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Allelopathic performance of common weed species grown in BRIS soil

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Laboratory experiments were conducted to evaluate the allelopathic activity of 15 common weed species of Beach Ridges Interspersed with Swales soil (BRIS) soil. The allelopathic activity was evaluated using Sandwich and Dish Pack methods. These methods were screened for testing the effect of leaf litter and leaf volatilization of the weed in three replications. The Sandwich method was carried out using three different amounts of leaf litter (i.e; 5 mg, 10 mg and 50 mg), whilst the Dish Pack method was carried out in four different distances (41, 58, 82 and 92 mm) from the samples to the receiver species. The pre-germinated *Lactuca sativa* (lettuce) was used as the receiver species. Among the 15 common weed species of BRIS soil, *Crotalaria pallida* showed the highest inhibition on elongation of the lettuce radicle (81.34%) in Sandwich bioassay compared to the control, followed by *Melastoma malabathricum* (51.31%) and *Asystasia gangetica* (50.60%). Meanwhile, *Mimosa pudica* showed the highest inhibition on elongation of the lettuce radicle (30.79%) in Dish pack bioassay compared to the control followed by *Lantana camara* (26.35%) and *Acroceras munroanum* (25.07%). The result obtained can be the guideline information for further research on the mechanism and allelochemicals involved in this allelopathic activity.

Keywords: Allelopathy; Sandwich Method; Dish pack method; Leaf litter; Leaf volatilization; Weed species.

INTRODUCTION

Allelopathy is a natural phenomenon that can be utilized as weed management strategies (Albuquerque et al., 2010). Allelopathy can affect many plant physiology processes such as plant ecology, occurrence, growth, dominant, communities, plant succession and plant productivity (Shankar et al., 2014). The term "allelopathy" was introduced by Hans Molisch (Fuji et al., 2004), and it is defined as the inhibitory and/or stimulatory effect of one plant to another plant due to the release of the chemical compounds into the environment (Rice, 1984). The chemical compounds, usually regarded as allelochemicals are released into the environment through four main pathways, which are leaching from above plant parts, volatilization,

decomposition and root exudation (Albuquerque et al., 2010). However, these pathways can vary in plant species and depending on the chemical nature of compounds (Scavo et al., 2019).

Allelochemicals are known to modify the growth and development of the plants, including the germination and early seedling growth of neighbour plants (Zhou and Yu, 2006). According to Li et al., (2010), allelochemicals like phenolic compounds, alkaloids and other secondary metabolites can lead to ecological and economic nuisance such as reduction in crop yield due to soil sickness, problem in regeneration of natural forest and also problem of replanting in the field. Contrary, allelopathy can be used as weed control strategies through crop rotation practices, by using as cover crop and mulching (Farooq et al.,

2011). Allelopathy can increase the quality of soil and yield production by minimizing the spread of plant pathogen and damaging the weed (Jabran and Farooq, 2013).

Allelopathy is one of the biological approaches that able to control the weed density by reducing the application of synthetic herbicide (Sodaiezadeh et al., 2012). The use of allelochemicals as natural herbicides is considered as environmental friendly because of their shorter half-lives in the environment as compared to the synthetic herbicides (Duke et al., 2000), easily being degraded to the environment by other organism and also less toxic than synthetic one (Rimando and Duke, 2006). Based on these advantages, it is vital to screen new species with high allelopathic potential to promote weed control practices using allelopathy. In particular, farmers should consider a non-herbicidal innovation of weed management to minimize the dependence on injurious synthetic herbicides (Dayan et al., 2009).

BRIS (Beach Ridges Interspersed with Swales) soil is lacked in many aspects such as too sandy, weakly structured, nutrient deficient, having low water retention capacity, limited ability to support plant growth and having a relatively high soil temperature (Toriman et al., 2009). BRIS soil arises from sediment or sand from the sea that was assembled from the erosion of steep cliffs layers by the sea during the monsoon seasons and has a component of coarse sand (Nossin, 1964). Based on these characteristics, allelopathic effects of weeds found in BRIS may be higher due to limited nutrient in soil. This supported by Bhowmik (2003) that the plant species that grown under low or moderate nutrient availability have high allelopathic activity. Thus, this research is developed to screen the allelopathic performance of common weed species found in BRIS soil which can help to reduce the problem of excessive use of synthetic herbicides and poor cultural practices.

