J. Agrobiotech. **Vol. 9**(1S), 2018, p. 260–268.

ISSN 1985-5133 (Press)
ISSN 2180-1983 (Online)

Evaluation for Allelopathic Activity of Selected Tree Species Grown in BRIS soil

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Keywords:
Allelopathic activity
Leaf litter leachates
Leaf volatilization
Mangifera indica
Manihot esculenta.

ABSTRACT

This study was carried out to evaluate the allelopathic activity of selected tree species grown in BRIS soil. The mature leaves from ten different tree species were collected as the material of donor plants. They were evaluated for the allelopathic activity of the leaf litter leachate and leaf volatilization by using Sandwich and Dish pack methods respectively, while *Lactuca sativa* (lettuce) was used as the receiver plant. Different amounts of leaf litter leachate were tested on the lettuce seedling growth for Sandwich method whilst, for Dish pack method the allelopathic activity was tested on different distances of the receiver plant from the donor plants. The results for Sandwich method showed that the tree species that gave the highest radical inhibition was *Mangifera indica* (mango) (60.05%) while the highest hypocotyl inhibition was *Peltophorum pterocarpum* (Yellow flame tree) (12.58%). Meanwhile, *Manihot esculenta* (cassava) showed the highest inhibition of radicle and hypocotyl by 86.50% and 76.63%, respectively, compared to control using Dish pack method. Based on this study, the evaluation for allelopathic activity of selected tree species grown in BRIS soil showed that there is significant presence of the allelochemicals in the tested tree species. The development of natural herbicides can be achieved with further studies to determine the effectiveness of allelochemicals in the tree species.

Keywords: Allelopathic activity, leaf litter leachates, leaf volatilization, *Mangifera indica*, *Manihot esculenta*.

ABSTRAK

Kajian ini adalah untuk menilai aktiviti alelopati bagi spesies pokok yang terpilih di tanah BRIS. Persampelan daun daripada sepuluh spesies pokok yang berbeza telah dijadikan sebagai tumbuhan penderma. Aktiviti alelopati pokok tersebut bagi larut resap daun dan pemeruapan daun telah diuji dengan menggunakan kaedah *Sandwich* dan *Dish pack* dan *Lactuca sativa* (salad) telah digunakan sebagai tumbuhan penerima. Jumlah daun yang berbeza telah digunakan untuk menguji pertumbuhan anak benih pokok salad bagi kaedah *Sandwich*, manakala, bagi kaedah *Dish pack*, aktiviti alelopati diuji melalui jarak yang berbeza daripada tumbuhan penderma terhadap benih salad. Keputusan untuk kaedah *Sandwich*, spesies pokok yang memberi perencatan radikal tertinggi ialah *Mangifera indica* (*mango*)(60.05%) dan untuk perencatan hipokotil tertinggi ialah *Peltophorum pterocarpum* (*Yellow flame tree*) (12.58%). Manakala, keputusan untuk kaedah *Dish pack*, spesies pokok yang memberi perencatan radikal dan hipokotil tertinggi ialah *Manihot esculenta* (*cassava*) dengan perencatan masing-masing sebanyak (86.50%) dan (76.63%). Berdasarkan kajian ini, penilaian untuk aktiviti alelopati bagi spesies pokok yang terpilih di tanah BRIS menunjukkan kehadiran alelokimia yang ketara di dalam sepuluh spesies pokok tersebut. Pembangunan herbisid semulajadi boleh dilakukan dengan menjalankan kajian selanjutnya dengan menentukan alelokimia yang berkesan dalam spesies pokok tersebut.

Kata Kunci: Aktiviti alelopati, larut resap daun, pemeruapan daun, *Mangifera indica, Manihot esculenta.*

INTRODUCTION

The term 'allelopathy' is used to express the meaning of the interaction between plants whether in inhibitory and/or stimulatory effects through the release of allelochemicals (Rice, 1984). The allelochemicals are released through exudations from roots, leaching of the leaf, volatilization and decomposition of plant tissue (Albuquerque et al., 2011; Rice, 1984). Secondary metabolites of allelopathy also known as allelochemicals act as the natural herbicides that can suppress the growth of nearby plants.

