Sains Malaysiana 45(4)(2016): 517–521

Allelopathic Potential of the Leaf and Seed of *Pueraria javanica* Benth. on the Germination and Growth of Three Selected Weed Species

(Potensi Alelopati Daun dan Benih *Pueraria javanica* Benth. ke atas Percambahan dan Tumbesaran Tiga Spesies Rumpai)

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ABSTRACT

Pueraria javanica *Benth. is one of the most common leguminous cover crop used in oil palm plantations of Malaysia. A study was conducted to determine the allelopathic potential of this plant, using the aqueous extract, sandwich and dish-pack methods, with the seed and leaf (of* P. javanica*) on three bioassay weed species namely,* Eleusine indica, Cyperus iria *and* Chromolaena odorata. *The aqueous extract experiment was conducted using 0 (control), 16.7, 33.3 and 66.7 g/L of the aqueous leaf and seed extracts while the sandwich method was carried out using 10 and 50 mg of each of the donour plant parts. Meanwhile, the dish-pack method was done using four different distances (41, 58, 82 and 92 mm) away from the donour plant. All experiments were replicated five times using the complete randomized design (CRD). The leaf extract exhibited 100% reduction on the fresh weight of* E. indica *and* C. odorata *while the seed extract exhibited 100% reduction on all parameters for* E. indica *and on the fresh weight of* C. iria *at 66.7 g/L concentration. The seed and leaf at 10 and 50 mg significantly reduced the radicle length of all the bioassay species. The dish-pack experiment also showed a reduction effect on the germination percentage and seedling growth parameters of all the bioassay species. However, the reduction effect was not totally in accordance to the distance from the donor species. More studies need to be conducted to determine the type of reduction mechanism involved in the allelopathic activity especially with respect to molecular and biochemical aspects.*

Keywords: Allelopathy; aqueous extract; dish-pack method; Pueraria javanica; *sandwich method*

ABSTRAK

Pueraria javanica *Benth. merupakan salah satu tumbuhan penutup bumi yang popular dan banyak digunakan di dalam ladang kelapa sawit di Malaysia. Suatu kajian telah dijalankan untuk menentukan potensi alelopati biji benih dan daun* P. javanica *terhadap tiga spesies bioasai rumpai iaitu* Eleusine indica, Cyperus iria *dan* Chromolaena odorata *melalui kaedah ekstrak akuas bertindih dan kotak petri. Eksperimen ekstrak akuas dijalankan dengan menggunakan tiga kepekatan ekstrak biji benih dan daun iaitu 16.7, 33.3 dan 66.7 g/L, manakala eksperimen bertindih menggunakan 10 dan 50 mg daripada setiap bahagian. Sementara itu, eksperimen kotak petri menggunakan empat jarak yang berbeza (41, 58, 82 dan 92 mm) daripada tumbuhan penderma. Semua eksperimen dilakukan sebanyak lima replikasi dengan menggunakan reka bentuk rawak lengkap. Ekstrak daun telah menunjukkan pengurangan sebanyak 100% terhadap berat basah* E. indica *dan* C. odorata, *manakala ekstrak biji benih pula menunjukkan 100% pengurangan terhadap semua parameter* E. indica *dan berat basah* C. iria *pada kepekatan 66.7 g/L. Biji benih dan daun pada kedua-dua timbangan telah mengurangkan panjang radikel semua spesies bioasai secara signifikan. Eksperimen kotak petri juga menunjukkan kesan pengurangan terhadap peratus percambahan dan semua parameter pertumbuhan anak benih spesies bioasai. Walau bagaimanapun, kesan pengurangan tersebut tidak selari dengan jarak daripada spesies penderma. Lebih banyak kajian perlu dijalankan untuk menentukan jenis mekanisme pengurangan yang terlibat dalam aktiviti alelopati terutamanya daripada aspek molekul dan biokimia.*

Kata kunci: Alelopati; ekstrak akuos; kaedah bertindih; kaedah kotak petri; Pueraria javanica

