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Full Length Article

Allelopathic Effects of Giant Sensitive Plant (*Mimosa pigra*) Leaf Powder on Germination and Growth of Popping Pod and Purslane

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Abstract

The most of invasive alien species possess allelopathic properties for beneficial success in site establishment. Giant sensitive plant (*Mimosa pigra* L.) is an important invasive species in nature. The objective of this study was to evaluate the allelopathic activity of *M. pigra* leaf residues on the germination, survival percentage and growth in two weeds, namely popping pod (*Ruellia tuberosa* L.) and purslane (*Portulaca oleracea* L.). Experiments were carried out under laboratory (agar medium) and greenhouse (soil medium) conditions with the addition the *M. pigra* leaf powder at varying concentrations of 1, 2, 3 and 4% (w/w) into the agar and soil medium. Negative and positive effects of *M. pigra* leaf residues were assessed. The results under two conditions showed the same trend, namely that the *M. pigra* leaf samples expressed inhibitory effects on the germination, survival percentage and growth of popping pod and exhibited stimulatory effects on purslane for all parameters. The soil experiment showed more prominent effects than the laboratory bioassay, with the dry biomass of popping pod being decreased up to 70.27% whereas the dry biomass of purslane sharply increased up to 1,562.50% and the effects became stronger when the concentration of *M. pigra* increased. The results indicated that the allelopathic effects of *M. pigra* were species-specific and concentration-dependent. The *M. pigra* leaf residues could be considered as a source of biologically active compounds.

Keyword: *Mimosa pigra* L.; Allelopathy; Popping pod; Purslane

Introduction

Plants are essential elements of the Earth's ecosystems and provide a rich source of biologically active substances (Field *et al*., 2006). In their communities, they compete together for space, water, light and nutrient. Many plants, including weeds, can interact by releasing secondary metabolites or allelochemicals into the growth environment and this phenomenon is called allelopathy (Einhelling, 1996). Allelopathy is defined as direct or indirect harmful or beneficial effects of one plant on another through the production of allelochemicals released into the environment (Rice, 1984). The allelochemicals are released into the environment through foliar and other parts by leaching, volatile emission, root exudation and plant residues decomposition, thereby affecting the growth of adjacent plants (Weir *et al*., 2004; Field *et al*., 2006). Allelochemicals offer a vast virtually untapped reservoir of chemical compounds with many potential uses (Li *et al*., 2010). Allelopathic plants, can be used either as a cover crop or for plant residues incorporation and they can resist biotic stress such as weed infestation, insect pests and disease pathogens and can additionally build up fertility and organic matter in the soil, thereby improving plant yield (Farooq *et al*., 2011; 2013). The hypothesis has been suggested that allelochemicals from weed species have the potential to be explored as natural herbicides (Bhowmik and Inderjit, 2003). Some plants provided excellent weed control in intercropping or as soil additives (Farooq *et al*., 2011). In addition, natural compounds are considered to be safe and beneficial to the environment and mankind compared to most synthetic compounds (Duke *et al*., 2000).

Most invasive plants or alien species can produce allelochemicals, which have a detrimental effect on the growth of competing for vegetation by providing a competitive advantage for the invader (Li *et al*., 2010). Allelopathic activity of various invasive plant species has been reported such as *Centaurea maculosa* Lam. (Bais *et al*., 2003), *Solidago canadensis* L. (Bing-yao *et al*., 2006) and *Tagetes minuta* L. (Sadia *et al*., 2015).

Mimosa pigra L. or giant sensitive plant belonging to the family Leguminosae is native to Central America but has invaded ecosystems worldwide, especially in parts of Southeast Asia and Australia and this species is listed in the 100 world's worst invasive species (Global Invasive Species Database, 2016). Phytochemical screenings of *M. pigra* showed the presence of flavonoid, quinone, saponin, sterol,

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tannin and phenolic compounds (Rosado-Vallado *et al*., 2000; Rakotomalala *et al*., 2013). Due to its potential for spread and the presence of specific phytochemicals, *M. pigra* could have allelopathic attributes. Being an allelopathic potential, it can play a vital role in controlling plant growth. To date, no research has been reported on the allelopathic characteristics of *M. pigra* residues. This study was, therefore, conducted to evaluate the allelopathic potential of *M. pigra* leaf residues on germination and growth of the other plants by mixing the leaf powder in the growth medium.