The widespread use of synthetic herbicides can increase the resistance in weed population. Moreover, weed control using synthetic herbicides have negative effect on the environment. These herbicides can pose health problems if it is not use properly and long-term exposures to the toxicity of these herbicides are potential health risks to the farmers. Allelopathic compounds have the potential to be used directly or indirectly as natural herbicides (Colquhoun, 2006). The use of allelochemicals is considered very beneficial and environmentally safe compared to synthetic herbicides because they have short half-life (Duke et al., 2002). Moreover, allelochemicals have high levels of specificity of target weeds, without the occurrence of weed resistance and no residue accumulation in the environment (Ahluwali, 2007). Further understanding of the physiological basis of allelopathy will allow researchers to develop new strategies for weed control (Weston, 1996).

Beach Ridges Interspersed with Swales soil, also known a BRIS soil is a type of soil that is considered as problematic in Malaysia. According to the studies by Muslim et al., (2004), they found that BRIS soil composed of more than 90% sand, weakly structured, low in nutrients, low water retention capacity with limited ability to support plant growth and have relatively high soil temperature. The trees grown on this type of soil are expected to have high allelopathic activity due to the stress conditions with limited supply of water and nutrients. BRIS soil probably has plant species with high allelopathic activity. This is correlated with previous study by Bhowmik (2003), where the contents of allelochemicals are usually higher when the allelopathic species grow under conditions of low or moderate nutrient availability compared to that of high availability. High concentration of allelochemicals is higher when the toxicity of plant residue of the donor plants increased (Pedrol et al., 2006).

In order to evaluate the allelopathic potential of plant species, several laboratory studies have been conducted either directly by using the aqueous, organic leachate or extracts of the donor plants; or indirectly by using the extraction of the leachate from the sand, agar or medium supporting the growth of the donor plants (Nornasuha & Ismail, 2017; Nurul et al, 2016; Ishak et al., 2016). The sandwich method was developed to test the allelopathic activity emitted from leaf litter leachate under controlled conditions in the laboratory. It is a less time-consuming bioassay method that can be used to screen a large number of samples (Morikawa et al., 2012). Whilst, the dish pack method had been developed to determine the allelopathic activity of volatile allelochemicals released from the donor plants to the target plant (Fujii et al., 2005). Therefore, this study was carried out to evaluate the allelopathic activity of selected tree species from BRIS soil using Sandwich and Dish pack methods.

MATERIAL AND METHODS

Plant Materials

The plant materials that were used for this study are donor plants from ten different tree species found in BRIS soil and the seeds of *Lactuca sativa* (lettuce) as the receiver plants. All of these plant materials (Table 1) were used in both Sandwich and Dish pack methods of this experiment.

Table 1 Plant materials used in the experiments.

Bil	Species name	Common name	Coordinate	
			Latitude	Longitude
1	*Peltophorum pterocarpum*	Yellow flame tree	5.755139	102.627222
2	*Acacia auriculiformis*	Earleaf acacia	5.755500	102.627222
3	*Elaeis guineensis*	Oil palm	5.772361	102.617778
4	*Cocos nucifera*	Coconut	5.773722	102.618056
5	*Bucida molineti*	Dwarf geometry tree	5.754861	102.627500
6	*Tabebuia rosea*	Pink pouis	5.754111	102.628611
7	*Pandanus odoratissimus*	*Mengkuang laut*	5.783306	102.621667
8	*Mangifera indica*	Mango	5.776833	102.618889
9	*Thespesia populnea*	*Baru laut*	5.783278	102.621944
10	*Manihot esculenta*	Cassava	5.753833	102.628056

Leaf Preparation

The fresh mature leaves of the donor plants were collected from the area around Besut District of Terengganu. The leaves of the plant samples were oven dried for 24 hours at 60 °C prior to being tested for their allelopathic activity.

Sandwich Methods

Three different amounts of dried leaves of the plant sample (5 mg, 10 mg, and 50 mg) were used for the analysis of leaf litter leachate through Sandwich method (Fujii et al., 2004) as in Figure 1. Agar powder (Nacalai Tesque, Kyoto, Japan) was used as the growth media. For the media preparation, the agar solution (0.75 % w/v) was prepared and autoclaved at 121 °C for 15 minutes. Each well of the multi-well dish plate was filled with 5 mg, 10 mg, and 50 mg of dried leaf samples, respectively, and this experiment was conducted in three replications (Fujii et al., 2004; Anjum et al., 2010). The first layer of agar (5 mL) was added to each well by using micropipette and allowed to solidify at room temperature.

