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ALLELOPATHY OF VELVET BEAN (*Mucuna cochinchinensis* (Wight) Burck) EXUDATES AND ITS INHIBITORY EFFECTS ON WEEDY RICE (*Oryza sativa* L.)

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Abstract
The experiment was conducted at the Toxicology laboratory, Faculty of Agriculture, University Putra Malaysia, Serdang, Malaysia in 2013. Allelopathic potential of aqueous methanol and water extracts of *Mucuna. cochinchinensis* leaves, seeds and roots were investigated on seed germination and seedlings growth of weedy rice (*Oryza sativa*); and biotest crop specie: lettuce (*Lactuca sativa*). The treatments consisted of five concentrations (100, 75, 50, 25, 0 %); plant parts (leaves, seed, root) and extraction solvents (methanol, water) were replicated three times and arranged as a completely randomized block (CRD) design. Germination, hypocotyl and radicle growth of all test plant species were inhibited at concentrations (100, 75, 50 and 25%). Inhibitory activity was dependent on the extraction solvents and extract concentrations as reported that methanol at higher extract concentration had the stronger inhibitory activity. The mean EC_{50} values of *M. cochinchinensis* leaves, seed and root of methanol extracts in relation to the germination inhibition of *O. sativa*, 86.06%, 416.32% and 72% respectively, and 30.66% 55.84% and 18.24%, respectively, in *Lactuca sativa*. Similar trend was observed with the varying concentration of the water extracts. The methanol extract of root showed a greater inhibitory effect on the hypocotyl and radicle growth of weedy rice than its water extract, and more effective on total germination.

Keywords: Allelopathy; velvetbean; weedy rice; lettuce; methanol extract; weed control

INTRODUCTION

The prominent feature of plants being sessile have made them to develop survival strategies and defense against herbivory, microbes and plant-weed relationship in ecological environment which led to the study of allelopathy. Allelopathy is the chemically mediated interference between co-habiting plants and includes a growth stimulation or inhibition of the target plant, mostly following a hormesis (Rice, 1979; Belz *et al*,. 2007).*Mucuna cochinchinensis* displayed a good activity against *Imperata cylindrica* in fields; *Striga hermonthica* and *Eleusine indica in vitro*(Avav *et al*., 2008; Ibrahim *et al.*, 2014; Ibrahim *et al.*, 2018). Previous phytochemical investigation showed that the plant has nitrogen-containing compounds with allelopathic potential such as L_DOPA that is a precursor of many alkaloids, catecholamines, and melanin and is released from velvet bean into soils, inhibiting the growth of nearby plant species (Anderson *et al.*, 2014). Nishihara *et al*. (2005) reported that large quantities (1% and 4–7%) of allelochemical are found in the leaves and seeds, respectively. It is estimated that velvet bean can release about 100–450 kg ha^{-1} of L-DOPA into the soil. Furthermore, its ability to control weeds and nematodes greatly reduces the need to apply synthetic chemicals to the crops (Vargas-Ayala *et al*., 2000; Fujii, 2003 and Nishihara *et al*., 2005). Reducing the impact of pesticide residue effect on crops, soil and underground water contamination and human health hazards. In Nigeria, yield increase of about 22 % and concomitant decrease in weed infestation have been observed in maize-velvetbean intercropped (Ahom *et al.*, 2017).

Weedy rice is a monotypic weed of cultivated rice (*Oryza sativa* L.). It originated from wild rice (*Oryza rufipogon* Griff.), landraces and interbreeding between cultivated and wild rice in south and southeast Asian countries and the USA (Huang *et al.* 2017;Qiu *et al.* 2017; Vigueira *et al.* 2017). Delouche *et al.*(2007) reported that weedy rice types are morphologically similar to cultivated rice varieties but are highly susceptible to seed shattering and greater seed dormancy. Seed dormancy can allow weedy *Oryza* sp. to persist in the soil for up to 10 years (Goss and Brown 1939; Teekachunhatean 1985). However, the dormancy mechanisms that underlie the ability of weedy rice to remain in the soil seedbank could be decimated via alleopathic-mediated interaction in the soil. The objectives of this study were to investigate the allelopathic potential of velvet bean on the suppression of weedy rice *in vitro* and to determine the related plant growth inhibitors secreting the allelopathic substances; mainly, phenolic compounds.