Materials and Methods

Plant Materials

Fresh mature green leaves of the entire tree of *Mimosa pigra* L. (age 2-3 years) were collected during its vegetative stage from its natural habitats, in areas of Kochan district, Chonburi province, Thailand. The leaves of *M. pigra* were air-dried in the shade with a well-ventilated place at an average temperature of 33 ± 3ºC then ground to a fine uniform texture and stored in a poly bag until further use. Popping pod (*Ruellia tuberosa* L.) and purslane (*Portulaca oleracea* L.) were chosen as the tested plant species. Seeds of popping pod and purslane were collected from a natural population in Nakhon Pathom province, Thailand, selecting healthy and vigorous plants with good fruit production.

Laboratory Bioassay

The allelopathic effects of *M. pigra* leaf samples were studied using the sandwich method developed by Fujii *et al*. (2003) with some modifications. Agar (0.4% w/v) was prepared as a growth medium and then 1 mL of agar solution was added into each well of six-well, multi-dish plastic plates (size 8.25 × 12.5 × 2.0 cm, Corning Incorporated, USA). After gelatinization of the agar, leaf samples were added into each well on the surface of the agar according to Table 1. Agar solution was then added in two layers (each 2 mL) onto the dried leaves. For the germination test, 10 and 20 seeds of popping pod and purslane, respectively, were sown in each well. Then, the plates were covered with a plastic lid and kept in dark condition with an average temperature of 28 ± 2ºC. The control was prepared exactly the same as described above but without plant samples between agar layers. After 7 d, the germination was determined in terms of the number and length of the radicle. A seed was considered germinated when its radicle was longer than 2 mm. The germination percentage was calculated according to the equation; (number of germinated seeds/total seeds) × 100%.

For the evaluation of seedling growth, 10 and 20 seedlings, each with 3 mm of radicle length, of popping pod and purslane, respectively were arranged in each well using the same procedure as for the germination bioassay. After 10 d, survival percentages were recorded using the formula (number of survival seedlings/total seedlings) × 100%. Sampling was conducted of the seedlings of popping pod and purslane (5 and 10 plants per replication, respectively) and the root and shoot lengths as well as fresh and dry weights were measured. Three replications were performed for each treatment.

Pot Experiment

Soil additive *M. pigra* leaf materials were prepared. Soil samples (300 g) were dried and crushed and mixed with the *M. pigra* leaf samples at the established dose rates (Table 1) and placed into plastic pots (diameter 10 cm). The control was applied similarly but without leaf samples. For the germination test, 10 and 20 seeds of popping pod and purslane, respectively were placed in each pot and adequate water was supplied. The pots were placed in a greenhouse under natural solar radiation with an average temperature of 35 ± 5ºC. After 10 d, germination percentages were recorded.

For the growth test, 10 and 20 seedlings each with 3 mm of radicle length, of popping pod and purslane, respectively, were placed in each pot. Soil moisture was maintained by applying daily irrigation with sufficient water. After 30 d, survival percentages were recorded using the equation previously described. The plants were carefully uprooted and washed with water. Then, using a sample of popping pod and purslane at 5 and 10 plants per replication, respectively, the root and shoot lengths as well as fresh and dry weights were measured. The experiments were carried out in four replications.

Statistical Analyses

Each experiment was staged in a completely randomized design (CRD) and each was repeated twice. All results were determined as means ± SE to indicate variation. Inhibition and stimulation percentages were calculated as follows: 100 - [(treatment × 100)/control], using - and + symbols to indicate inhibitory and stimulatory effects, respectively and were presented in parentheses. For statistical analyses, data were analyzed using ANOVA and relationships were considered to be significant when $P < 0.05$. Differences between the control and samples were determined using Duncan's multiple range test in the R program (R Core Team, 2015).

Results

Allelopathic Effects of *M. pigra* Leaf Residues on Germination

Effects of *M. pigra* leaf residues on popping pod and purslane germination are shown in Fig. 1. Leaf residues of *M. pigra* had allelopathic effects by inhibiting the germination in popping pod but increasing the germination percentage in purslane in both the laboratory (Fig. 1A) and greenhouse (Fig. 1B) experiments.

Table 1: Residue concentrations of *M. pigra* applied to weeds

Leaf weight (g)		Equivalent concentration (%)
Laboratory experiment (agar volume 5 mL)	Greenhouse experiment (soil amount 300 g)	
0.05	3.0	1
0.10	6.0	2
0.15	9.0	3
0.20	12.0	4

The inhibitory and promotive effects of the leaf litter were concentration-dependent, with higher concentrations showing significantly greater effectiveness (Fig. 1). The greatest effect was shown at the highest concentration (4%). The germination percentage of popping pod was decreased up to 81.47% and 52.17% compared with the control under laboratory and greenhouse conditions, respectively. The germination percentage of purslane was increased up to 39.99 and 86.92% compared with the control under laboratory and greenhouse conditions, respectively.

