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Evaluation of the Allelopathic Potential of Fifteen Common Malaysian Weeds

(Penilaian Potensi Alelopati ke atas Lima Belas Rumpai Biasa di Malaysia)

NURUL AIN, M.B., NORNASUHA, Y. & ISMAIL, B.S.*

ABSTRACT

The use of allelopathy concept in weed management has received attentions to minimize extensively the reliance on herbicide applications on the agriculture industry in Malaysia. A laboratory study was conducted to evaluate the allelopathic potential of 15 Malaysian common weed species of different morphological characteristics (broadleaves, sedges and grasses). They were screened using the Sandwich method (from leaf litter leachate) and the Dish pack method (for testing the presence and content of volatile compounds in weeds). Among the 15 weed species tested, the leaf litter leachate of Centrosema pubescens *was observed to be the most sensitive plant material inhibiting the growth of lettuce radicle (84%) and hypocotyl (55%) in the Sandwich bioassay compared to the control. This was followed by* Asystasia gangentica *(81%) and* Cynodon dactylon *(80%) inhibiting the lettuce radicle growth. In the Dish pack bioassay,* Rhynchelytrum repens *demonstrated maximum inhibition on the radicle and hypocotyl elongations by 44% and 29%, respectively, (over control) at 41 mm distance from the source well. Meanwhile, at the same distance,* Cynodon dactylon *was observed to have the least inhibitory effect on lettuce radicle growth by 12%. The results presented can be utilized as benchmark information for further research on the identification and isolation of allelochemicals for weed control strategies.*

Keywords: Allelochemical; allelopathy; dish pack method; sandwich method

ABSTRAK

Penggunaan konsep alelopati dalam pengurusan rumpai telah mendapat perhatian untuk meminimumkan kebergantungan secara meluas terhadap penggunaan racun rumpai ke atas ladang pertanian di Malaysia. Kajian makmal telah dijalankan untuk mengenal pasti potensi alelopati terhadap 15 spesies rumpai biasa di Malaysia yang berbeza secara fizikal luarannya (daun lebar, rusiga dan rumput) dan ia disaring menggunakan kaedah Sandwich (daripada sarap daun) dan kaedah Dish pack (untuk menguji kehadiran dan kandungan bahan meruap dalam rumpai). Daripada 15 spesies rumpai yang diuji, sarap daun Centrosema pubescens *diperhati sebagai bahan tumbuhan yang paling sensitif dalam merencatkan pertumbuhan akar selada (84%) dan pucuk selada (55%) dalam bioasai Sandwich berbanding kawalan, diikuti dengan* Asystasia gangentica *(81%) dan* Cynodon dactylon *(80%) yang merencatkan akar selada. Dalam bioasai Dish pack,* Rhynchelytrum repens *menunjukkan perencatan maksimum ke atas pemanjangan akar dan pucuk masing-masing sebanyak 44% dan 29% (berbanding kawalan) pada jarak 41 mm dari lubang punca tumbuhan penderma. Walau bagaimanapun, pada jarak yang sama,* Cynodon dactylon *diperhatikan mengalami kesan perencatan paling sedikit terhadap pertumbuhan akar sebanyak 12%. Keputusan yang dibentangkan dapat digunakan sebagai maklumat penanda aras untuk kajian selanjutnya ke atas pengenalpastian dan pengasingan alelokimia terhadap strategi pengawalan rumpai.*

Kata kunci: Alelokimia; alelopati; kaedah dish pack; kaedah sandwich

INTRODUCTION

Malaysia has been classified as one of the top countries rich in biodiversity. There are 15,000 plant species of which more than 100 weed species in the Malaysian agro-ecosystems are invasive (Baki 2004). The warm tropical climate coupled with adequate rainfall throughout the year is very conducive to the luxuriant growth of weeds. Weed has been found to colonize and invade new farming areas, intensive agricultural sites and abandoned farmlands (Baki 2004). Weed infestation is one of the major constraints of the plantation industry in Malaysia since weeds compete with the cultivated crops for available space, nutrients and moisture. Thus, weeds reduce productivity and lower the quality of the produce (Sahid & Chan 2000). A weed can be defined as a plant growing in a place where it is not desired and weeds compete for the limited space and available resources with the crop plant (Altieri 1988). Depending on their morphological characteristics weeds are classified into four groups namely grasses, sedges, broadleaved weeds and ferns (Barnes & Chan 1990).