MATERIALS AND METHODS

Plant Materials

In this research, 15 samples of common weed species found in BRIS area were used as the donor plants to determine its allelopathic activity potential by using the Sandwich Method and Dish Pack Method. Seeds of *Lactuca sativa* (lettuce) were used as the receptor plants. The leaf of each plant sample was collected from BRIS soil at Besut District of Terengganu. The collected fresh

leaves samples were then dried in the oven at 60 °C for 24 hours and kept at room temperature (28 °C ± 2) for further usage.

Sandwich Method

The sandwich method (Fujii et al., 2004) was used to evaluate allelopathic activity of leaves litter leachates from the 15 selected donor plant (Appiah et al., 2015). The dried leaves were weighed into 5 mg, 10 mg and 50 mg. All amounts were prepared in three replications. For media preparation, agar powder from Nacalai Tesque, Kyoto, Japan was solidified at 30-31 °C and used as media. The solution of agar (0.75 % w/v) was prepared and autoclaved for 15 minutes at 121 °C.

A total of 5 mL of the agar was weighted into each well of the multi-dish containing dried leaves as the first layer. Another 5 mL of agar medium was added into each well of the multi-dish as the second layer. The agar medium was let to solidify. Five seeds of pre-germinated *Lactuca sativa* were sown on the agar surface of each dish at equal distances. The multi-dishes were incubated in complete darkness at 21 °C. After four days, the percentage of germination and length of radicle and hypocotyl were measured and recorded.

Dish pack method

The dish pack method as proposed by Yoshiharu Fujii (Fujii et al., 2005) was used to evaluate the presence of volatile substances from donor plant species. The distance from the source well (where dried samples were placed) to the other wells were set at 41, 58, 82 and 92 mm. The source well was filled with 200 mg of dried leaves while the other wells were added with 0.7 mL of distilled water containing filter paper in each well.

The experiments were conducted in three replications. For control treatment, no dried leaves were added. Five seeds of pre-germinated lettuce seed were placed on the filter paper in each well. The multi-well dishes were sealed tightly with cellophane tape to prevent the losses of volatile compounds. The multi-well dishes were also wrapped with aluminium foil to provide dark condition and placed it in plant growth chamber at 21 °C. After four days, the lengths of radicle and hypocotyl were measured, recorded and compared to that of control.

Statistical Analysis

All the experiments were carried out in three replicates and were conducted by using Completely Randomized Design (CRD). The

experimental data were subjected to the one-way variance analysis (ANOVA). The means were compared by using the Duncan Multiple range test at the 5 % level of significance. The software SPSS version 14.0 and Microsoft Excel were used for the statistical analysis.

RESULTS

Allelopathic Performance of Leaf Litter from Common Weed species on Lettuce in Sandwich Method

Table 1 and Table 2 show that the elongation of radicle and hypocotyl are significantly decreased ($p < 0.05$) as the amount of leaf litter leachates from different weed species increased. The donor plants inhibited the radicle length more compared to the hypocotyl length of lettuce (Table 1 and Table 2).

Radicle growth:

Table 1 showed that the highest inhibition of radicle was recorded for *Crotalaria pallida* (67.64%), which inhibited the radicle elongation up to more than 50%, when the lettuce seedlings were grown with 5 mg dry leaf of weed. Leaf litter of all other species exhibited low allelopathic

activity, i.e which was less than 50%. When the weed species were screened at moderate leaf concentration (10 mg dish^{-1}), *C. pallida* exhibited the maximum inhibition (82.84%) of radicle. Marked reduction of 50.02% was recorded for *Ageratum conyzoides* compared with the control. When 50 mg dish^{-1} of leaf litter incorporated, the inhibition magnitude was observed more than 50% for all species with the exception of *Lantana camara* and *Lygodium microphyllum*. It was found that 50 mg dish^{-1} leaves concentration of *C. pallida* was enough to inhibit the radicle elongation up to more than 90%.

Based on the average inhibition percentage in Table1, the highest radicle inhibition compared to control was by *C. pallida* (81.34%), followed by *Melastoma malabathricum* (51.31%) and *Asystasia gangetica* (50.60%). The level of radicle inhibition of selected weed species from BRIS soil at Besut District can be classified into five levels; which are less than 30%, 30-39%, 40-49%, 50-69% and more than 70%, and were labeled as the first, second, third, fourth and fifth most effective levels causing inhibition of lettuce radicle (Figure 1).