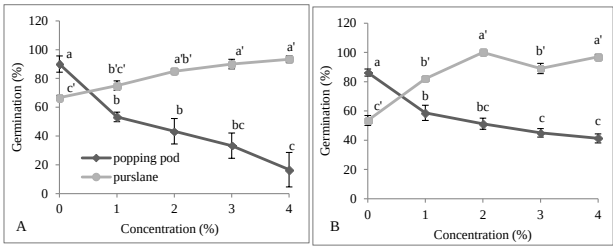

Fig. 1: Effects of *M. pigra* leaves on germination of popping pod and purslane in laboratory (A) and greenhouse (B) experiments. Data points marked with the same letters are not significantly different at P≥0.05

Allelopathic Effects of *M. pigra* Leaf Residues on Survival Percentage

Using the sandwich method, the activity of *M. pigra* leaves significantly decreased the survival percentage of popping pod and significantly increased it in purslane (Fig. 2A). The effect increased as the concentration increased and the strongest effect was observed in the concentration of 4%, 80.00% decreased and 45.94% increased in popping pod and purslane, respectively. Similar results were produced in the soil test, survival percentage of popping pod was significantly decreased whereas it increased in purslane after treatment using a concentration of 2, 3 and 4% (Fig. 2B). The survival percentage of popping pod was reduced up to 55.00% after being treated with the highest concentration and the survival percentage of purslane rose to 53.85% in the treatment of using 2% dry matter.

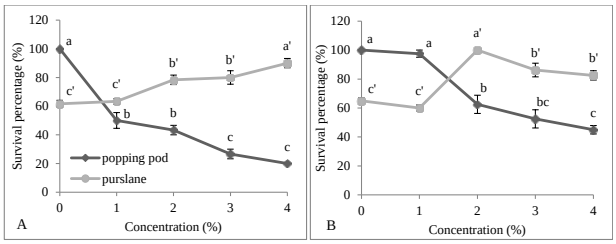

Fig. 2: Effects of *M. pigra* leaves on survival percentage of popping pod and purslane in laboratory (A) and greenhouse (B) experiments. Data points marked with the same letters are not significantly different at P≥0.05

Allelopathic Effects of *M. pigra* Leaf Residues on Plant Growth

Under laboratory conditions, the *M. pigra* leaves retarded the growth of popping pod and significantly inhibited shoot and root length by 44.71% and 85.19%, respectively, after treatment with the 4% concentration of leaf litter but no significant different in fresh and dry weights (Table 2). On the contrary, the leaf triggered the growth of purslane, with a significantly increased shoot length up to 30.13% and fresh weight up to 98.58% in the culture of 2% and 3%, respectively (Table 3). Similar to the bioassay results, the soil supplemented with the *M. pigra* leaf powder apparently hindered the growth of popping pod (Table 4). This reduction depended on the amount of the applied plant materials, with the highest value of residues (4%) producing 46.13, 57.96, 73.91 and 70.27% reduction in shoot length, root length, fresh weight and dry weight, respectively (Table 4). In the pot experiment of purslane, similar observations to the bioassay results were also recorded. The *M. pigra* leaf residues clearly significantly improved the growth of purslane and the degree of promotion became larger as the amount of leaf residue increased. The plant height and weight were significantly increased more than 300 and 700%, respectively compared with the control and the highest concentration of soil supplemented with leaf powders raised the dry weight up to 1,562.50% (Table 5).

Discussion

The experiment clearly demonstrated that *M. pigra* leaf residues contained potent allelopathic substances and could secrete into the medium, agar and soil. Allelopathic activity of the leaf residues had both inhibitory and stimulatory effects depending on the target plant species.

Table 2: Effects of *M. pigra* leaves on popping pod growth in laboratory experiment

Concentration (%)	Shoot length (mm)	Root length (mm)	Fresh weight (mg)	Dry weight (mg)
0	9.04 ± 0.92ab[1]	23.22 ± 1.24a	17.60 ± 1.39[ns]	0.70 ± 0.38[ns]
1	11.11 ± 2.51ab (+22.85)[2]	9.56 ± 1.64b (-58.83)	14.95 ± 3.48 (-15.06)	0.80 ± 0.27 (+14.28)
2	14.33 ± 2.31a (+58.45)	8.78 ± 2.11bc (-62.19)	16.30 ± 3.47 (-7.39)	0.60 ± 0.58 (-14.28)
3	6.67 ± 2.19b (-26.28)	4.44 ± 1.13cd (-80.88)	13.95 ± 3.56 (-20.74)	0.60 ± 1.41 (-14.28)
4	5.00 ± 0.96b (-44.71)	3.44 ± 0.11d (-85.19)	13.17 ± 0.82 (-25.17)	0.70 ± 0.09 (0.00)