Weeds are the major component that interferes in the oil palm production system. In Malaysia, 60 to 70 weed species have been reported to be growing under young oil palms, and of these 20 to 30 species are found under the older trees (Mohamad et al. 2010). Weeds also invade estates and rice cultivation areas. The use of herbicides to control weeds is a common practice and chemical herbicides are used in most extensively agricultural plantations of Malaysia. The annual global cost of herbicides in rice cultivation system is expected to reach US$3 billion by the year 2025 (Zhang et al. 2004). The constant use of chemical herbicides also leads to an increased risk of herbicide-resistance, environmental pollution, unsafe agricultural products, and negative effects on human health (Vyvyan 2002). Thus, there has been an increased effort globally to replace synthetic herbicides with other alternatives to minimize reliance on herbicides. The use of the allelopathy concept in weed management has recently received much attention. It can be carried out by utilizing the allelopathic interactions either directly or indirectly through the use of allelochemicals as alternative tools for weed control (Dayan et al. 2000; Duke et al. 1996; Kong et al. 2002). Allelopathy refers to the direct or indirect chemical effect of one plant on the germination, growth or development of neighbouring plants (Bond 2002). This phenomenon happens when the donor plant releases some allelochemicals into the surrounding environment through volatilization, leachate and root exudates during growth and decomposition of the plant parts (Pimentel et al. 2001). The allelopathic concept itself has been applied particularly in the form of cultural practices and cropping patterns decades ago to suppress weed emergence (De Albuquerque et al. 2010). Allelochemicals are considered valuable in current research methods for the development of new natural herbicides for weed management and control (Saxena et al. 2016). Therefore, the incorporation of invasive weeds with strong allelopathic activity into studies on agro-ecosystems would be an effective weed control strategy (Khan et al. 2010).

Most of the existing research studies on allelopathy have been focused more on specific plants to investigate their allelopathic potential rather than on exploring the allelopathic potential of plants on a broad spectrum. One important matter that needs to be explored is the potential that allelopathic species have on the suppression of weed seed germination and weed seedling emergence (Duke et al. 2002). Several earlier studies focused on selected plant species such as, the potential allelopathic effects of *Mikania micrantha* on the seed germination and seedling growth of *Coix lacryma-jobi* (Li & Jin 2010); the allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae) (Prati & Bossdorf 2004); the allelopathic effects of *Chromolaena odorata* (L.) King and Robinson on seed germination and seedlings growth of paddy and banyard grass (Suwal et al. 2010). However, studies on the screening of a broad spectrum of plants for identifying their allelopathic potential are still limited. In view of the importance of discovering the allelopathic potential of several weed species, the present study was undertaken to investigate the allelopathic potential of the leaf litter leachate and volatile compounds from three categories of morphologically different Malaysian weed species on the germination and growth of *Lactuca sativa* (lettuce), the receptor plant.

Materials and Methods

PLANT MATERIALS

A total of 15 weed samples namely; *Asystasia gangetica*, *Blumea lacera*, *Centrosema pubescens*, *Costus speciosus*, *Impatiens balsamina*, *Cyperus kyllingia*, *Cyperus iria*, *Cyperus rotundus*, *Fimbristylis miliacea*, *Rhynchospora corymbosa*, *Cynodon dactylon*, *Eragrostis atrovirens*, *Panicum maximum*, *Pennisetum purpureum* and *Rhynchelytum repens* were collected from various localities in Malaysia. They were divided according to their different morphological characteristics namely broadleaves, sedges and grasses. The fresh leaves were put in bags until the time of use. *Lactuca sativa* (lettuce) seeds were purchased from Takii Seed Co. Ltd, Kyoto, Japan, and used as the bioassay species because of its reliability in germination and its sensitivity to changes.

SANDWICH METHOD

The sandwich method developed by Fujii et al. (2003), was used to evaluate the allelopathic potential of the leaf litter leachate obtained from the leaves of the selected donor plants. Two different concentrations of the leaf leachate at 10.0 and 50.0 mg were added into separate wells in the 6-well dish (Nalga Nunc. International, Tokyo). Agar (Nacalai Tesque, Kyoto, Japan) was used as the medium for the lettuce seedling growth. About 5.0 mL of autoclaved agar was added onto the dried leaf samples in each well. Thereafter, another 5.0 mL of autoclaved agar was poured over as a second layer and allowed to solidify at room temperature. Every treatment was replicated thrice. For the untreated control, 10.0 mL of agar was poured into each empty well of the multi-dish (containing no plant material). In each well, five *L. sativa* seeds were placed vertically on the agar and were evenly spaced. The germination and seedling growth (radicle and hypocotyl length) were recorded after incubation in the dark at 20°C for four days.