Table 1: Allelopathic effects of different amount of leaf applied on the lettuce radicle growth using the Sandwich method

Species Name	Treatment			Means inhibition (%)	Rank
	5 mg	10 mg	50 mg		
<i>Crotalaria pallida</i>	67.63 _j	82.84 _g	93.56 _h	81.34	1
<i>Melastoma malabathricum</i>	38.58 _{hi}	42.45 _e	72.93 _f	51.32	2
<i>Asystasia gangetica</i>	40.96 _i	49.88 _f	61.05 _{de}	50.63	3
<i>Ageratum conyzoides</i>	28.28 _{fg}	50.02 _f	64.39 _e	47.56	4
<i>Imperata cylindrica</i>	24.27 _{ef}	38.81 _{de}	75.51 _{fg}	46.19	5
<i>Mitracarpus hirtus</i>	33.14 _{gh}	39.25 _{de}	56.24 _d	42.88	6
<i>Mimosa pudica</i>	19.09 _{bcde}	37.49 _{de}	60.77 _{de}	39.12	7
<i>Macroptilium atropurpureum</i>	20.75 _{cde}	35.88 _{de}	57.85 _d	38.16	8
<i>Stachytarpheta jamaicensis</i>	13.27 _{bc}	17.88 _b	78.48 _g	36.54	9
<i>Acroceras munroanum</i>	19.86 _{bcde}	37.20 _{de}	50.97 _c	36.01	10
<i>Lantana camara</i>	23.50 _{def}	34.03 _d	49.65 _c	35.73	11
<i>Rubus moluccanus</i>	16.20 _{bcd}	25.80 _c	58.44 _d	33.48	12
<i>Phyllanthus niruri</i>	2.70 _a	16.96 _b	79.25 _g	32.97	13
<i>Tridax procumbens</i>	12.51 _b	25.71 _c	57.58 _d	31.93	14
<i>Lygodium microphyllum</i>	16.79 _{bcde}	15.13 _b	30.76 _b	20.89	15
Control	0.00 _a	0.00 _a	0.00 _a	0.00	-

Note: Means within the rows followed by the same alphabet were not significantly different ($p < 0.05$) according to DMRT. Values given in the table were inhibition percentage over that of the control. Plants were ranked in order of their inhibitory activity.

Table 2: Allelopathic effects of different amount of leaf applied on the lettuce hypocotyl growth using the Sandwich method

Species Name	Treatment			Means inhibition (%)	Rank
	5 mg	10 mg	50 mg		
<i>Crotalaria pallida</i>	29.23 _g	38.62 _l	47.22 _h	38.35	1
<i>Stachytarpheta jamaicensis</i>	9.59 _f	15.92 _j	55.32 _h	26.94	2
<i>Melastoma malabathricum</i>	2.46 _{ef}	7.32 _{hi}	35.13 _g	14.97	3
<i>Ageratum conyzoides</i>	10.52 _f	27.31 _k	5.59 _{cde}	14.48	4
<i>Phyllanthus niruri</i>	-11.74 _{bc}	-7.63 _{cdef}	52.35 _h	10.99	5
<i>Imperata cylindrica</i>	-5.28 _{cd}	-5.99 _{def}	34.12 _g	7.62	6
<i>Asystasia gangetica</i>	-1.29 _{de}	10.02 _{ij}	3.13 _{bcd}	3.95	7
<i>Acroceras munroanum</i>	-3.91 _{cde}	4.38 _{ghi}	10.80 _{de}	3.76	8
<i>Mitracarpus hirtus</i>	4.19 _{ef}	1.25 _{fghi}	5.24 _{cde}	3.56	9
<i>Mimosa pudica</i>	-5.56 _{cd}	-4.15 _{efg}	3.33 _{bcd}	-2.13	10
<i>Lantana camara</i>	-16.31 _{ab}	-15.45 _{bc}	23.83 _f	-2.65	11
<i>Tridax procumbens</i>	-12.60 _{abc}	-9.04 _{cde}	13.22 _e	-2.80	12
<i>Lygodium microphyllum</i>	-2.50 _{de}	-13.97 _{bcd}	2.54 _{bcd}	-4.64	13
<i>Macroptilium atropurpureum</i>	-15.10 _{ab}	-20.50 _b	-13.89 _a	-16.50	14
<i>Rubus moluccanus</i>	-21.36 _a	-29.62 _a	-4.77 _b	-18.58	15
Control	0.00 _{de}	0.00 _{efgh}	0.00 _{bc}	0.00	-

Note: Means within the rows followed by same alphabet were not significantly different ($p < 0.05$) according to DMRT. Values given in the table were inhibition percentage over that of the control. Plants were ranked in order of their inhibitory activity.

