Evaluation on Allelopathic Potential of Velvet Bean (*Mucuna cochinchinensis*) on Germination of Goosegrass (*Eleusine indica* L.)

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Abstract: The experiment was conducted at the Toxicology laboratory, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia in 2013. Allelopathic potential of aqueous methanol and water extracts of *M. cochinchinensis* leaves, seed and root was investigated on seed germination and seedling growth of goosegrass and biotest crop species: 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, radicle and hypocotyl growth of all test plant species were inhibited at concentrations (100, 75, 50 and 25%). The total germination percentage was lowest with the methanolic extracts of leaf, seed and root of *M. cochinchinensis* at 100 % concentration in the order of 0.00, 30.66 and 4.66%, respectively. Concomitantly, the radicle length inhibition percentages of methanolic extracts at higher concentration of 100% were 100, 88.5 and 94.4% of the leaf, seed and root extracts, respectively. The water extracts recorded the highest germination percentage and lower inhibitory activity of the radicle and hypocotyl length. The study confirmed plant growth-inhibitory compounds of *M. cochinchinensis* is dependent on the extraction solvents and extract concentrations as expressed that methanolic solvent at higher extract concentration had the stronger inhibitory activity.

Keywords: *Allelopathy, Velvet bean, Concentration, Goosegrass, Inhibition, Weed control.*

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INTRODUCTION

The current trend of organic farming and natural agriculture getting a pride-of-place to counteract heavy reliance on agrochemical such as pesticides and inorganic fertilizers in conventional agricultural production has led to the study of allelopathy. Allelopathy is defined as "a beneficial or detrimental effect from a donor plant to the recipient by chemical pathway" (Rice 1984). This development has encouraged the exploitation of phytotoxic activity of allelopathic plants in weed management through naturally occurring plant chemicals as a sustainable strategy that reduced concerns about pesticide residues in food, environmental pollutions, harmful side-effects on human health and the evolution of herbicide resistant weed biotypes. This is particularly important in sustainable agriculture where the use of synthetic herbicides is prohibited.

Several plant based herbicides such as mesotrione and glufosinate were originally derived from allelopathic compounds (Coloquhoun, 2006). The compounds involved in allelopathic interference being secondary plant metabolites include a host of phenolics- flavonoids, coumarins, terpenoids and organic acids from diverse botanical families (Céspedes et al., 2006, 2013; Muñoz et al., 2013), which could be considered to exhibits either inhibitory or stimulatory role in the plant environment depending on the concentration of the compounds. Allelochemicals are typically known to suppress germination, causing dysfunctions of root and seedling growth. More importantly, they alter several plant physiological processes, such as water utilization, mineral uptake, photosynthesis, cell morphology, membrane permeability, protein synthesis and enzyme activities, among countless others (Weir et al., 2004).

Velvet bean (*Mucuna cochinchinensis*), a non-edible and wild tropical legume is grown generally for its beneficial nitrogen-fixing potential with nodulating N_2-fixing rhizobial bacteria. It produces high amount of aromatic non-protein amino acid, L-3,4-dihydroxyphenylalanine (L-Dopa) (Fujii, 1991). Previous studies have demonstrated that intercropping subsistence maize with velvet bean potentially reduces *Striga* parasitism and smothers speargrass (*Imperata cylindrica*) with concomitant yield increases, improved soil nitrogen and reduced soil erosion (Avav et al., 2008; Nwaichi and Ayalogu 2010; Akal et al., 2012). Adaptable strategies using allelopathic plants in crop rotation, as mulches, residues, cover crops and companion crops have been utilized to contained the effects of noxious weeds in the farming systems of the south, south-east Asia and sub-Saharan Africa. *Mucuna, Desmodium, Pueraria,* and *Stylosanthes* species are widely used in between row crops of rubber and oil palm in Malaysia and cereal field crops endemic to *Striga* parasitism in Africa to smother weeds while at the same time fixing nitrogen to the soils (Shaharuddin and Jamaluddin, 2007; Hooper et al., 2010). Besides improving soil fertility by fixing nitrogen and making it available to the main crop and reduce competition from noxious weeds, it also improves palm growth and reduces the immaturity period (Hasnol et al., 2012).

The current level of our understanding of *mucuna* is that it fixes atmospheric N through a symbiotic relationship with soil microorganisms - making the plant an efficient source of N. In addition, it was observed that few other weeds cohabitate with velvetbean in the tropics, and those that grow along with it are usually found in limited amounts and are generally along the fringes of a field (Ibrahim, personal observation). Nowadays research is being geared up to exploit the interface between chemical dynamics in this underutilized legume that exhibits allelopathic potential and biological control of weeds to contribute to sustainable weed management in agro-ecosystem. Therefore, the objective of this research was to utilize the allelochemicals in different plant parts of *Mucuna* - leaf, seed and root for its effects on the germination of common test species (lettuce) and goosegrass assayed under laboratory conditions.