DISH PACK METHOD

The dish pack experiment was conducted to determine the effect of volatile materials from the leaves of the donor plant on the receptor plant (adopted from Appiah et al. 2015; Fujii et al. 2005). The weed leaf samples were cut approximately to 3.0 mm^2 to enhance the release of volatile compounds. Multi-well plastic dishes were used with each well measuring 36.0 × 18.0 mm^2 in size. The source well distances (where plant sample was placed) to the other wells were 41, 58, 82 and 92 mm^2, respectively, based on the well of the multi-well plastic dish used. The first well (source well) of each multi-well plastic dish was filled with 200.0 mg of the oven-dried weed leaf samples. The remaining five wells had Whatman No. 6 filter paper laid in them. Then, 0.70 mL of distilled water was pipetted into each of the wells containing the filter paper. Five lettuce seed (the bioassay species) were sown and evenly spaced on the filter paper. For the control treatment, there was no plant sample in the source well. Three replicates were done for each leaf weed sample. The multi-well dishes were tightly sealed with cellophane tape to avoid loss of volatile compounds and labeled appropriately. The germination and seedling growth (radicle and hypocotyl length) were recorded after incubation in the dark at 20°C for four days.

STATISTICAL ANALYSIS

The experiment was carried out using a complete randomized design (CRD) with three replications for each type of weed leaf sample. Evaluation of the mean values of the length of the lettuce radicle and hypocotyl were subjected to the analysis of variance, using the software SPSS version 23.0 (Statistical Programme for Social Science) and Microsoft Excel. The mean values of inhibition for each species were compared using the Duncan Multiple Range Test (DMRT) at the 5% level of significance.

RESULTS AND DISCUSSION

ALLELOPATHIC EFFECTS OF THE LEAF LEACHATE OF COMMON MALAYSIAN WEED SPECIES (AS DETERMINED BY THE SANDWICH METHOD)

The sandwich method presented benchmark information on the type of chemicals involved in the allelopathy phenomenon in nature by the leaching off mechanism (Shinwari & Fujii 2013). The effects of the radicle and hypocotyl length of the lettuce seedlings (bioassay species) from two different concentrations of the dried leaves of 15 weed species are shown in Tables 1 and 2. It can be seen that the radicle and hypocotyl length of the lettuce seedlings were reduced significantly ($p>0.05$) by the leaf leachate of the different donor species. The leaf leachate of the different weed species inhibited the radicle and hypocotyl elongation of the lettuce seedlings.

Based on the mean percentage inhibition results obtained, it was observed that, both radicle and hypocotyl growth of lettuce were affected by the leaf litter leachate of the different weed species ranging from 53-85% and 10-55%, respectively. The radicle elongation is the most sensitive growth variable measured in allelopathic studies. Inhibition of lettuce radicle elongation ranging from 80-89% occurred in three species (*Centrosema pubescens*, *Asystasia gangetica* and *Cynodon dactylon*), 70-79% in two species (*Rhynchelytrum repens* and *Fimbristylis miliacea*), 60-69% in six species (*Panicum maximum*, *Pennisetum purpureum*, *Impatiens balsamina*, *Eragrostis atrovirens*, *Costus speciosus* and *Blumea lacera*) and 50-59% in four species (*Cyperus rotundus*, *Cyperus kyllingia*, *Cyperus iria* and *Rhynchospora corymbosa*).

The inhibitory effect on radicle elongation by each weed species was more than 50% whereby the inhibitory effect on the hypocotyl growth was less than 50%, with the exception of *C. pubescens* (55.03%). Four species namely, *F. miliacea*, *A. gangetica*, *R. repens* and *E. atrovirens* demonstrated lettuce inhibition in the range of 40-49%. The species *C. dactylon*, *C. speciosus*, *I. balsamina* and *P. purpureum* demonstrated lettuce hypocotyl inhibition in the range of 30-39%. The species *C. iria*, *P. maximum*, *R. corymbosa* and *C. kyllingia* inhibited lettuce hypocotyl growth in the range of 20-29% whereas, *C. rotundus* (11.48%) and *B. lacera* (10.97%) showed the least inhibitory effect on the lettuce hypocotyl growth.