INRODUCTION

The excessive use of pesticides in agriculture has led to the contamination of surface and ground water sources (Worsham 1989). Thus, researchers are currently paying more attention to sustainable and environmentally-friendly farming methods. This can be achieved by applying the allelopathy concept into farming activities. Allelopathy is the effect of one plant onto the other directly or indirectly (including microbes) via the production of chemical compounds that are released into the environment (Rice 1984). Allelopathic plants can suppress weeds with their own naturally occurring plant chemicals. Recently, studies on cover crops have been getting attention due to their ability to suppress weeds as well as, provide other beneficial effects such as nutrient cycling and soil improvement. The ability of cover crops

to suppress weeds is believed to be due to the release of biologically active compounds found in plants that are fundamental in allelopathy (Putnam & De Frank 1979; White et al. 1989). *Pueraria javanica* is one of the tropical leguminous cover crops which is commonly planted in oil palm plantations to reduce soil erosion, fix nitrogen (Magdoff & Weil 2004) and suppress weed growth (Deuss 1967). It is an evergreen, perennial, low herbaceous plant mainly found in Southeast Asia (Imam Purwanto 2007). It has been reported that plants of the genus *Pueraria* possess some biologically active ingredients that have been found to be useful Chinese herbal medicine for the relief of fever, vomiting, non-specific intoxication and bleeding wounds (You et al. 2002). They are reported to contain a high level of isoflavone (You et al. 2002) and phenolic acid (Samedani et al. 2013). However, more studies are needed in order to assess the allelopathic activity of *P. javanica* and its potential for development as a natural herbicide. Thus, the present study was carried out to investigate the allelopathic potential of the leaves and seeds of *P. javanica* on the germination and growth of three of the world's most noxious weeds, through the aqueous extraction, the sandwich and the dish-pack methods.

MATERIALS AND METHODS

PLANT MATERIALS

The leaves of *Pueraria javanica* were collected at the flowering stage from the Ulu Bernam Estate of the United Plantations in Selangor, Malaysia while the seeds of *P. javanica* were obtained from the National Farmers Organization of Malaysia in March 2014, and were dried in a hot-air oven at 45°C for 3 days. Seeds of the bioassay species namely *Eleusine indica* and *Cyperus iria* were purchased from 'Herbiseeds' (www.herbiseeds.com) located at W End, Reading RG10 0NJ in the United Kingdom while *Chromolaena odorata* seeds were collected from Sungkai in Perak, Malaysia.

EFFECT OF THE LEAF AND SEED AQUEOUS EXTRACTS OF *P. JAVANICA* ON THE GERMINATION AND GROWTH OF THE BIOASSAY SPECIES

The following experiment was conducted to determine whether the leaf and seed of *P. javanica* contained water-soluble phytotoxic constituents. Ten grams of the dried leaf/seed of *P. javanica* were immersed in a flask containing 150 mL distilled water and agitated for 24 hours using an orbital shaker at room temperature (28°C). The extract was centrifuged for 15 minutes and filtered through Whatman No.2 filter paper and the micropore filter (0.2 µm) using a filter pump. Three concentrations of the aqueous extract (66.7, 33.3 and 16.7 g L^{-1}) were prepared for the experiment (Ishak et al. 2016). Ten seeds each of the bioassay species namely *E. indica, C. iria* and *C. odorata* were placed separately in petri dishes (9 cm diameter) lined with filter paper (Whatman No.1), moistened with 5 mL of each extract concentration, while distilled water was used for the control. The petri dishes were placed in a completely randomized design in an incubator set at temperature 28°C (±2) and photoperiod of 12 h light followed by 12 h darkness. The treatments were replicated five times. Germination percentage, fresh weight and seedling length of the bioassay species were recorded after 7 days of incubation. The fresh weight, root and shoot length of the seedlings were expressed as percentage of the control (distilled water).