Materials and Methods

Mature velvetbean plants that were cultivated in the glass house of Faculti Pertanian, Universiti, Putra Malaysia in 2013 were harvested and separated into leaves, seeds and roots. These plant portions were thoroughly washed and rinsed with distilled water, oven-dried at 50 ^{o}C for 72 hours, grounded with a Wiley mill in order to pass through a 1-mm screen mesh, and stored in a refrigerator at 4 ^{o}C until when required.

The dried leaves, seeds and roots extract was carried out by soaking 0.5 kg in 1 L of methanol and distilled water to generate two fractions from each part and placed on a shaker for 48 hours at room temperature. The aqueous extracts were filtered through four layers of cheesecloth to remove the fiber debris and then filtered once again through a filter paper (no. 1; Whatman International, Maidstone, UK). Each extract was dried *in vacuo* on a rotary evaporator at 45 ^{o}C and then weighed. The methanol and water-extracted fractions were redissolved with 100 ml of sterile distilled water. The final concentration of each extract was 50 g L^{-1}. The aqueous solutions were described as 100 % and distilled water was added to the solutions to make different dilution (75, 50 and 25 %). The pH of the extracts ranges from 6.0 to 6.6. Extracts were stored in a refrigerator at 8 ^{o}C until further used for bioassay tests.

Allelopathic effect of methanol and water-soluble extracts from velvet bean on seedling germination and growth.

The germination test was carried out in an incubator according Hussain *et al*. (2008). The germination was assessed after 7 days by counting the number of germinated seeds. Germination was considered as the rupture of the seed coat and radicle emergence of ≥1 mm.

The total germination (TG) was determined, as described by Siddiqui (2007), and the percentage inhibition (I) as:

$$\mathrm{I} = 1 - \frac{Lt}{Lc}\, x\, 100$$

Lt = radicle length of the germinated seeds exposed to treatment, and Lc = radicle length of control germinated seed) computed.

All data were subjected to ANOVA and statistically analyzed by using a one-way ANOVA in JMP SAS statistical software (v. 9; SAS, Cary, USA) and the Tukey-Kramer HSD test was used to determine the differences between the treatment means at the 5 % probability level.

Germination bioassay

Weedy rice (*Oryza sativa*) was used as representative species because of their noxious effects in rice production. Lettuce (*Lactuca sativa*), was selected as a general biotest specie because it is frequently used as a model specie in allelopathic bioassay (Macias *et al.*, 2000). The seeds were surface- sterilized with 1.5 % (v/v) sodium hypochloride for 1 minute before they were washed (three times) with sterile distilled water. Empty and undeveloped weed seeds were discarded by floating in tapwater. Ten seeds each of lettuce and weedy rice were placed in the petri dishes to which 4 ml of each extract solutions of varying concentrations were added. Sterile distilled water was used as the control. The petri dishes were sealed with paraffin wrappers to prevent water loss by evaporation and to avoid contamination. The petri dishes were kept in an incubator at 28 ^{o}C for one week. The experiment was laid out as a 2 x 3 x 5 factorial comprised of extraction solvent (methanol and water), plant parts (leaves, seed and root) and concentration (0, 25, 50, 75 and 100 %) in a Completely Randomized Design (CRD) with 3 repetitions. Germination was considered to have occurred as the rupture of the seed coat and the radicle protrusion beyond the seed coat by at least 1 mm.

The mean LC_{50}value (the dose for 50 % inhibition of seedling growth) was calculated by using a probit analysis, as described by Finney (1971). A logistic equation was fitted to the germination data as a function of the logarithm of the concentrations of the *M. cochinchinensis* leaves, seed and root extracts by using SPSS for Windows (v. 19.0; SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Allelopathic effects of dried aqueous extracts from *Mucuna Cochinchinensis* on germination and seedling growth of *Lactuca sativa*.

The inhibitory effect of both the methanol and water extracts on the total seed germination and radicle inhibition is depended on the extract concentration and the plant species. For *L. sativa*, the seed germination was completely inhibited by the *M. cochinchinensis* root and leaves extracts at 75 and 100 % concentrations with lower inhibition as the concentration decreased (Table 1), which significantly affected the radicle inhibition of the plant. Both seed germination and radicle inhibition were less sensitive to the seed extract at different concentration when compared with the leaves and root extracts. The leaves and root extracts exhibited higher inhibition of seed germination and shorter radicle irrespective of the concentration and the extraction solvents, whereas, the seed extract showed little or no inhibition of seed germination. This might be attributed to higher rate of the allelochemicals presence in the leaf and root