TABLE 1. Allelopathic effects (growth inhibition) of different concentrations of the weed leaf litter leachate (% control) on lettuce radicle growth as determined by the sandwich method

Morphological characteristics	Weed species	Treatments		Mean Percentage inhibition	Rank
		10 mg	50 mg		
Broadleaf weeds	*Asystasia gangetica*	78.15 a	83.48 c	80.81	2
	Blumea lacera	44.37fgh	75.78 f	60.07	11
	Centrosema pubescens	77.04 ab	91.26 a	84.15	1
	Costus speciosus	47.48fg	78.81 d	63.15	10
	Impatiens balsamina	54.74 e	76.30ef	65.52	8
Sedges	*Cyperus kyllingia*	47.11fgh	68.37 h	57.26	13
	Cyperus iria	43.04 gh	71.48 g	56.00	14
	Cyperus rotundus	47.04fgh	64.96i	57.74	12
	Fimbristylis miliacea	56.59 de	86.22 b	71.41	5
	Rhynchospora corymbosa	42.59 h	64.81 i	53.70	15
Grasses	*Cynodon dactylon*	73.63 b	86.44 b	80.04	3
	Eragrostis atrovirens	47.93 f	81.78 c	64.85	9
	Panicum maximum	59.41 d	78.59 de	69.00	6
	Pennisetum purpureum	58.59 de	78.00ef	68.30	7
	Rhynchelytrum repens	65.19 c	90.74 a	77.96	4

Means values followed by the same alphabet within each column are not significant at $p<0.05$ according to Duncan's multiple range test
Values given in the table are inhibition percentage over that of the control
*Plant were ranked in order of their inhibitory activity

TABLE 2. Allelopathic effects (growth inhibition) of different concentrations of the weed leaf litter leachate (% control) on lettuce hypocotyl growth as determined by the sandwich method

Morphology characteristic	Weed species	Treatments		Mean Percentage inhibition	Rank
		10 mg	50 mg		
Broadleaf weeds	*Asystasia gangetica*	39.74 a	47.74 cde	43.74	3
	Blumea lacera	- 9.55 h	31.48 fg	10.97	15
	Centrosema pubescens	39.87 a	70.19 a	55.03	1
	Costus speciosus	25.03 cd	49.03 cd	37.03	7
	Impatiens balsamina	26.84 bcd	41.03 def	33.94	8
Sedges	*Cyperus kyllingia*	14.19 ef	32.39 fg	23.29	13
	Cyperus iria	21.68 cde	35.61 fg	28.65	10
	Cyperus rotundus	9.55 f	13.42 h	11.48	14
	Fimbristylis miliacea	35.48 ab	59.35 b	47.42	2
	Rhynchospora corymbosa	18.45 def	29.29 g	23.87	12
Grasses	*Cynodon dactylon*	29.16 bc	49.03 cd	39.10	6
	Eragrostis atrovirens	25.81 cd	56.77 bc	41.29	5
	Panicum maximum	20.52 cde	32.00 fg	26.26	11
	Pennisetum purpureum	21.55 cde	38.84 efg	30.19	9
	Rhynchelytrum repens	24.26 cde	61.03 b	42.65	4

Means values followed by the same alphabet within each column are not significant at $p<0.05$ according to Duncan's multiple range test
Values given in the table are inhibition percentage over that of the control
*Plants were ranked in order of their inhibitory activity

The results showed that, the application of the leaf leachate of plant materials from each weed species significantly increased the level of inhibition of the radicle and hypocotyl elongation of the bioassay plants as the concentration of leaf leachate used increased from 10 to 50 mg (compared to control). The allelopathic effect in terms of radicle and hypocotyl elongation appeared to be concentration dependent. The laboratory experiment showed that, the percentage of inhibition on lettuce growth was dependent on the concentration of plant leaf leachate applied. For both concentration of 10 and 50 mg of the oven-dried leaf treatments, the lettuce radicle growth was suppressed more severely than the hypocotyl growth. The radicle elongation is the most sensitive growth variable and it is measured in allelopathic studies since the effect on radicle elongation is more dependent on the physiological characteristics (Haugland & Brandsaeter 1996).