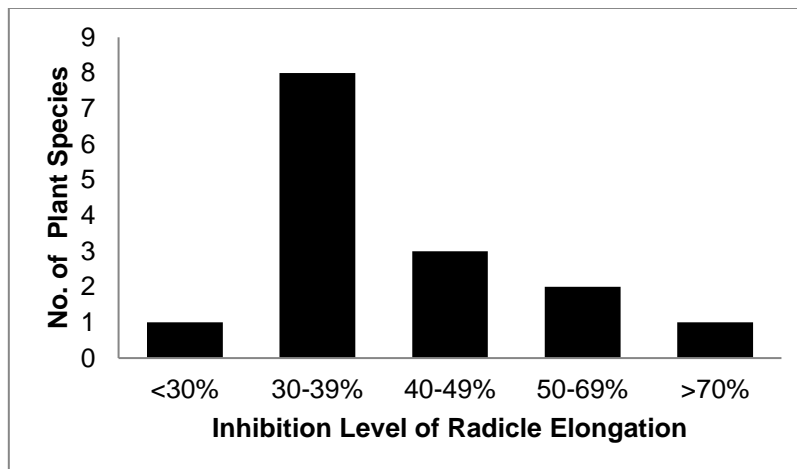


Figure 1; Frequency distribution of plant species for allelopathic activity against lettuce radicle as determined by Sandwich method. Each bar chart represented the number of weed species in each level of inhibition percentage.

Based on Table 1 and Figure 1, the inhibition rate of lettuce radicle ranged from more than 70% were observed in one species (*C. pallida*), 50-69% in two species (*Melastoma malabathricum* and *Asystasia gangetica*), 40-49% in three species (*A. conyzoides*, *Imperata cylindrica*, and *Mitracarpus hirtus*), 30-39% in eight species (*Stachytarpheta jamaicensis*, *Mimosa pudica*, *Macroptilium atropurpureum*, *Acroceras munroanum*, *L. camara*, *Rubus moluccanus*, *Phyllanthus niruri* and *Tridax procumbens*), and less than 30 % in one species (*L. microphyllum*).

Hypocotyl growth:

Table 2 showed that the growth and inhibition on the hypocotyl length were seemed to depend on the different amount of leaves applied. Hypocotyl elongation by each plant was less than 60% in all concentration (Table 2). At 5 mg dish⁻¹, *C. pallida*, *S. jamaicensis* and *A. conyzoides* showed significant inhibitory effects compared to control. However, at 10 mg dish⁻¹, several weed species exhibited higher hypocotyl inhibition. Overall *C. pallida* resulted in the strongest inhibition of hypocotyl length (38.62%). At 10 mg dish⁻¹ the inhibitory effect of *S. jamaicensis* and *P. niruri* was more than 50%. All other species exhibited less than 50% inhibitory effect except of *M. atropurpureum* and *R. moluccanus* at 50 mg dish⁻¹. Both species showed significant stimulatory effect compared to control at this high concentration of leaves.

Subsequently the inhibitory effect on the hypocotyl length was ranked into four levels; more than 30 %, 10-29 %, 0-9 % and less than 0 % (Figure 2). Based on Table 2 and Figure 2, the inhibition rate of lettuce hypocotyl elongation ranging from more than 30 % inhibition was observed in one species (*C. pallida*), 10-29 % in four species (*S. jamaicensis*, *M. malabathricum*, *A. conyzoides* and *P. niruri*), 0-9 % in four species (*I. cylindrica*, *A. gangetica*, *A. munroanum*, *M. hirtus*), and less than 0 % in five species (*M. pudica*, *L. camara*, *T. procumbens*, *L. microphyllum*, *M. atropurpureum*, and *Rubus moluccanus*).

Allelopathic Performance of Leaf Volatilization from Common Weed species on Lettuce in Dish pack Method

Table 3 and Table 4 indicated the allelopathic inhibitory results of 15 common weed species using dish pack method. The radicle and hypocotyl elongation of lettuce germination were significantly inhibited ($p < 0.05$) by the leaf

volatilization from different weed species at different distance. The negative value in the inhibition percentage represented a stimulatory effect on the growth of the lettuce elongation compared to control (Table 3 and Table 4).

Radicle growth:

Based on Table 3, all species showed significantly less than 50 % of inhibition ($p < 0.05$) on the lettuce radicle elongations for all the distance of leaf sample from the source well (Table 3). At the shortest distance from source well (41 mm), *Mimosa pudica* exhibited the highest inhibition value on lettuce radicle (30.18%). In contrast, *C. pallida* showed the lowest inhibition value (6.27%) compared to control. Several species such as *A. gangetica* and *M. hirtus* exhibited decrement trends in inhibition magnitude as the distance increased (from 41 mm to 92 mm). However, other species showed non-significant pattern for radicle inhibition among them but still significant with the control.