extracts compared to the seed extract. Although, the water aqueous extracts showed lower inhibition of germination and seedlings growth when compared to the methanol aqueous extracts, there was significantly lower germination and subsequent inhibition. At the highest extract concentration of 100 %, both methanol leaves and root extracts completely inhibited the germination and radicle length of *L sativa*, indicating their suppressive effects on the seed and seedlings growth at higher concentration. Similarly, results obtained for the germination and seedlings growth of *O. sativa* showed that methanol and water aqueous seed extract exhibited lower germination and inhibition of radicle and hypocotyl growth of *O. sativa* (Table 2). However, there was significant inhibition of the radicle length at any of the tested concentrations.
Generally, the level of inhibition of seed germination and radicle length decreased were increased with the increasing concentration of the extracts. The increased inhibitory rate with the increasing concentration was in accordance with previous reports (Fujii, 1991; Chon *et al.,* 2003; Meksawat and Pornprom, 2010; Hussain *et al.,* 2011) for other allelopathic species.

Germination bioassays

The effect of the *M. cochinchinensis* leaves, seeds and root and extracts on the germination of the weed species and *L. sativa* after the probit analysis is presented on (Table 3).The total number of seeds, ungerminated seeds, expected response, and probability were determined against the four different concentrations (100, 75, 50, and 25%) of the methanol and water aqueous extracts. The data of the ungerminated seeds were fitted to the probit model after log transformation of the data. The result of thex^2-tests for goodness-of-fit was d=10 (at the 95% confidence limit) for the ungerminated seeds. The regression equations were Y=-6.25 + 4.207X and Y = -5.72 + 3.149X in relation to the *L. sativa* germination after exposure to both the methanol and water aqueous leaves extracts of *M. cochinchinensis* respectively. The concentration of 30.66 % and 60.96 % were diagnosed concentration of the methanol and water aqueous leaves extracts of that inhibited 50%of the seed germination of *L. sativa*. However, the seed and root of both the methanol and water aqueous extracts were analyzed (Table 3), with their corresponding diagnostic concentration of 55.84 % and 99.73 % in relation to the methanol and water aqueous seed extracts and 18.24 % and 56.40 % in the root extracts that inhibited 50%of the seed germination of *L. sativa*, respectively. According to Zhang *et al.* (2020), reported that dose-dependent concentration of allelochemical (isoliquiritigenin) caused a 40 % reduction of radicle elongation in *L. sativa.*

In respect to *O. sativa*, the regression equations obtained in the leaves and seed of the methanol and water aqueous extracts were Y=-12.08 + 6.245X and Y = -8.427 + 4.091X and Y=-4.612 + 1.763X with their corresponding diagnostic concentration of 86.06 % and 114.77 % and 416.32 % respectively as presented on Table 4. No regression equation was computed in relation to *O. sativa* after exposure to the seed water aqueous extract of *M. cochinchinensis* because there was complete seed germination. The root aqueous extracts of *M. cochinchinensis* also exhibited lower inhibition of germination. The regression equations were Y= -9.646 + 5.194X and Y= -11.57 + 5.824X following exposure to the root extracts. The concentration of 72 % of the methanol root extract and 96.93 % of the water aqueous extract of *M. cochinchinensis* of inhibited 50 % of the seed germination of *O. sativa*. The aqueous extracts of the leaves and root of *M. cochinchinensis* suppressed the seed germination and seedlings growth of *L. sativa* and *O. sativa.* The methanol root extract of *M. cochinchinensis* significantly inhibited *L. sativa* germination at varying concentration tested, however, it demonstrated a very lethal because it caused 18.24 % phytotoxic effect on the seed germination. Hussain *et al*. (2020) documented that *Acacia phyllodes* extract at 100 % resulted in a lethal reduction and caused 50.78 % phytotoxicity in *L. sativa* shoot length.
The marginal improvement in the germination at low concentrations of the *M. cochinchinensis* extracts could be the result of the activity of the extraction solvents (methanol and water), concentration and the amount of phytotoxins present in the plan parts.

Table 1: Effects of velvetbean extracts on germination and seedling growth of *Lettuce (L. sativa)*.