In terms of lettuce growth suppression, it can be highlighted that *C. pubescens* ranked top among the 15 weed species tested. *C. pubescens*, a broadleaved weed which caused 84% percentage inhibition, had the strongest allelopathic potential among the species evaluated using the sandwich method. The leaf leachate from *C. pubescens* had the strongest inhibitory effect in reducing the lettuce radicle and hypocotyl growth compared to the leachate of the other weed species evaluated. It was observed that, lettuce seedlings grown with 10 and 50 mg leaf litter leachate of *C. pubescens* recorded remarkable reduction in radicle length of more than 70% and 90%, respectively. The suppression of the hypocotyl growth of more than 35% and 70% was obtained when treated with 10 and 50 mg concentration of the leaf litter leachate, respectively.

Centrosema pubescens also known as Centro or butterfly pea which belongs to the family Fabaceae originated from Central and South America. It is abundant in humid tropical environments. *C. pubescens* is a vigorous, trailing, twining and climbing perennial herbaceous species with trifoliate leaves and which is fairly drought tolerant (Skerman et al. 1988). *C. pubescens* has been used as a forage crops and is a great source of protein to grazing cattle (Offor & Wariboko 2013). During the nineteenth century, *C. pubescens* was introduced to Malaysia and Indonesia as a cover crop. According to Offor and Wariboko (2013), the combination of grass and *C. pubescens* (a legume) has better suppressive effect on weeds by creating a dense ground cover and spreading naturally to cover a large surface area. *C. pubescens* is grown as a cover crop because it is able to suppress weeds biologically as it has a twining vigorous growth habit. Leguminous cover crop species commonly used in Malaysia are *Pueraria javanica*, *Centrosema pubescens*, *Calopogonium mucunoides*, *Calopogonium caeruleum* and *Mucuna bracteata* (Mathews & Saw 2007).

The study showed that *C. pubescens* exhibited the strongest allelopathic potential in inhibiting the growth of the radicle and hypocotyl of the lettuce plant (bioassay plant). The bioassay seedlings were in stress due to the application of the leaf leachate of *C. pubescens*. The factor that influences the allelopathic activity of a plant is the chemical content or allelochemicals that are present is the donor species. Secondary plant metabolites or allelochemicals suppressed seed germination and seedling emergence, therefore, these are some of the compounds that may be useful directly as herbicides or as templates for herbicide development. *C. pubescens* has been reported to be rich in isoflavone glycoside such as pubescidin, sitosterol, stigmasterol and sitosterol 3-O-β-D-glucopyranoside from HPLC analysis (Tostes et al. 1997). Isoflavone is always present in the Fabaceae family. Isoflavone is a secondary metabolite that affects plant physiology, biochemistry and ecological functions (Taylor & Grotewold 2005). Therefore, these allelochemicals in *C. pubescens* might have caused growth reduction in lettuce.

ALLELOPATHIC EFFECTS OF THE LEAF LEACHATE OF COMMON MALAYSIAN WEED SPECIES (AS DETERMINED BY THE DISH PACK METHOD)

The dish pack method presents the route of action for evaporation of the volatile allelochemicals from the leaves of plant species. This experiment gives crucial benchmark information for analyzing volatile chemical compounds released through allelopathy in nature. Tables 3 and 4 represent the growth inhibition of the radicle and hypocotyl of the lettuce seedling (bioassay plant) at different positions and distances from the source well. The position and distances were based on the distance between test plant and lettuce seeds in the Multi-well dish plastic in order to check the intensity of volatile compounds activities in non-contact system (Fujii et al. 2005). About 15 plant species of different morphological characteristics were tested and from different plant families. The dish pack experiment showed that, the radicle and hypocotyl elongation of the lettuce seedlings were significantly reduced by the volatile allelochemicals released from the different donor weed species. The dried leaf of the 15 weed species inhibited the radicle and hypocotyl elongation of the lettuce seedlings compared to that of the corresponding control.

Based on the dish pack experiment, the observed rate of inhibition of the lettuce radicle growth (Table 3) ranged from 10-40% after treatment with 15 common Malaysian weed species of different morphological characteristics. Radicle elongation is the most sensitive growth variable measured in allelopathic evaluations (Sairah et al. 2014). The allelopathic effects on lettuce growth in terms of radicle elongation depended on the concentration of volatile chemicals from the donor species. The weed, *R. repens* was observed to produce the strongest inhibitory effect on the lettuce radicle growth with percentage inhibition up to 39%. Three other species (*C. pubescens*, *A. gangetica* and *C. speciosus*) caused radicle growth reduction in the range of 20-30%. Growth inhibition between 10-20% was observed after treatment with seven weed species (*C. rotundus*, *E. atrovirens*, *I. balsamina*, *R. corymbosa*, *B. lacera*, *C. kyllingia* and *F. miliacea*). The species *C. iria*, *P. maximum*, *P. purpureum* and *C. dactylon* suppressed lettuce radicle elongation by 10-15%.