The average radicle inhibition percentage by dish method is shown in Table 3, with the highest radicle inhibition compared to control was observed in *Mimosa pudica* (30.79%), followed by *Lantana camara* (26.35%) and *Acroceras munroanum* (25.07%). The radicle inhibition level of selected weed species through Dish pack method can be classified into four levels; less than 15 %, 15-20 %, 20-25 % and more than 25 % (Figure 3). From Table 3 and Figure 3, the inhibition rate of the lettuce radicle elongation ranging from more than 25% was observed in three species (*M. pudica*, *L. camara* and *A. munroanum*), 20-25 % in five species (*T. procumbens*, *A. gangetica*, *M. hirtus*, *A. conyzoides* and *M. malabathricum*), 15-20 % in three species (*P. niruri*, *S. jamaicensis* and *R. moluccanus*), and less than 15 % in four species (*C. pallida*, *I. cylindrica*, *L. microphyllum* and *M. atropurpureum*).

Hypocotyl growth:

Table 4 showed that *M. pudica* exhibited the highest inhibition value (17.67%) at the shortest distance from source well. However, there was no weed species showed more than 50 % inhibition of hypocotyl elongation at all the distances from the source well in the Table 4. *Phyllanthus niruri* and *C. pallida* showed stimulatory effects on the hypocotyl length but not significant from the control at 41 mm distance of source well.

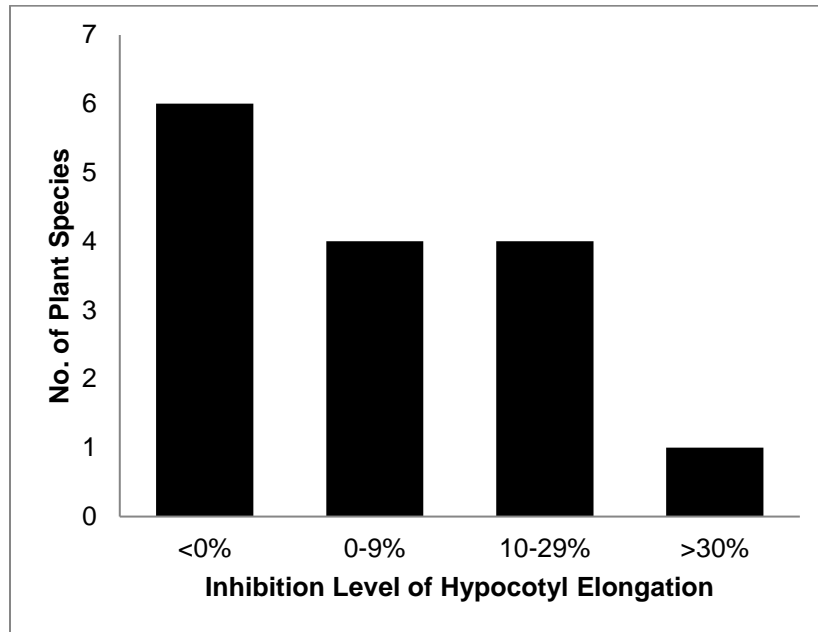


Figure 2: Frequency distribution of plant species for allelopathic activity against lettuce hypocotyl as determined by Sandwich method. Each bar chart represented the number of weed species in each level of inhibition percentage.

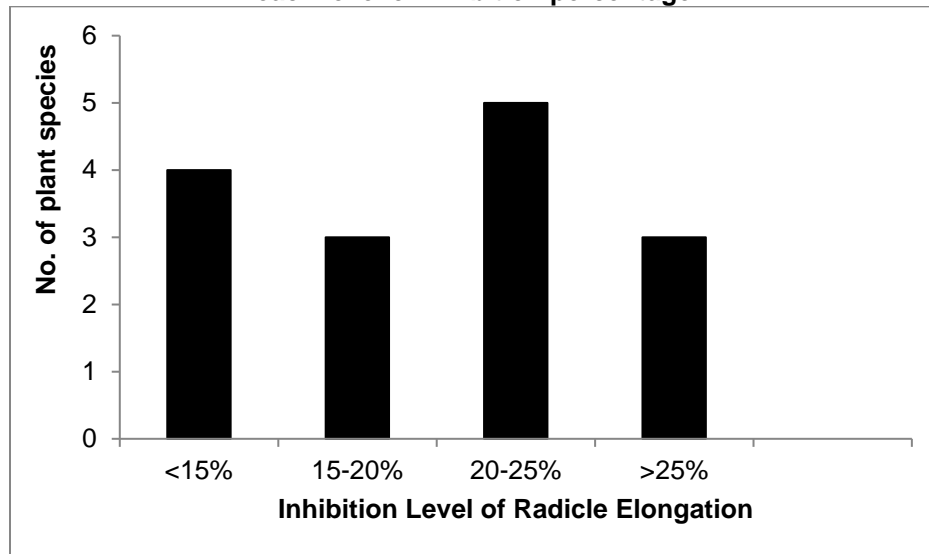


Figure 3: Frequency distribution of plant species for allelopathic activity against lettuce radicle as determined by Dish pack method. Each bar chart represented the number of weed species in each level of inhibition percentage.