[1]Value represent mean ± SE and the same lowercase letter in the same column are not significantly different at P≥0.05, [ns] indicates not significant
[2]Numbers in parentheses with – and + are the inhibition and stimulation percentage compared with the control, respectively

Table 3: Effects of *M. pigra* leaves on purslane growth in laboratory experiment

Concentration (%)	Shoot length (mm)	Root length (mm)	Fresh weight (mg)	Dry weight (mg)
0	6.97 ± 0.62b[1]	12.05 ± 0.52[ns]	14.10 ± 2.64b	0.50 ± 0.06[ns]
1	8.21 ± 2.11ab (+17.79)[2]	12.16 ± 1.51 (+0.91)	23.10 ± 0.95ab (+63.83)	0.66 ± 0.08 (+32.00)
2	9.07 ± 0.95a (+30.13)	13.39 ± 3.21 (+11.12)	20.00 ± 0.58ab (+41.84)	0.50 ± 0.08 (0.00)
3	8.87 ± 1.12a (+27.26)	13.44 ± 0.89 (+11.53)	28.00 ± 0.99a (+98.58)	0.64 ± 0.09 (+28.00)
4	8.80 ± 1.11ab (+26.26)	13.39 ± 1.67 (+11.12)	24.80 ± 3.21a (+75.89)	0.76 ± 0.13 (+52.00)

[1]Value represent mean ± SE and the same lowercase letter in the same column are not significantly different at P≥0.05, [ns] indicates not significant
[2]Numbers in parentheses with + is the stimulation percentage compared with the control

Table 4: Effects of *M. pigra* leaves on popping pod growth in greenhouse experiment

Concentration (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
0	1.84 ± 0.15a[1]	9.92 ± 0.45a	1.12 ± 0.10a	0.11 ± 0.02a
1	1.90 ± 0.06a (+2.96)[2]	7.16 ± 0.31b (-27.77)	0.69 ± 0.06b (-38.20)	0.10 ± 0.01a (-14.41)
2	1.36 ± 0.11b (-25.94)	5.39 ± 0.39c (-45.61)	0.36 ± 0.03c (-67.94)	0.04 ± 0.00b (-66.67)
3	1.06 ± 0.07bc (-42.66)	4.29 ± 0.39c (-56.73)	0.31 ± 0.04c (-72.13)	0.03 ± 0.01b (-69.37)
4	0.99 ± 0.07c (-46.13)	4.18 ± 0.73c (-57.96)	0.29 ± 0.02c (-73.91)	0.03 ± 0.00b (-70.27)

[1]Value represent mean ± SE and the same lowercase letter in the same column are not significantly different at P≥0.05
[2]Numbers in parentheses with – and + are the inhibition and stimulation percentage compared with the control, respectively

Table 5: Effects of *M. pigra* leaves on purslane growth in greenhouse experiment

Concentration (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
0	1.03 ± 0.02c[1]	1.11 ± 0.03c	0.25 ± 0.01d	0.01 ± 0.00c
1	4.13 ± 0.30b (+307.75)[2]	5.22 ± 0.23b (+371.95)	3.27 ± 0.13c (+1,229.27)	0.07 ± 0.13b (+737.50)
2	5.33 ± 0.18a (+418.39)	7.25 ± 0.67a (+555.66)	6.69 ± 0.43a (+2,617.48)	0.11 ± 0.03ab (+1,225.00)
3	4.80 ± 0.16ab (+366.73)	5.34 ± 0.07b (+383.26)	4.95 ± 0.12b (+1,910.16)	0.13 ± 0.02ab (+1,475.00)
4	4.32 ± 0.26b (+319.75)	5.77 ± 0.31b (+421.90)	5.78 ± 0.77ab (+2,250.81)	0.14 ± 0.02a (+1,562.50)

[1]Value represent mean ± SE and the same lowercase letter in the same column are not significantly different at P≥0.05
[2]Numbers in parentheses with + is the stimulation percentage compared with the control