EFFECT OF THE LEAF AND SEED LEACHATE OF *P. JAVANICA* ON THE GERMINATION AND GROWTH OF THE BIOASSAY SPECIES USING THE SANDWICH METHOD

The following experiment was conducted to determine the allelopathic activity of the leachate from the donor plant leaves (Fujii 1994), as well as the seeds of *P. javanica*. A total of 10 mg per well (of dried leaves) were placed into five wells of the six-well (around 10 cm^2 area per well) multi-dish plastic plate (35 × 18 mm, Therma Fisher Scientific Inc.). The above mentioned procedure was repeated using 10 mg of seeds and 50 mg of leaves and seeds. Agar with no treatment was used as control. Agar powder (Nacalai Tesque Inc.) was used as the growth medium (0.75% w/v). 5 mL of agar was added into each well causing the donor plant materials to rise up and the agar to solidify before another 5 mL of agar was added over it. Five seeds of each bioassay species were placed on the surface. The multi-dish was sealed with cellophane adhesive tape, labelled and incubated at temperature 28°C (± 2) and photoperiod of 12 h light followed by 12 h darkness. The treatments were replicated five times. The germination percentages as well as the hypocotyl and radicle lengths of the seedlings were recorded after 7 days. Data on the length of seedlings was used to calculate the percentage elongation (compared to control).

EFFECT OF LEAF AND SEED VOLATILIZATION OF *P. JAVANICA* ON THE GERMINATION AND GROWTH OF THE BIOASSAY SPECIES (DISH-PACK METHOD)

The dish-pack experiment was carried out to determine the presence of volatile compounds from the donor plant. 200 mg of dried leaves and seeds were placed into one of the six-well-multi-dish plastic plate (35 × 18 mm, Thermo Fisher Scientific Inc.). Filter paper was placed into each of the other five wells followed by 0.7 mL of distilled water and five seeds of each of the bioassay species. Each well represented a different distance (41, 58, 82 and 92 mm) from the donor plant. The multi-dish was sealed with cellophane adhesive tape, labelled and incubated at temperature 28°C (±2) and photoperiod of 12 h light followed by 12 h darkness. The treatments were replicated five times. The germination percentages as well as the length of the hypocotyl and radicle (bioassay seedlings) were measured on the fifth day. The hypocotyl and radicle lengths were expressed as percentage of the control.

Statistical Analysis

The experimental data recorded was subjected to the analysis of variance (one-way ANOVA) using the software SPSS Version 20 and Microsoft Excel. Means were compared using the Duncan Multiple range test (at 5% level of significance).

Results and Discussion

Effects of the aqueous extracts of the leaf and seed of *P. javanica* on the three bioassay weed species

As shown in Table 1, the aqueous extracts of the leaf and seed of *P. javanica* showed progressive reduction effects on the seedling growth of all the three bioassay species as the concentration increased. The leaf extract exhibited 97.8% and 97% reduction on the fresh weight of *E. indica* and *C. odorata* while the seed extract exhibited 100% reduction on the shoot length, radicle length and fresh weight of *E. indica* and 97.9% on the fresh weight of *C. iria* at the concentration of 66.7 g/L. However, at 16.7 g/L concentration, the seed extract significantly increased the shoot length and fresh weight of *C. odorata* by 49.5 and 31%, respectively (compared to control). Germination percentage of the bioassay species was also progressively reduced by both the leaf and seed extracts of *P. javanica* as the concentrations increased. The seed extract showed complete reduction of the germination percentage of *E. indica* at 66.7 g/L concentration while the leaf extract significantly reduced the germination percentage of *E. indica* and *C. odorata* at 66.7 g/L concentration. The above results indicated that the leaf and seed of *P. javanica* contain some water-soluble allelochemicals that can affect the growth and germination of the bioassay species. The degree of reduction by both extracts became progressively higher as the concentration increased. Both extracts significantly reduced the growth of all the bioassay species at the highest concentration but there were some stimulationary effects at the 16.7 g/L concentration. This showed that the effect of the extracts was concentration dependent as reported by Singh et al. (2006), where biological activities of the receiver plants to allelochemicals were found to be concentration dependent with a response threshold. The results obtained were congruent with the reported data of Ismail and Nornasuha (2014) where the lowest concentration of the leaf extract of *M. micrantha* caused significant increase in fresh weight of *E. indica*. The stimulation of weed growth at 16.7 g/L concentration might also be due to the hormetic effect whereby a low dose of toxicants can cause stimulatory effects (Calabrese & Baldwin 2002). In the present study, the three weed species responded differently to each extract. *Chromolaena odorata* was more sensitive to the leaf extract while *C. iria* was more sensitive to the seed extract. This demonstrated that the allelopathic constituents of the plant species showed selective activity. The data indicated that the leaf extract possessed more inhibitory effects than the seed extract. This may be due to the water-soluble allelochemicals present in the leaf extract that could have caused greater inhibitory effects on the bioassay species compared to those from the seed extract. Further studies need to be carried out in order to determine the type and content of the allelochemicals involved.

