

Effect of *Hyptis suaveolens* Poit Leaf Extracts on Seed Germination and Subsequent Seedling Growth of *Pennisetum setosum* (Swartz.)

L.C. Rich and *Mimosa invisa* Mart

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Abstract: Dry leaf of *Hyptis suaveolens* Poit was extracted with water and methanol and the effect on seed germination and seedling growth were determined in *Pennisetum setosum* and *Mimosa invisa*. The water extract significantly reduced *P. setosum* seed germination and strongly inhibited shoot and root lengths of both *P. setosum* and *M. invisa*. The inhibition percentages increased with the increasing of dry leaf ratios of the extracts. Shoot growth of *P. setosum* and *M. invisa* seedling was less sensitive to the extract than root growth was. The lower leaf ratios extracts (1:80 and 1:40) did not reduce shoot length of both weeds, in contrast, the 1:80 ratio extract promoted *P. setosum* shoot length 16.97% of untreated control. In this study, *P. setosum* was more susceptible to *H. suaveolens* leaf water extract than *M. invisa*. The methanol extracts of *H. suaveolens* leaf significantly inhibited seed germination and seedling growth of both *P. setosum* and *M. invisa* and the inhibitory effects were higher than those of the water extracts. This might indicate solubility of the allelochemicals in *H. suaveolens* leaf better in methanol than in water. It is interesting to study further on allelopathy of this plant for the practical use in agricultural pest management.

Key words: *Hyptis suaveolens*, *Pennisetum setosum*, *Mimosa invisa*, water extract, methanol extract, germination, seedling growth

INTRODUCTION

Hyptis suaveolens Poit, an annual weed of the family Lamiaceae is generally found in upland area in Thailand both in crop and non-crop areas. This weed species was reported to have inhibition potential on seed germination and seedling growth of some crops and weeds species (Kapoor, 2011). Besides that activity, the extracts and oils of *H. suaveolens* were also reported to have anti-microbial activities to some bacteria and fungi (Mandal *et al.*, 2007; Moreira *et al.*, 2010) and insecticidal effects on stored-grain coleopteran pests and larvae of the yellow fever mosquito (Tripathi and Upadhyay, 2009; Amusan *et al.*, 2005).

From a broad spectrum on allelopathic effects to plant pests, *H. suaveolens* may have high potential to be used in agriculture, especially in organic farming to reduce the costs of pests management. The purpose of this study was to determine the effect of leaf extract of *H. suaveolens* on seed germination and subsequent

seedling growth of *Pennisetum setosum* (Swartz.) L.C. Rich and *Mimosa invisa* Mart in order to confirm its herbicidal activity. The data of this experiment will be used as basic knowledges for the further study on practical use of *H. suaveolens* in agricultural pest management.

MATERIALS AND METHODS

Mature leaf of *H. suaveolens* collected from Srinakharinwirot University, Ongkarak Campus was air-dried and kept in refrigerator. The appropriate amount of the leaf were grinded before using in each trial. Seeds of *Pennisetum setosum* (Swartz) L.C. Rich. and *Mimosa invisa* Mart were collected, cleaned, tested on germination and kept in refrigerator.

Effect of *H. suaveolens* leaf-water extract on seed germination and subsequent seedling growth: The grinded dry leaf of *H. suaveolens* was soaked in distilled water at

the ratio of 1:10 (dry leaf (g): water (mL)) and kept in refrigerator for 24 h. The mixer was filtrated through Whatman No. 1 filter paper and the extract was diluted to 1:20, 1:40 and 1:80 ratios with distilled water. For germination test, 5 mL of each of water extracts was added to petri dish laid with germination paper and twenty seeds of each test plant were placed on the paper in each plate. The plates were covered and kept on shelf with 3800 luxes artificial light, 13 h photoperiod. Germination percentage of the test plants were examined daily and shoot and root lengths were determined 7 days after treatment.

Effect of *H. suaveolens* leaf-methanol extract on seed germination and subsequent seedling growth:

H. suaveolens leaf-methanol extracts at the ratios of 1:10, 1:20, 1:40 and 1:80 (dry leaf (g): methanol (mL)) were prepared by the same method of the leaf-water extract, using methanol instead of water. For germination test, 5 mL of each of methanol extract and unextracted methanol (blank control) was added throughout the filter paper in each petri dish and the plates were left opened for 24 h under room conditions in order to remove the methanol solvent. Then, 5 mL of distilled water was added to each plate and 20 seeds of each test plant were placed on filter paper in each petri dish. The determination of seed germination and subsequent seedling growth of the test plants were the same as in leaf-water extract trial.

Statistical analysis: The experiments were conducted as randomized complete block design with 3 replications, using distilled water as untreated control. All data were subjected to the analysis of variance and the Duncan's new Multiple Range Test (DMRT) was used to compare the treatment means.