Overall, *M. pigra* leaf litter showed effective suppression of germination, survival percentage (Fig. 1 and 2) and growth of popping pod (Table 2 and 4) but improved germination and survival percentage (Fig. 1 and 2) as well as prominently increasing the biomass of purslane by the stimulation of all measured growth parameters (Table 3 and 5). In most cases, the allelopathic effects of *M. pigra* were dependent on the concentration, with inhibition and stimulation increased as the amount of leaf powder increased. These findings coincided with Gilmore (1999) who claimed that allelopathy includes both inhibitory and promoting activities and is a concentration-dependent phenomenon. The growth of the two target plant species in the presence of *M. pigra* leaf samples at different concentrations indicated the response was species-specific and concentration-dependent as well as depending on the properties of the allelochemicals. Nekonam *et al*. (2014) suggested that variability in allelopathic expression in a plant might be due to the different nature of allelochemicals released by the species. There are various direct modes of action of allelochemicals on plant growth and metabolism, cell division, cell elongation, membrane permeability, mineral uptake, photosynthesis, respiration and specific enzyme activity, whereas there are indirect effects on soil properties and nutrient cycling (Rice, 1984; Weir *et al*., 2004; Farooq *et al*., 2013; Hernández-Aro *et al*., 2016).

From the results, *M. pigra* leaf powder showed both inhibition and stimulation effects on the germination and plant growth. Allelopathic plant residues mixed in the soil as powder obstructed seed germination and inhibited the growth of weeds through the release of allelochemicals (Teasdale and Mohler, 2000; Lin *et al*., 2003) or owing to excess soil moisture and increased nitrogen immobilization (Bradow, 1993). On the other hand, the

addition of plant residues into the soil might be providing nutrients, adding organic matter, improving the soil structure, regulating the soil temperature, conserving soil moisture and enhancing biological activities resulting in the stimulation of plant germination and growth (Farooq *et al*., 2011; 2013).

Based on the current results, a comparison of the plant growth parameters of the two experiments indicated that the allelopathic effects of leaf samples in the pot experiment were stronger than in the laboratory experiment. This might have been due to an association between the allelochemicals and the soil properties (Bhadoria, 2011). Hernández-Aro *et al*. (2016) reported that allelochemicals from plant residues were changed by soil microbial populations and this affected soil physicochemical properties including an organic material, electrical conductivity and the pH value. Therefore, the allelopathic effects on germination and plant growth by the *M. pigra* leaf litter might be attributed to both allelopathic effects and their physical presence in the soil or to soil properties associations. However, the fact that the results of the laboratory bioassay and the soil experiment showed the same trend, suggested that the mechanisms involved were principally from allelopathic effects rather than due to an impact on soil properties.

Several studies have shown that various allelopathic plants caused both suppression and stimulation of growth of the tested species (Hong *et al*., 2004; Batish *et al*., 2007; Hernández-Aro *et al*., 2016). However, the results obtained in this study are the first report on the allelopathic potential of *M. pigra*. Many legume species, including sunhemp (*Crotalaria juncea* L.), yellow sweet clover (*Melilotus officinalis* (L.) Pall.), cowpea (*Vigna unguiculata* (L.) Walp.), alfalfa (*Medicago sativa* L.), velvet bean (*Mucuna pruriens* (L.) DC.) and red clover (*Trifolium pratense* (Linn.)) are mainly considered as a cover crop in agrosystems to control weeds, to conserve soil, to suppress insects, nematodes and other disease pathogens and to enhance nutrient recycling (Farooq *et al*., 2011).

The results indicated that *M. pigra* had strong allelopathic activity and may secrete some allelopathic substance into the environment. Thus, *M. pigra* may be a candidate for the source of novel biologically active compounds. At present, the active allelochemicals of *M. pigra* have not yet been identified. However, Rosado-Vallado *et al*. (2000) and Rakotomalala *et al*. (2013) reported the presence of flavonoid, quinone, saponin, sterol, tannin and phenolic compounds in *M. pigra*. Khaliq *et al*. (2011) recommended that allelochemicals are usually more effective at influencing the target in a mixture than as individual compounds and Jabran *et al*. (2015) also suggested that allelopathic activity and allelopathic effects are often due to the synergistic activity of allelochemicals rather than to a single compound because of the diversity of allelochemicals in plants. However, further isolation of the individual allelopathic substances in *M. pigra* responsible for the allelopathic effect of weed is a challenging and meaningful task. Moreover, further studies of the nature of allelopathic compounds present in the soil and their effects should be clarified before these compounds are recommended for large-scale application under field conditions.

Conclusion

M. pigra leaf powder decreased germination and growth of popping pod but increased it in purslane. The responses were species-specific and concentration-dependent. This revealed that the *M. pigra* leaf residues have the potent allelopathic activity that effectively controlled germination and plant growth through both inhibitory and stimulatory effects. Accordingly, *M. pigra* may be a promising candidate for sourcing novel, biologically active compounds as well as for soil additive materials.

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