Effect of the leaf and seed leachate of *P. javanica* (sandwich method) on the three bioassay weed species

The radicle length of all the bioassay species were significantly reduced (compared to control) by 10 and 50 mg of both parts of the donor species (Table 2). The reduction by

TABLE 1. Effect of the aqueous extracts of the leaf and seed of *P. javanica* on the germination and seedling growth (% of the control) of *E. indica*, *C. iria* and *C. odorata*

Extract conc. (g/L)	Shoot length		Radicle length		Fresh weight		Germination percentage	
	L	S	L	S	L	S	L	S
E. indica								
0 (control)	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	80a	80a
16.7	136.0a	88.4a	84.2a	113.4a	68.2b	41.0b	70a	38a
33.3	49.4b	43.9b	24.1b	56.5a	31.0c	20.0c	22c	14b
66.7	6.1c	0.0c	1.6b	0.0b	2.2d	0.0c	4d	0c
C. iria								
0 (control)	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a	96a	96a
16.7	107.8a	62.1b	69.4b	104.8a	88.2a	42.1b	74b	48b
33.3	36.7b	38.4c	33.3c	41.3b	31.0b	26.3b	26c	38b
66.7	11.5c	1.5d	6.7d	1.6c	13.1b	2.1c	14c	4c
C. odorata								
0 (control)	100.0a	100.0b	100.0a	100.0a	100.0a	100.0b	76a	76a
16.7	118.0a	149.5a	18.5b	68.5b	104.0a	131.0a	62a	72ab
33.3	42.7b	83b	8.2b	28.2c	26.0b	67.2c	32b	50ab
66.7	3.9c	75.7b	0.6c	13.3c	3.0b	42.0c	4c	48b

Means within column and species followed by same alphabet are not significantly different ($p<0.05$) according to the Duncan Multiple range test. L=leaves, S=seeds

TABLE 2. Effect of the leaf and seed leachate of *P. javanica* (sandwich method) on the germination and seedling growth (% of the control) of *E. indica*, *C. iria* and *C. odorata*

Dry weight of donor sp. (mg)	Shoot length		Radicle length		Germination percentage	
	L	S	L	S	L	S
E. indica						
0 (control)	100.0a	100.0a	100.0a	100.0a	83.2a	83.2a
10	86.9a	84.8a	53.9b	36.9b	66.0b	63.6b
50	87.2a	42.7b	37.0 b	12.6c	64.8b	34.4c
C. iria						
0 (control)	100.0a	100.0a	100.0a	100.0a	56.4a	56.4a
10	56.5b	87.7a	28.4b	53.0b	21.2b	29.6b
50	49.9b	85.6a	13.8b	26.5c	14.8b	27.6b
C. odorata						
0 (control)	100.0a	100.0a	100.0a	100.0a	88.0a	88.0a
10	94.7a	75.2b	26.3b	19.1b	78.4a	60.8b
50	57.9b	66.9b	15.3b	14.6b	46.4b	34.8c

Means within column and species followed by same alphabet are not significantly different (p<0.05) according to the Duncan Multiple range test. L=leaves, S=seeds

50 mg of the seeds on the radicle length of all the bioassay species ranged from 73.5 - 87.4% while the reduction by 50 mg of the leaves ranged from 63.0 - 86.2%. The shoot length of *C. iria* was significantly reduced by 43.5 and 50.1% by 10 and 50 mg of the leaves, respectively. Higher reduction in germination percentage of the bioassay species was obtained from the 50 mg treatment compared to that from the 10 mg treatment for both plant parts. *Cyperus iria* showed higher germination percentage reduction compared to that of the other two weeds species tested. Based on the results, the radicle length showed higher percentage reduction compared to that of the shoot length. The higher root growth reduction might have been due to greater permeability of the allelopathic substances into the root rather than that into the shoot tissue (Nishida et al. 2005) and also the direct contact between the root and the phytotoxic compounds could inhibit cell division (Rietjens & Alink 2003), which is very active at the meristimatic tissue of the growing root tip. The data showed that the higher amount of the donor plant parts caused higher reduction of the radicle length, thus demonstrating the presence of more phytotoxic compounds leaching into the agar. The experiment also showed that allelopathy is a plant-species selective activity since different weeds gave distinctively different reactions.