RESULTS AND DISCUSSION

Effect of *H. suaveolens* leaf-water extract on seed germination and subsequent seedling growth:

H. suaveolens leaf-water extracts at all ratios tested in this study significantly reduced number of germinated seeds of *P. setosum* 7 days after treatment (Table 1). The extracts at 1:20 and 1:10 ratios (leaf:water) completely inhibited seed germination and those at 1:80 and 1:40 ratios provided 26.41 and 56.61% inhibition. Root growth of *P. setosum* was also significantly affected by the extract at all ratios, their length were inhibited 46.46 and 49.01% by the extracts of 1:80 and 1:40 ratios, respectively (Table 1). *P. setosum* shoot growth was less effected from *H. suaveolens* leaf extract. The lower leaf ratios extracts (1:80 and 1:40) did not reduce shoot length, in contrast, the 1:80 ratio extract promoted shoot length 16.97% of untreated control (Table 1).

Table 1: Effect of *H. suaveolens* leaf-water extract on seed germination and subsequent seedling growth of *P. setosum* and *M. invisa* 7 days after treatment

Leaf: water ratios (g:mL)	<i>P. setosum</i>		<i>M. invisa</i>	
	S.G. ^{2f}	I.P. ^{4f}	S.G. ^{2f}	I.P. ^{4f}
Seed germination				
Control (distilled water)	88.33 ^{afj}	-	100.00 ^{afj}	-
1:80	65.00 ^b	26.41	100.00 ^g	0.00
1:40	38.33 ^c	56.61	100.00 ^g	0.00
1:20	0.00 ^d	100.00	100.00 ^g	0.00
1:10	0.00 ^d	100.00	100.00 ^g	0.00
CV (%)	23.87	-	1.30	-
Root growth				
Control (distilled water)	3.53 ^{afj}	-	4.95 ^{afj}	-
1:80	1.89 ^b	46.46	2.55 ^b	48.48
1:40	1.80 ^b	49.01	2.44 ^b	50.70
1:20	0.00 ^e	100.00	1.74 ^c	64.84
1:10	0.00 ^e	100.00	1.22 ^d	75.35
CV (%)	7.63	-	4.13	-
Shoot growth				
Control (distilled water)	2.77 ^{afj}	-	4.67 ^{afj}	-
1:80	3.24 ^a	-16.97	4.29 ^a	8.85
1:40	2.85 ^b	2.88	4.21 ^a	9.85
1:20	0.00 ^e	100.00	3.24 ^b	30.62
1:10	0.00 ^e	100.00	0.80 ^e	82.86
CV (%)	5.63	-	8.67	-

^{1f}Means in the same column followed by the same letter are not significantly different. According to DMRT 0.05; ^{2f}Seed germination (%); ^{3f}Root or shoot lengths in millimeters (mm); ^{4f}Inhibition percentage of seed germination, root or shoot lengths (%) calculated from I.P. = C-T/C×100 where, C = seed germination or plant growth in control; T = seed germination or plant growth in each treatment

M. invisa seed germination was not affected by *H. suaveolens* leaf-water extracts at all dry leaf ratio, however, its root and shoot were significantly inhibited by the extracts, especially at the higher leaf ratios (Table 1). *H. suaveolens* leaf-water extracts at the ratios of 1:80, 1:40, 1:20 and 1:10 (leaf:water) significantly reduced root length 48.48, 50.70, 64.84 and 75.35%, respectively (Table 1). Shoot growth of *M. invisa* seedling was less sensitive to the extract than root growth was, it was affected only by the extracts of the high leaf ratios (1:20 and 1:10) and the inhibition percentages were 30.62 and 82.86%, respectively (Table 1).

Effect of *H. suaveolens* leaf-methanol extract on seed germination and subsequent seedling growth:

H. suaveolens leaf-methanol extract provided higher inhibitory effects on seed germination and subsequent seedling growths than the water extract did in both test plants. The methanol extracts at higher ratios inhibited *P. setosum* seed germination similar to the water extracts (96.30 and 100.00% by 1:20 and 1:10 ratios extracts, respectively) (Table 2). The lower ratio methanol extracts

Table 2: Effect of *H. suaveolens* leaf-methanol extract on seed germination and subsequent seedling growth of *P. setosum* and *M. invisa* 7 days after treatment

Leaf: methanol ratios (g:mL)	<i>P. setosum</i>		<i>M. invisa</i>	
	S.G. ^{3f}	I.P. ^{5f}	S.G. ^{3f}	I.P. ^{5f}
Seed germination				
Blank control ^{1f}	90.00 ^{2d}	-	100.00 ^{2d}	-
1:80	30.00 ^b	66.67	100.00 ^a	0.00
1:40	15.00 ^c	83.33	80.00 ^b	20.00
1:20	3.33 ^{cd}	96.30	48.33 ^c	51.67
1:10	0.00 ^d	100.00	0.00 ^d	100.00
CV (%)	22.98	-	13.66	-
Leaf: methanol ratios (g: mL)	<i>P. setosum</i>		<i>M. invisa</i>	
	Length ^{4f}	I.P. ^{5f}	Length ^{4f}	I.P. ^{5f}
Root growth				
Blank control ^{1f}	2.98 ^{2d}	-	5.68 ^{2d}	-
1:80	0.88 ^b	70.47	2.32 ^b	59.16
1:40	0.55 ^c	81.54	1.26 ^c	77.82
1:20	0.26 ^d	91.28	0.37 ^d	93.49
1:10	0.00 ^d	100.0	0.00 ^d	100.00
CV (%)	15.26	-	17.26	-
Shoot growth				
Blank control ^{1f}	3.37 ^{2d}	-	3.67 ^{2d}	-
1:80	2.14 ^b	36.50	3.06 ^b	16.62
1:40	1.77 ^b	47.48	2.61 ^c	28.88
1:20	0.40 ^c	88.13	0.84 ^d	77.11
1:10	0.00 ^c	100.00	0.00 ^e	100.00
CV (%)	22.63	-	8.18	-