Table 3: Allelopathic effects of different distance from source well on the lettuce radicle growth using the Dish pack method

Species Name	Distance from Source Well				Mean Inhibition (%)	Rank
	41 mm	58 mm	82 mm	92 mm		
<i>Mimosa pudica</i>	30.18 _{de}	43.14 _g	19.81 _{abcde}	30.02 _{cd}	30.79	1
<i>Lantana camara</i>	24.93 _{cde}	28.29 _{ef}	20.99 _{bcdef}	31.20 _{cd}	26.35	2
<i>Acroceras munroanum</i>	22.75 _e	16.90 _{abcdef}	21.86 _{bcdef}	38.78 _d	25.07	3
<i>Tridax procumbens</i>	30.46 _e	24.77 _{cdef}	16.61 _{abcde}	26.53 _{bcd}	24.59	4
<i>Asystasia gangetica</i>	23.33 _{cde}	20.41 _{abcdef}	33.25 _f	17.79 _{bc}	23.69	5
<i>Mitracarpus hirtus</i>	23.62 _{cde}	23.90 _{cdef}	30.91 _{ef}	15.74 _{abc}	23.54	6
<i>Ageratum conyzoides</i>	20.13 _{bcd}	23.04 _{bcdef}	26.24 _{cdef}	20.99 _{bc}	22.60	7
<i>Melastoma malabathricum</i>	22.88 _{cde}	29.15 _f	7.58 _{ab}	21.28 _{bc}	20.22	8
<i>Phyllanthus niruri</i>	18.08 _{bc}	16.90 _{abcdef}	13.99 _{abcd}	25.37 _{bcd}	18.58	9
<i>Stachytarpheta jamaicensis</i>	20.70 _{bcde}	20.99 _{abcdef}	12.83 _{abcd}	18.08 _{bc}	18.15	10
<i>Rubus moluccanus</i>	7.14 _a	11.07 _{abc}	26.82 _{def}	18.66 _{bc}	15.92	11
<i>Crotalaria pallida</i>	6.27 _a	26.53 _{def}	10.50 _{ab}	11.07 _{ab}	13.59	12
<i>Imperata cylindrica</i>	14.72 _{abc}	14.56 _{abcde}	11.65 _{abc}	12.25 _{ab}	13.30	13
<i>Lygodium microphyllum</i>	15.59 _{abc}	13.12 _{abcd}	11.36 _{abc}	9.32 _{ab}	12.35	14
<i>Macroptilium atropurpureum</i>	11.65 _{ab}	7.29 _a	5.25 _a	19.81 _{bc}	11.00	15
Control	0 _a	0 _a	0 _a	0 _a	0.00	-

Note: Means within the rows followed by the same alphabet were not significantly different ($p < 0.05$) according to DMRT. Values given in the table were inhibition percentage over that of the control. Plants were ranked in order of their inhibitory activity

Table 4: Allelopathic effects of different distance from source well on the lettuce hypocotyl growth using the Dish pack method