TABLE 3. Effect of the leaf and seed volatilization of *P. javanica* (dish-pack method) on the germination and seedling growth (% of the control) of *E. indica, C. iria* and *C. odorata*

Distance from donor sp. (mm)	Shoot length		Radicle length		Germination percentage	
	L	S	L	S	L	S
E. indica						
0 (control)	100.0a	100.0a	100.0a	100.0a	96a	96a
41	81.2a	58.5b	65.8a	71.4ab	80b	88ab
58	100.9a	66.1b	84.8a	106.8a	88ab	92a
82	106.8a	57.0b	77.2a	59.8b	88ab	72b
92	97.4a	65.2b	85.4a	88.0ab	80b	80ab
C. iria						
0 (control)	100.0a	100.0a	100.0a	100.0a	92a	92a
41	58.1b	84.4a	40.4b	65.8b	48b	72b
58	67.6b	70a	36.8b	55b	68ab	68b
82	47.8b	75.3a	29.8b	54.4b	48b	56b
92	61.5b	86.2a	29.8b	38.6b	68ab	64b
C. odorata						
0 (control)	100.0a	100.0a	100.0a	100.0a	84a	84a
41	50.7b	83.9ab	57.4ab	54.3b	40b	84a
58	37.1b	69.3ab	38.7b	46.2b	28b	52b
82	37.1b	67.3ab	38.2b	47.9b	28b	52b
92	73.2ab	57.6b	92.3a	36.5b	52b	48b

Means within column and species followed by same alphabet are not significantly different (p<0.05) according to the Duncan Multiple range test. L=leaves, S=seeds

EFFECT OF THE LEAF AND SEED VOLATILIZATION OF *P. JAVANICA* (DISH-PACK METHOD) ON THE THREE BIOASSAY WEED SPECIES

Based on the results from Table 3, there was reduction in the shoot and radicle length of all the bioassay species compared to that of the control. The leaves of *P. javanica* significantly caused reduction of the radicle length of *C. iria* at all the tested distances while the seeds significantly reduced the radicle length of *C. odorata* at all the distances tested. The leaf and seed caused significant reduction in the germination percentage of *C. odorata* and *C. iria* at all the distances tested. However, the reduction on the growth parameters of all the bioassay species did not correspond fully to the distance from the donor species (further distance from the donor plant did not necessarily show lower reduction). A possible reason could be that the bioassay species were not sufficiently sensitive to respond fully to the difference in distances but the donor plant did cause reduction over all the parameters of the bioassay species.

CONCLUSION

The aqueous extract of the leaf and seed of *Pueraria javanica* significantly reduced the germination and growth parameters of the three bioassay species at 66.7 g/L concentration. The sandwich method showed significant reduction to the radicle length of all the bioassay species tested at the 50 mg treatment of leaves and seeds. The dish-pack method also exhibited a reduction effect on all the three bioassay species (from both parts of the donor plant). Based on the results of the present study, it can be concluded that *Pueraria javanica* has allelopathic influence on the germination and growth of the three tested weeds species. The results obtained may be an important reference for further investigations on the allelopathic effects of *P. javanica* in weed control studies.

ACKNOWLEDGEMENTS

This research was sponsored by Research Grant No. ERGS/1/2013/STG03/UKM/01/1 (STWN) from the Ministry of Education, Malaysia. Thanks are also extended to United Plantations Berhad for giving us permission to collect samples from Ulu Bernam Estate and to the National Farmers Organization for supplying the necessary seed samples.

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Received: 23 June 2015
Accepted: 2 October 2015