Materials and Methods

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

Mature velvetbean plants that were grown in the glass house of Faculti Pertanian, Universiti, Putra Malaysia in 2013 were harvested and separated into leaves, seed and roots. These plant portions were thoroughly washed and rinsed with distilled water, oven-dried at 50 ^{o}C for 72 hours, ground with a Wiley mill in order to pass through a 1-mm screen mesh, and stored in a refrigerator at 4 ^{o}C until required. The dried leaves, seed and roots were extracted by soaking 0.5 kg in 1l 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 cheese cloth 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**.** 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 6.02 to 6.56. Extracts were stored in a refrigerator at 8 ^{o}C until further used for bioassay tests.

Goosegrass (*Eleusine indica*) was used as representative weed species because of its noxious effects in arable crop production. Lettuce (*Lactuca sativa*), was selected as a general biotest specie because it is frequently used as a model specie in allelopathic bioassay (Macìas 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 tap water. The seeds of goosegrass were treated with solution of KNO_3 (0.2 %) for 48 hours in the dark to break their dormancy and washed many times with distilled water. Glass petri dishes (9 cm diameter) were used and underlain with two sterile filter papers (Whatman No. 2). Ten seeds of lettuce

and fifty seeds of googegrass 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 2 x 5 factorial in a completely randomized design 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 total germination (TG) was determined, as described by Siddiqui (2007), and the percentage inhibition (1 – 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.

Results and Discussion

The inhibitory effect of both the methanol and water extracts on the total seed germination and radicle inhibition 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.

Table 1. Effects of methanol extract from different parts of on germination and seedling growth of *L. sativa*

Concentration (%)	Total germination (%)	Radicle Length (cm)	% Radicle inhibition	Hypocotyl length (cm)
Leaves				
0	100a	6.66a	0.00	2.49ab
25	63.33b	5.09ab	23.57	3.01a
50	20.00c	3.59b	46.10	2.53ab
75	6.67c	0.32c	95.52	1.30bc
100	0.00c	0.00c	100.00	0.00c
SE±	4.94	0.35		0.33
F-ratio	74.00	68.02		13.85
Prob> F	<.0001	<.0001		0.0004
Seed				
0	100a	6.66a	0.00	2.49ab
25	76.69ab	6.00a	9.91	2.79a
50	53.33bc	3.98b	40.24	2.23abc
75	40.00c	3.69b	44.59	2.04bc
100	30.00c	2.56b	61.56	1.82c
SE±	6.15	0.42		0.14
F-ratio	21.32	16.43		7.82
Prob> F	<.0001	0.0002		0.0040
Root				
0	100.00a	6.66a	0.00	2.49a
25	26.67b	3.43b	48.50	2.22a
50	3.33c	0.27c	95.95	0.17b
75	0.00c	0.00c	100.00	0.00b
100	0.00c	0.00c	100.00	0.00b
SE±	4.22	0.63		0.38
F-ratio	103.25	21.81		11.02
Prob> F	<.0001	<.0001		0.0011

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

Although the aqueous extracts showed lower inhibition of germination and seedling growth when compared to the methanol aqueous extracts, there was significantly lower germination and subsequent inhibition (Table 2). The results of our findings collaborated earlier reports enunciated by Fujii et al. (1991) that velvetbean (Mucuna pruriens) increased the graminaceous crop yield in mixed culture, but smother the growth of noxious weeds such as nutsedge (Cyperus spp.) and cogongrass (Imperata cylindrica).

Table 2. Effects of water extract from different parts of M. cochinchinensis on germination and growth of L. sativa

Concentration (%)	Total germination (%)	Radicle Length (cm)	% Radicle inhibition	Hypocotyl length (cm)
Leaves				
0	100.00a	6.66a	0.00	2.49ab
25	96.97a	5.83ab	12.46	3.94a
50	53.33b	5.07ab	23.87	3.64a
75	50.00b	2.75b	58.71	3.39a
100	20.00c	2.41b	63.81	1.00b
SE±	5.57	0.76		0.42
F-ratio	37.04	6.15		7.99
Prob> F	<.0001	0.0092		0.0037
Seed				
0	100.00a	6.66a	0.00	2.49a
25	96.67a	5.94ab	10.81	2.60a
50	86.67ab	5.33b	19.97	2.92a
75	63.33bc	3.07c	53.90	2.54a
100	50.00c	2.88c	56.76	1.09b
SE±	5.16	0.38		0.20
F-ratio	17.79	20.35		12.18
Prob> F	0.0002	<.0001		0.0007
Root				
0	100.00a	6.66a	0.00	2.49ab
25	86.67a	4.47b	32.88	3.47a
50	56.67b	3.17bc	52.40	4.17a
75	43.33b	2.57bc	61.41	2.40ab
100	13.33c	2.03c	69.52	1.22b
SE±	5.96	0.46		0.47
F-ratio	33.59	16.15		5.76
Prob> F	<.0001	0.0002		0.0114

