0.20d (44.69)	10.13 ± 0.52c (27.68)	3.89 ± 0.16e (61.39)	0.59 ± 0.06f (74.51)	0.30 ± 0.03b (54.24)

[1] The same lowercase letters in the same column are not significantly different at $P \geq 0.05$.
[2] Values are shown as mean ± SE and numbers in parentheses show the inhibition percentage (%) compared with the control.

Table 2. Effects of *M. pigra* extract on *E. crus-galli* growth and the chlorophyll content.

Solvent	Concentration ($g\ L^{-1}$)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Chlorophyll content ($mg\ g^{-1}$)
Control		18.82 ± 0.25a[1]	15.67 ± 0.22a	15.92 ± 1.02a	2.35 ± 0.14a	1.21 ± 0.06a
Water	1	17.24 ± 0.24b (8.40)[2]	13.07 ± 0.27b (16.61)	13.60 ± 0.66a (14.60)	1.60 ± 0.06cd (32.00)	1.19 ± 0.11a (2.00)
	10	16.90 ± 0.15bc (10.20)	10.70 ± 0.40de (31.72)	11.69 ± 0.33ab (26.57)	1.36 ± 0.04de (41.96)	0.89 ± 0.03bcd (26.78)
	100	16.88 ± 0.30bc (10.33)	10.96 ± 0.36cde (30.06)	12.18 ± 1.22ab (23.49)	1.26 ± 0.16de (46.49)	0.95 ± 0.07abc (21.14)
80% MeOH	1	17.78 ± 0.48ab (31.25)	12.18 ± 0.21bc (22.30)	13.92 ± 0.98a (12.59)	1.66 ± 0.07bcd (29.47)	1.21 ± 0.04ab (0.00)
	10	16.84 ± 0.41bc (10.52)	12.00 ± 0.29bcd (23.42)	14.88 ± 1.15a (6.56)	1.46 ± 0.12cde (37.87)	1.01 ± 0.05ab (16.63)
	100	16.62 ± 0.15bc (11.69)	10.04 ± 0.07e (35.93)	14.87 ± 1.31a (6.62)	1.70 ± 0.04bcd (27.74)	0.84 ± 0.06bcd (31.20)
MeOH	1	17.50 ± 0.42ab (7.01)	11.90 ± 0.16bcd (24.06)	15.82 ± 0.92a (0.64)	2.19 ± 0.12ab (6.89)	0.88 ± 0.03bcd (27.71)
	10	15.74 ± 0.13cd (16.37)	10.82 ± 0.33cde (30.95)	15.04 ± 0.65a (5.55)	2.00 ± 0.12abc (14.98)	0.68 ± 0.02cd (43.69)
	100	15.00 ± 0.41d (20.30)	10.60 ± 0.31de (32.35)	9.11 ± 0.44b (42.76)	1.02 ± 0.07e (56.51)	0.63 ± 0.06d (48.03)

[1] The same lowercase letters in the same column are not significantly different at $P \geq 0.05$.
[2] Values are shown as mean ± SE and numbers in parentheses show the inhibition percentage (%) compared with the control.

Table 3. Comparison of different solvents of *M. pigra* extracts on *R. tuberosa* and *E. crus-galli* growth.

Solvent	*R. tuberosa*					*E. crus-galli*				
	SL (cm)	RL (cm)	FW (g)	DW (g)	Ch ($mg\ g^{-1}$)	SL (cm)	RL (cm)	FW (g)	DW (g)	Ch ($mg\ g^{-1}$)
Water	5.49a[1]	12.97a	8.08a	1.68a	0.50[ns]	17.49a	12.16[ns]	13.53[ns]	1.66b	1.05a
80% MeOH	4.67b	11.78b	6.13b	1.24b	0.47	17.52b	12.12	14.90	1.86a	1.05a
MeOH	4.39b	11.74b	5.80b	1.12b	0.44	16.82b	11.87	13.98	1.89a	0.85b

SL, shoot length; RL, root length; DW, dry weight; FW, fresh weight; Ch, chlorophyll content
[1] The same lowercase letters in the same column are not significantly different at $P \geq 0.05$, ns indicate not significant

Figure 1. Effects of *M. pigra* methanolic extract (100% MeOH) at different concentrations on *R. tuberosa* growth. Arrow indicates necrosis and chlorosis.

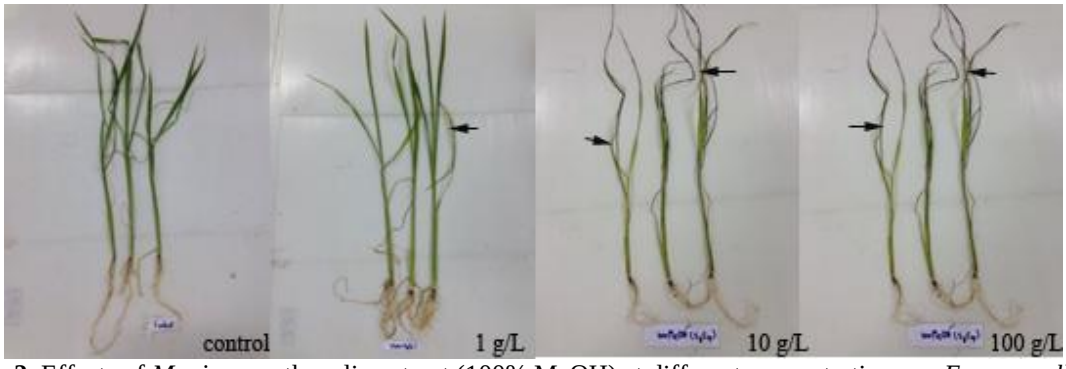

Figure 2. Effects of *M. pigra* methanolic extract (100% MeOH) at different concentrations on *E. crus-galli* growth. Arrow indicates necrosis and chlorosis.

5. Conclusion

The present study provided evidence of the phytotoxic potential of *M. pigra* extract. Methanol was a suitable solvent to extract the phytotoxic compounds. Accordingly, *M. pigra* extract may be a promising candidate for controlling weed species. Further application of *M. pigra* extract as a potential bio-herbicide for weed control should be tested on more different weed and crop species.

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