Species Name	Distance from Source Well				Mean Inhibition (%)	Rank
	41 mm	58 mm	82 mm	92 mm		
<i>Mimosa pudica</i>	17.67 _e	29.81 _g	24.32 _f	20.64 _{cde}	23.11	1
<i>Lantana camara</i>	14.00 _e	22.01 _{fg}	15.61 _{def}	30.26 _e	20.47	2
<i>Acroceras munroanum</i>	17.88 _e	15.11 _{cdef}	12.39 _{def}	27.50 _{de}	18.22	3
<i>Melastoma malabathricum</i>	12.59 _e	17.88 _{efg}	16.52 _{ef}	12.84 _{bcd}	14.96	4
<i>Lygodium microphyllum</i>	10.32 _{cde}	16.06 _{def}	5.49 _{abcde}	7.35 _{abc}	9.81	5
<i>Tridax procumbens</i>	10.78 _{cde}	7.80 _{abcde}	2.27 _{abcd}	8.26 _{abc}	7.28	6
<i>Asystasia gangetica</i>	3.88 _{abcd}	2.73 _{abcd}	7.80 _{cde}	14.20 _{bcd}	7.15	7
<i>Mitracarpus hirtus</i>	4.34 _{abcd}	12.39 _{bcdef}	6.85 _{bcde}	0.45 _{ab}	6.01	8
<i>Imperata cylindrica</i>	8.46 _{bcde}	4.58 _{abcde}	6.40 _{abcde}	3.67 _{ab}	5.78	9
<i>Macroptilium atropurpureum</i>	4.34 _{abcd}	4.58 _{abcde}	5.04 _{abcde}	4.58 _{ab}	4.63	10
<i>Ageratum conyzoides</i>	4.58 _{abcd}	-1.86 _{ab}	4.58 _{abcde}	1.82 _{ab}	2.28	11
<i>Rubus moluccanus</i>	0.45 _{ab}	-3.22 _a	5.95 _{abcde}	0.91 _{ab}	1.02	12
<i>Stachytarpheta jamaicensis</i>	1.16 _{abc}	1.36 _{abc}	-3.22 _{abc}	-5.53 _a	-1.56	13
<i>Phyllanthus niruri</i>	-2.97 _a	-0.45 _{ab}	-7.35 _a	1.36 _{ab}	-2.35	14
<i>Crotalaria pallida</i>	-0.25 _{ab}	0.00 _{ab}	-6.90 _{ab}	-7.80 _a	-3.74	15
Control	0 _{abcd}	0 _{ab}	0 _{abcde}	0 _{ab}	0	

Note: Means within the rows followed by the same alphabet were not significantly different ($p < 0.05$) according to DMRT. Values given in the table were inhibition percentage over that of the control. Plants were ranked in order of their inhibitory activity

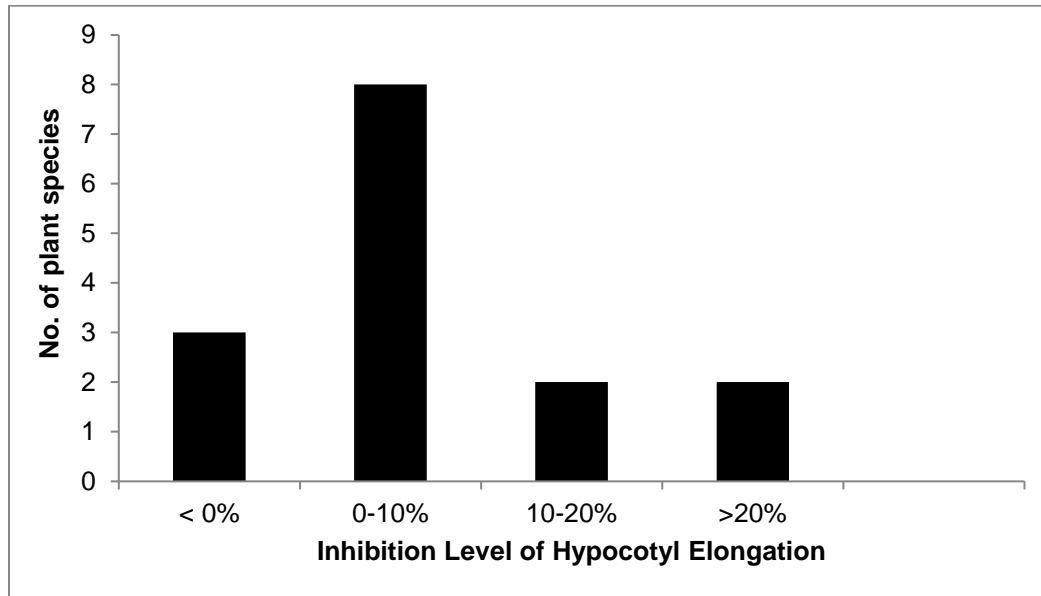


Figure 4: Frequency distribution of plant species for allelopathic activity against lettuce hypocotyl as determined by Dish pack method. Each bar chart represented the number of weed species in each level of inhibition percentage.

Based on the average hypocotyl inhibition percentage (Table 4), the highest hypocotyl inhibition compared to control was by *M. pudica* (23.11%), followed by *L. camara* (20.47%) and *A. munroanum* (18.22%). The hypocotyl inhibition level of selected weed species through Dish pack method was then classified into four levels; less than 0, 0-10 %, 10-20 %, and more than 20 % (Figure 4). From Table 4 and Figure 4, the inhibition rate of the lettuce hypocotyl elongation ranging from less than 0% in three species (*S. jamaicensis*, *P. niruri* and *C. pallida*), 0-9 % in eight species (*L. microphyllum*, *T. procumbens*, *A. gangetica*, *M. hirtus*, *I. cylindrica*, *M. atropurpureum*, *A. conyzoides* and *R. moluccanus*), 10-20 % inhibition was observed in two species (*A. munroanum* and *M. malabathricum*) and more than 20 % inhibition was observed in two species which was *M. pudica* and *L. camara*.