Table 4 represents the percentage inhibition on lettuce hypocotyl growth at four different distance positions from the source well (where weed samples were placed). Based on the results obtained, the mean percentage inhibition occurred on each weed sample was less than 30%. An inhibition of lettuce hypocotyl elongation using the dish pack method was as follows: Inhibition of 20-30% occurred in three species (*R. repens*, *A. gangetica* and *E. atrovirens*); inhibition of 15-20% occurred in three species (*C. rotundus*, *P. maximum* and *C. kyllingia*); inhibition of 10-15% occurred in five species (*R. corymbosa*, *C. speciosus*, *F. miliacea*, *C.*pubescens and *C. iria*) and inhibition of 1-10% occurred in four species (*B. lacera, I. balsamina*, *C. dactylon* and *P. purpureum*).

TABLE 3. Allelopathic effects (growth inhibition) of different distances of plant volatile compounds (% control) on lettuce radicle as determined by the dish pack method

Morphological characteristics	Weed species	Distances from the source well				Mean percentage of inhibition	Rank
		41 mm	58 mm	82 mm	92 mm		
Broadleaf weeds	*Asystasia gangetica*	35.55 ab	15.42 bcd	17.58 bcde	30.96 ab	24.83	3
	Blumea lacera	29.92 bc	17.33 bcd	13.39 cde	10.46 def	17.77	9
	Centrosema pubescens	25.52 cd	23.43 b	26.36 ab	32.63 ab	26.99	2
	Costus speciosus	21.34 dce	15.48 bcd	34.73 a	15.48 cde	21.76	4
	Impatiens balsamina	24.48 cd	15.90 bcd	17.99 bcd	20.50 cde	19.72	7
Sedges	*Cyperus kyllingia*	23.64 cd	17.16 bcd	15.06 bcde	10.46 def	16.58	10
	Cyperus iria	20.08 de	18.41 bc	13.81 cde	6.69 ef	14.75	12
	Cyperus rotundus	20.50 de	17.99 bc	14.64 bcde	26.36 abc	19.87	5
	Fimbristylis miliacea	17.99 de	12.97 bcd	15.06 bcde	15.48 cde	15.38	11
	Rhynchospora corymbosa	18.41 de	19.66 bc	25.52 abc	10.04 def	18.41	8
Grasses	*Cynodon dactylon*	14.23e	9.62 cd	5.02 ef	17.16 cde	11.51	15
	Eragrostis atrovirens	23.43	19.66 bc	13.39 cde	22.59 abcd	19.77	6
	Panicum maximum	18.62	7.11 de	16.32 bcde	15.48 cde	14.38	13
	Pennisetum purpureum	14.23 e	14.64 bcd	8.37 def	15.48 cde	13.18	14
	Rhynchelytrum repens	42.68 a	37.24 a	33.05 a	34.73 a	36.92	1

Means values followed by the same alphabet within each column are not significant at p<0.05 according to Duncan's multiple range test
Values given in the table are inhibition percentage compound to that of the control
*Plants were ranked in order of their inhibitory activity

TABLE 4. Allelopathic effects (growth inhibition) of different distances of plant volatile component (% control) on lettuce hypocotyl as determined by the dish pack method