Concentration (%)	Total Germination (%)	Radicle Length (cm)	% Radicle Inhibition	Hypocotyl length (cm)	Total Germination (%)	Radicle Length (cm)	% Radicle Inhibition	Hypocotyl length (cm)
	Methanol extract				Water extract			
Leaves								
0	100a	6.66a	0.00	2.49ab	100.00a	6.66a	0.00	2.49ab
25	63.33b	5.09ab	23.57	3.01a	96.97a	5.83ab	12.46	3.94a
50	20.00c	3.59b	46.10	2.53ab	53.33b	5.07ab	23.87	3.64a
75	6.67c	0.32c	95.52	1.30bc	50.00b	2.75b	58.71	3.39a
100	0.00c	0.00c	100.00	0.00c	20.00c	2.41b	63.81	1.00b
SE±	4.94	0.35		0.33	5.57	0.76		0.42
F-ratio	74.00	68.02		13.85	37.04	6.15		7.99
Prob> F	<.0001	<.0001		0.0004	<.0001	0.0092		0.0037
Seed								
0	100a	6.66a	0.00	2.49ab	100.00a	6.66a	0.00	2.49a
25	76.69ab	6.00a	9.91	2.79a	96.67a	5.94ab	10.81	2.60a
50	53.33bc	3.98b	40.24	2.23abc	86.67ab	5.33b	19.97	2.92a
75	40.00c	3.69b	44.59	2.04bc	63.33bc	3.07c	53.90	2.54a
100	30.00c	2.56b	61.56	1.82c	50.00c	2.88c	56.76	1.09b
SE±	6.15	0.42		0.14	5.16	0.38		0.20
F-ratio	21.32	16.43		7.82	17.79	20.35		12.18
Prob> F	<.0001	0.0002		0.0040	0.0002	<.0001		0.0007
Root								
0	100.00a	6.66a	0.00	2.49a	100.00a	6.66a	0.00	2.49ab
25	26.67b	3.43b	48.50	2.22a	86.67a	4.47b	32.88	3.47a
50	3.33c	0.27c	95.95	0.17b	56.67b	3.17bc	52.40	4.17a
75	0.00c	0.00c	100.00	0.00b	43.33b	2.57bc	61.41	2.40ab
100	0.00c	0.00c	100.00	0.00b	13.33c	2.03c	69.52	1.22b
SE±	4.22	0.63		0.38	5.96	0.46		0.47
F-ratio	103.25	21.81		11.02	33.59	16.15		5.76
Prob> F	<.0001	<.0001		0.0011	<.0001	0.0002		0.0114

Values in the column with same letter are not significantly different at P<0.05

Table 2: Effects of velvetbean extracts on germination and seedling growth of weedy rice *(O. sativa)*.

Concentration (%)	Total Germination (%)	Radicle Length (cm)	% Radicle Inhibition	Hypocotyl length (cm)	Total Germination (%)	Radicle Length (cm)	% Radicle Inhibition	Hypocotyl length (cm)
	Methanol extract				Water extract			
Leaves								
0	100.00a	12.21a	0.00	8.08a	100.00a	12.21a	0.00	8.08a
25	100.00a	9.03b	25.55	8.12a	100.00a	9.70ab	20.56	7.60a
50	86.67a	5.12c	58.07	5.64b	90.00ab	6.97b	42.92	7.05a
75	83.33a	1.67d	86.32	3.36c	83.33ab	3.13c	74.37	5.70a
100	23.33b	1.26d	89.68	2.12c	56.67b	1.91c	84.36	1.98b
SE±	4.47	0.67		0.49	8.03	0.73		0.66
F-ratio	50.72	50.14		7.06	4.95	35.05		13.92
Prob> F	<.0001	<.0001		0.0057	0.018	<.0001		0.0004
Seed								
0	100.00a	12.21a	0.00	8.08a	100.00a	12.21a	0.00	8.08a
25	100.00a	9.39ab	23.10	8.17a	100.00a	12.48a	-2.21	8.52a
50	90.00a	7.44b	39.07	7.03ab	100.00a	1.70a	12.37	7.63a
75	93.33a	3.25c	73.38	5.26b	100.00a	4.84b	60.36	7.25a
100	86.67b	2.00c	83.62	4.94b	100.00a	3.00b	75.43	5.33b
SE±	4.94	0.77		0.57	0.00	0.76		0.90
F-ratio	1.45	30.15		7.06		25.02		1.88
Prob> F	0.2867	<.0001		0.0057		<.0001		0.19
Root								
0	100.00a	12.21a	0.00	8.08a	100.00a	12.21a	0.00	8.08a
25	90.00ab	7.27b	40.46	6.99ab	100.00a	9.95a	18.51	6.29b
50	80.00b	3.53c	71.09	6.81ab	93.33ab	6.08b	45.29	7.62ab
75	53.33c	1.51c	87.63	4.63bc	80.00b	4.84bc	60.36	8.45a
100	13.33d	0.76c	93.78	3.27c	43.33c	1.92c	84.28	7.33ab
SE±	4.22	0.60		0.62	4.22	0.63		0.75
F-ratio	68.25	61.18		9.94	31.87	41.46		1.22
Prob> F	<.0001	<.0001		0.0016	<.0001	<.0001		0.36

Values in the column with same letter are not significantly different at P<0.05

Table 3: Probit analysis for the seed germination of *L. sativa*, exposed to four concentration of *M. cochinchinensis* aqueous extracts.