¹Added unextracted methanol in plates laid with filter paper, evaporated for 24 h; ²Means in the same column followed by the same letter are not significantly different. According to DMRT 0.05; ³seed germination (%); ⁴root or shoot lengths in millimeters (mm); ⁵Inhibition percentage of seed germination, root or shoot lengths (%) calculated from I.P. = C-T/C×100 where, C = seed germination or plant growth in control; T = seed germination or plant growth in each treatment

(1:80 and 1:40) provided 66.67 and 83.33% inhibition, respectively which were much higher than those of the water extracts effect (Table 1 and 2). Similar to the effect on seed germination, the *H. suaveolens* leaf-methanol extracts at all ratios significantly inhibited both root and shoot lengths of *P. setosum* seedling. The 1:80, 1:40, 1:20 and 1:10 ratios (leaf:methanol) extracts showed 70.47, 81.54, 91.28 and 100.00% root inhibition and 36.50, 47.48, 88.13 and 100.00% shoot inhibition, respectively (Table 2). *M. invisa* was also more sensitive to *H. suaveolens* leaf-methanol extract than the water extract, its seed germination were significantly decreased by the methanol extracts at 1:40, 1:20 and 1:10 ratios (20, 51.67 and 100% inhibition, respectively (Table 2). The methanol extract at all ratios significantly inhibited root and shoot lengths of *M. invisa*. At 1:80, 1:40, 1:20 and 1:10 ratios of the methanol extract, root length of *M. invisa* was inhibited 59.16, 77.82, 93.49 and 100.00% and shoot length was inhibited 16.62, 28.88, 77.11 and 100.00%, respectively when compared to blank control (Table 2).

Water extract of *H. suaveolens* leaf significantly reduced *P. setosum* seed germination and strongly

inhibited shoot and root lengths of both *P. setosum* and *M. invisa*. The inhibition percentages increased with the increasing of dry leaf ratios of the extracts. Water extract of many plant species such as mesquite, annonaceae plants, black mustard and neem were reported to inhibit seed germination and seedling growth of test plants and the degree of inhibition increased with the increase of extract concentrations (Siddiqui *et al.*, 2009; Turk *et al.*, 2005; Charchafchi *et al.*, 2007).

In this study, the higher leaf ratios of *H. suaveolens* leaf water extract strongly inhibited both shoot and root lengths, in contrast, the lower leaf ratios extracts promoted shoot growth. This results were similar to the research on Annonaceae plants leaves water extract that inhibited seedling growth of the test plants at higher leaf ratios but promoted growth at lower leaf ratios. Al-Zahrani and Al-Robai (2007) and Randhawa *et al.* (2002) also reported on the shoot promotion effect of the water extract of *Calotropis procera* and sorghum at low concentrations in their studies.

H. suaveolens leaf water extracts exhibited higher inhibitory effect on *P. setosum* than on *M. invisa*. Chatiyanon and Wongwattana (2006) studied on inhibitory effect of water extract from different plant parts of *Murraya paniculata* L. Jack. and found that the extracts significantly inhibited seed germination and seedling growth of *Mimosa invisa* and *Pennisetum setosum* and *P. setosum* was more sensitive to the extracts than *M. invisa* was. The methanol extracts of *H. suaveolens* leaf significantly inhibited seed germination and seedling growth of both *P. setosum* and *M. invisa* and the inhibitory effects were higher than those of the water extracts. Maighany *et al.* (2007) study on allelopathic potential of *Trifolium resupinatum* L. (Persian clover) and *Trifolium alexandrinum* L. (Berseem clover) in four test weeds and found that seedling growth of the weed species declined with the increasing concentrations of the extracts and the methanolic extract caused greater decline in the seedling growth than the aqueous extract. Studies on allelopathic effects of extracts of *Impatiens* species (Vrchotova *et al.*, 2011) and *Chenopodium ambrosioides* (Hegazy and Farrag, 2007) also showed stronger inhibition on germination and growth of test plants of methanol extract than water extract. This indicated that the allelochemicals in *H. suaveolens* leaf dissolved better in methanol than in water.

CONCLUSION

From the results of this experiment, it could be concluded that *H. suaveolens* leaf has allelochemicals which dissolved better in methanol than in water and the

chemical exhibited herbicidal activities on seed germination and seedling growth of *P. setosum* and *M. invisa*. Allelopathy of *H. suaveolens* should be studied more for the practical use in agricultural pest management.

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