Morphological characteristics	Weed species	Distances from the source well				Mean percentage of inhibition	Rank
		41 mm	58 mm	82 mm	92 mm		
Broadleaf weeds	*Asystasia gangetica*	21.02ab	28.53a	18.92abc	18.92abc	21.85	2
	Blumea lacera	12.31bcdefg	12.31bcd	11.11bcde	3.90ef	9.91	12
	Centrosema pubescens	12.01bcdefg	11.11bcde	11.71bcde	17.12bcdef	12.99	10
	Costus speciosus	11.41cdefg	11.71bcde	18.32abc	12.31bcdef	13.44	8
	Impatiens balsamina	9.91defg	9.91cde	9.31cdef	5.71cdef	8.71	13
Sedges	*Cyperus kyllingia*	18.62bcde	12.31bcd	17.22abc	17.72abcd	16.59	6
	Cyperus iria	8.71efg	14.71bcd	7.51defg	11.71bcdef	10.66	11
	Cyperus rotundus	15.31bcdef	14.71bcd	22.52a	21.12ab	18.17	4
	Fimbristylis miliacea	10.81cdefg	12.91bcd	18.32abc	11.11bcdef	13.29	9
	Rhynchospora corymbosa	16.52bcde	18.32abc	14.11abcd	5.10def	13.51	7
Grasses	*Cynodon dactylon*	7.21fgh	9.91cde	2.70efg	2.70f	5.63	14
	Eragrostis atrovirens	19.22bc	19.52abc	15.91abcd	26.73a	20.35	3
	Panicum maximum	15.02bcdefg	11.11bcde	21.32abc	20.72ab	17.04	5
	Pennisetum purpureum	3.60gh	3.90de	-1.5g	5.71cdef	2.93	15
	Rhynchelytrum repens	28.53a	22.52ab	24.32a	23.72ab	24.77	1

Means values followed by the same alphabet within each column are not significant at $p<0.05$ according to Duncan's multiple range test
Values given in the table are inhibition percentage compared to that of the control
*Plants were ranked in order of their inhibitory activity

Among the 15 weed species tested, the volatile allelochemicals from *R. repens* resulted in the strongest inhibitory effects on both the radicle and hypocotyl of the bioassay plant at almost all the four distances from the source well. Therefore, *R. repens* has strong allelopathic potential based on the inhibitory activity on both the radicle and hypocotyl growth of lettuce seedlings. The dish pack method showed that the volatile allelochamicals from *R. repens* species were the highest compared to that of the other weed species tested. This could be due to the rapid inhibiting effects caused by the volatile compounds present in *R. repens*.

Rhynchelytrum repens (Willd.) C.E. Hubb., is a short-lived perennial grass, commonly known as Natal Red-top Grass or by the Malay name, Rumput dawai (Gould 1994). It occurs widespread on dry, open areas and often on sandy soil. It can be easily found in the rubber, oil palm, sugar cane, coconut, tea and cocoa plantations (Padgett 1972). *R. repens* is a persistent grass that belongs to the family Poaceae and is dispersed efficiently via rhizomes and wind-borne seeds. *R. repens* is widespread in tropical areas and has been consistently observed as an invasive species in pastures, mainly in open areas and at roadsides. Possley and Joyce (2006) observed that, high densities of *R. repens* were associated with a significant reduction in native species diversity due to suppression by *R. repens*.

Conclusion

The results of the study provide insight on the allelopathic potential of some weeds species in Malaysia as well as the presence of allelochemicals in the leaf litter leachate and volatile compound of fifteen Malaysian weed species. The interaction between the chemicals released from the tested weed species with those of the bioassay species (lettuce) created conditions that were not favourable for its growth. It was observed that, the leaf litter leachate from 15 common weed species produced more positive allelopathic effects than the volatile compounds based on the results from the two methods used.

The ranking of the screened plants, in order of their inhibitory activity, gives information on the allelopathic potential of the weed species. The weed that ranked the highest in the production of inhibiting effects on the lettuce seeds was *C. pubescens* (from the sandwich method). This weed has the potential to be exploited for potent allelochemicals. On the other hand, *R. repens* showed strong allelopathic potential based on the inhibitory activity on the lettuce radicle and hypocotyl growth when dish pack method was used.

Based on the results, it is suggested that *C. pubescens* and *R. repens* have strong potential for development as natural herbicides in weed management. However, further studies need to be carried out in order to determine the content and type of allelochemicals present in *C. pubescens* and *R. repens*. The results presented can be utilized as benchmark information for further research in the development of new environment-friendly natural herbicides.

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Nurul Ain, M.B. & Ismail, B.S.*
School of Environmental and Natural Resource Sciences
Faculty of Science and Technology
Universiti Kebangsaan Malaysia

43600 UKM Bangi, Selangor Darul Ehsan
Malaysia

Nornasuha, Y.
School of Agricultural Sciences and Biotechnology
Faculty of Bioresources and Food Industry
Universiti Sultan Zainal Abidin, Kampus Besut
22200 Besut, Terengganu Darul Iman
Malaysia

*Corresponding author; email: ismail@ukm.edu.my

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