Extract Concentration (%)	Total number of seeds	Number of ungerminated seeds	Expected response	Probability
		Leave		
Methanol leaf				
100	10	10	9.85	0.985
75	10	9	9.49	0.949
50	10	8	8.14	0.814
25	10	4	3.55	0.355
Water leaf				
100	10	8	7.51	0.751
75	10	5	6.12	0.612
50	10	5	3.93	0.393
25	10	0	1.14	0.114
		Root extract		
Methanol root				
100	10	10	10.00	0.999
75	10	10	9.97	0.997
50	10	10	9.74	0.974
25	10	7	7.29	0.729
Water root				
100	10	9	7.98	0.798
75	10	6	6.61	0.661
50	10	4	4.30	0.430
25	10	1	1.18	0.118
		Seed extract		
Methanol seed				
100	10	7	7.00	0.700
75	10	6	6.00	0.604
50	10	5	4.61	0.461
25	10	2	2.35	0.235
Water seed				
100	10	5	5.02	0.502
75	10	4	3.43	0.343
50	10	1	1.64	0.164
25	10	0	0.25	0.025

Regression line parameters (methanol leaf extract): Y = a + bX; Y = -6.25 + 4.207X; diagnostic concentration = 30.66%.
Regression line parameters (water leaf extract): Y = a + bX; Y = -5.72 + 3.149X; diagnostic concentration = 60.96%.
Regression line parameters (methanol root extract): Y = a + bX; Y = -5.611 + 4.45X; diagnostic concentration = 18.24%.
Regression line parameters (water root extract): Y = a + bX; Y = -5.88 + 3.357X; diagnostic concentration = 56.40%.
Regression line parameters (methanol seed extract): Y = a + bX; Y = -3.611 + 2.067X; diagnostic concentration = 55.84%.
Regression line parameters (water seed extract): Y = a + bX; Y = -6.517 + 3.26X; diagnostic concentration = 99.73%

Table 4: Probit analysis for the seed germination of weedy rice, exposed to four concentration of *M. cochinchinensis* aqueous extracts.

Extract Concentration (%)	Total no. of seeds	Number of ungerminated seeds	Expected response	Probability
Methanol leaf				
100	10	8	6.58	0.658
75	10	2	3.55	0.355
50	10	1	0.71	0.70
25	10	0	0.00	0.00
Water leaf				
100	10	4	4.03	0.403
75	10	2	2.25	0.225
50	10	1	0.70	0.070
25	10	0	0.03	0.003
Root extract				
Methanol root				
100	10	9	7.71	0.771
75	10	4	5.37	0.537
50	10	2	2.05	0.205
25	10	0	0.85	0.009
Water root				
100	10	6	5.31	0.531
75	10	2	2.58	0.258
50	10	1	0.47	0.047
25	10	0	0.00	0.000
Seed extract				
Methanol seed				
100	10	1	1.37	0.137
75	10	1	0.95	0.095
50	10	1	0.52	0.052
25	10	0	0.16	0.016

Regression line parameters (methanol leaf extract): Y = a + bX; Y = -12.08 + 6.245X; diagnostic concentration = 86.06%.
Regression line parameters (water leaf extract): Y = a + bX; Y = -8.427 + 4.091X; diagnostic concentration = 114.77%.
Regression line parameters (methanol root extract): Y = a + bX; Y = -9.646 + 5.194X; diagnostic concentration = 72.00%.
Regression line parameters (water root extract): Y = a + bX; Y = -11.568 + 5.824X; diagnostic concentration = 96.93%.
Regression line parameters (methanol seed extract): Y = a + bX; Y = -4.612 + 1.763X; diagnostic concentration = 416.32%.

Conclusion

The results obtained in this study showed that the methanol and water aqueous extracts of the leaves, seed and root of *M. cochinchinensis* possess allelochemicals that suppressed seed germination and root growth of *L. sativa* and *O. sativa* and that the inhibition was concentration percentage and extraction solvent-dependent.

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