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

Similarly, the methanol leaf extract (100, 75, 50, and 25 %) significantly suppressed the germination and radicle growth of E. indica (Table 3), while the root extract of M. cochinchinensis significantly decreased the germination at the 100% and 75% concentrations. The radicle and hypocotyl also responded to the different levels of concentration of the extracts of M. cochinchinensis as expressed with corresponding decreased in length. However, aqueous seed extract did not significantly inhibit the germination of E. indica at 0, 25, 50 and 75 % concentration except at 100 % concentration (Table 4). There was significant inhibition of the radicle length at any of the tested concentrations. The two plant parts (seed and root) extracts irrespective of the extraction solvent (methanol and water) did not differed

significantly on the hypocotyl length. However, the leaf extracts of the methanol and water solvent recorded significant difference with respect to the hypocotyl length.

Generally, the level of inhibition of seed germination and radicle length decreased were increased with the increasing concentration of the extracts. At the highest extracts concentration of 100 %, both methanol leaves and root extracts completely inhibited the germination and radicle length of L sativa and E. indica, indicating their suppressive effects on the seed and seedling growth at higher concentration. The increasing 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.

Table 3. Effects of methanol extract from different parts of *M. cochinchinensis* on germination and growth of *E. indica*

Concentration (%)	Total Germination (%)	Radicle Length (mm)	% Radicle inhibition	Hypocotyl length (mm)
Leaves				
0	76.00a	13.90a	0.00	35.00a
25	32.00b	6.43b	53.74	3.67b
50	18.00b	0.25c	98.20	3.00b
75	2.00c	0.00c	100.00	0.00b
100	0.00c	0.00c	100.00	0.00b
SE±	3.35	0.96		1.78
F-ratio	85.96	40.98		71.32
Prob> F	<.0001	<.0001		<.0001
Seed				
0	76.00a	13.90a	0.00	35.00
25	77.33a	9.96ab	28.35	36.00
50	37.33b	4.73bc	65.97	34.33
75	35.33b	2.97bc	78.63	33.33
100	30.66b	1.60c	88.49	36.33
SE±	2.94	1.14		2.57
F-ratio	62.73	20.37		0.23
Prob> F	<.0001	<.0001		0.9169
Root				
0	76.00a	13.90a	0.00	35.00
25	36.00b	2.72b	80.43	32.00
50	24.00b	1.99b	85.68	35.00
75	24.00b	1.57b	88.71	25.33
100	4.66c	1.33b	90.43	28.00
SE±	3.66	0.84		2.80
F-ratio	52.58	41.12		2.37
Prob> F	<.0001	<.0001		0.1226

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

Table 4. Effects of water extracts from different parts of *M. cochinchinensis* on germination and growth of *E. indica*

Concentration (%)	Total germination (%)	Radicle Length (mm)	% Radicle inhibition	Hypocotyl length (mm)
Leaves				
0	76.00a	13.90a	0.00	35.00
25	54.00b	8.00b	42.45	23.33
50	32.67c	7.70b	44.60	30.67
75	11.33d	5.18b	62.73	34.33
100	4.67d	4.37b	68.56	35.00
SE±	3.50	0.91		2.72
F-ratio	71.84	16.78		3.37
Prob> F	<.0001	0.0002		0.0542
Seed				
0	76.00a	13.90a	0.00	35.00
25	74.00ab	11.43ab	17.77	31.67
50	70.00ab	10.03ab	27.84	34.00
75	56.00bc	6.03c	56.62	32.67
100	38.67c	5.50c	60.43	33.00
SE±	3.98	1.44		1.94
F-ratio	15.49	6.17		0.43
Prob> F	0.0003	0.0009		0.7825
Root				
0	76.00a	13.90a	0.00	35.00
25	46.00b	7.47b	46.26	31.00
50	32.67b	3.67b	73.60	30.67
75	14.00c	3.17b	77.19	34.67
100	9.33c	3.03b	78.20	31.67
SE±	3.75	1.17		1.86
F-ratio	51.72	15.76		1.24
Prob> F	<.0001	0.0003		0.3546

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

Conclusion

The results from this study showed that velvet bean possesses a strong allelopathic potential and exhibits strong inhibition of goosegrass germination in the bioassay. It revealed that the inhibition was concentration and extraction solvent-dependent. The inhibitory magnitude of the plant laef was greater than the root and stem and the extent of inhibition of the plant parts was proportional to the increase of applied concentration.

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