Based on the result (Table 3 and Table 4), the allelopathic effect of selected weed species through Dish pack method did not produce satisfactory results because percentage inhibition was less than 50 % compared to the control.

DISCUSSION

The average inhibition percentage inhibited the radicle elongation of lettuce, ranging from 21-

81%, suggested that the common weed species of BRIS soil had different allelopathic performance. Oolfsdotter et al., (2002) proposed that the allelopathic plants have characteristics that can inhibit the growth of other plants at different concentrations.

The inhibitory activities of the selected weed species increased with the amount of leaf litter leachate applied. It is because; the inhibitory activity depended on the concentration of chemical extraction (Mousavi et al., 2013). The result is in the agreement with findings by Syed et al., (2014b), mentioned that plant which have allelopathic potential, possess high toxicity level at high concentration of plants applied. This was also demonstrated by Gilani et al., (2002) in other plant that inhibitory activity of leaf litter increased as the applied concentration increased.

The results indicated that there were different behavior of lettuce seedlings; both inhibitory and stimulatory effect by the leaf litter of the selected weed species that had been applied. This is attributed to the presence of allelochemicals in different amounts (Chon et al., 2005; Syed et al. 2014a). When all three concentration of leaf litter were applied, different allelopathic behavior had been observed.

In the allelopathic analysis, the radicle elongation is the most sensitive growth variable measured compared to hypocotyl (Syed et al.,

2014a). This is because the result showed that the leaf litter leachate of all weed species inhibited the radicle elongation of lettuce higher compared to hypocotyl at all three used concentrations.

When the weed species were ranked according to the mean inhibition value on the lettuce radicle, *Crotalaria pallida* had the highest inhibitory effect. *Crotalaria pallida* belonging to Fabaceae has medicinal uses and is used as a ground cover and green manure crop in the limited scale in the humid area (Tropical Plants Database, 2019). Previous study by Ishak et al. (2016) indicates that the family of Fabaceae has potential to inhibit the growth of other weeds due to its allelopathic activity.

Based on the result, the allelopathic effect of selected weed species using Dish pack method did not show satisfactory result when compared with the control. This could be due to the presence of lower amounts of volatile chemical substances in the selected weed species. There were positive and negative effects on the growth of lettuce after treatment of the weed species compared to control. Some of the weed species showed inhibition on the lettuce radicle and hypocotyl growth while others stimulated the growth of lettuce. Moreover, it was shown that the inhibitory effect of weed species increases with the distance of lettuce, which was from 92 mm, 82 mm, 58 mm and 41 mm. Nevertheless, some weed species did not correspond fully to the distance from the weed species. The donor plant did not show significant reduction over all the parameters.

Based on the result, *M. pudica* significantly caused reduction on the lettuce elongation at all the tested distance. Thus, *M. pudica* was ranked top among the weed species tested, suggesting the presence of volatile allelochemical compounds in *M. pudica*. Phytochemical analyses of the plant have indicated the existence of chemical constituents like terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins (Ghandiraja et al., 2009). In addition, high concentration of allelochemicals presence in *M. pudica* may also be responsible for the inhibition effect.

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CONCLUSION

The screening tests suggest the presence of allelochemicals in the leaf litter leachate and leaf volatilization of 15 screened weed species. Weed that have high allelopathic activity caused inhibition and/ stimulation in the radicle and hypocotyl length of lettuce. Leaf litter of tested weed species have higher allelopathic activity compared to the leaf volatilization. This might be contributed by the presence of high allelochemicals in the leaf litter and different amount and types of allelochemicals released through leaf litter and leaf volatilization. Sandwich method shows that *Crotalaria pallida* has the highest allelopathic performance. Whilst, *Mimosa pudica* exhibits the highest allelopathic performance when tested using Dish pack method. The results presented can be used as guideline to further elucidate chemicals that are involved in the allelopathy phenomenon. In addition, application of water soluble extract of these plants under greenhouse and field conditions are recommended for further evaluate the allelopathic effect.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

NY designed the experiments, performed data analysis, wrote and review the manuscript. NHH and NAS performed the experiments. NHH also performed data analysis and wrote the manuscript. All authors read and approved the final version.

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