Exactly 5 mL of the agar was added again for the second layer and five pre-germinated lettuce seeds (*Lactuca sativa*) were placed vertically after the agar is solidified. The multi-well plastic plate was covered with plastic tape and labelled appropriately and than incubated in the dark for 4 days at 21 °C. The parameters that were measured are radicle and hypocotyl length and all the data was recorded for analysis (Fujii et al., 2004).

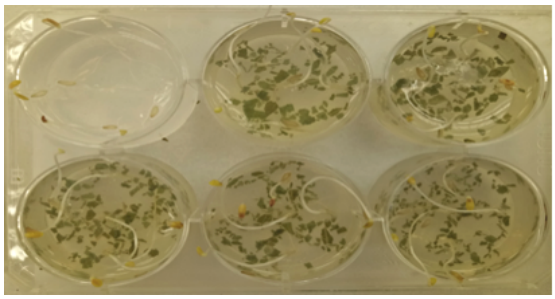

Figure 1 View from the top of multi-well dish plate used to test the allelopathy of tree species using leaves litter leachates (Sandwich method).

Dish Pack Methods

A multi-well dish plate with six wells was used and 200 mg of oven dried samples was placed into one well. The other 5 wells were placed with moistened filter paper (0.7 mL) and five pre-germinated lettuce seeds as in Figure 2, and then the multi-well dish plate was sealed. The multi-well dish plate was incubated in the dark for 4 days at 21 °C. The radicle and hypocotyl length of the seeds were measured and the data was recorded for analysis (Fujii et al., 2005).

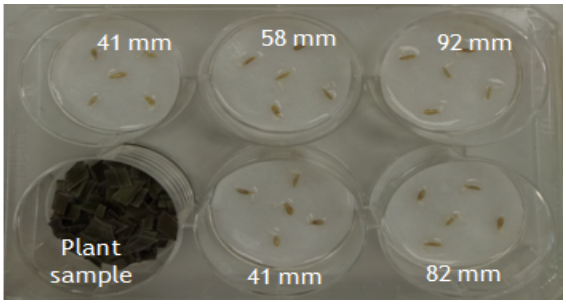

Figure 2 View from the top of multi-well dish plate used for Dish pack method.

Statistical Analysis

Both experiments were conducted in Completely Randomized Design (CRD) with three replicates. All the data was analyzed through SPSS software version 21, using one way Analysis of Variance (ANOVA) at 5 % level of significance. The means were compared by using Duncan Multiple range test (DMRT).

RESULTS AND DISCUSSION

Allelopathic Effects as Determined by the Sandwich Method

The percentage of inhibitions for radicle and hypocotyl elongation of lettuce seedlings by the ten selected tree species of BRIS soil were shown in Tables 2 and 3 and the percentage of inhibitions were obtained through Sandwich method. The inhibition for radicle length of lettuce by the leaf litter leachates of all the ten tree species were significantly different ($p<0.05$) at all the three amounts of dried samples used which were 5 mg, 10 mg and 50 mg (Table 2). The allelopathic effect on the radicle length for all the tree species depended on the amount of samples used. The least sample amount which was at 5 mg, gave the minimum percentage of inhibition, while for the highest sample amount which was at 50 mg gave the maximum percentage of inhibition when compared to control as shown in Tables 2 and Table 3.

Table 2 showed the frequency distribution of plant species for allelopathic activity against the radicle of lettuce seedlings. Five species of the plants were observed to have inhibition levels ranging from 20 – 39 %, followed by four other species ranging from 40 – 59 % and only one species had inhibition level of more than 60

%. Based on the results in Table 2, *Mangifera indica* is the only species recorded with an inhibition level of more than 60%. Meanwhile, research done by Yan et al. (2006) discovered that this species contained allelochemicals with high polarity. Studies done by Sahoo et al. (2010) and Khan et al. (2013) also reported that *Mangifera indica* does have the inhibitory effect on the growth of receptor plants.

In contrast, Table 3 showed the frequency distribution of plant species for allelopathic activity against hypocotyls of lettuce seedlings. Five of the tree species were recorded to have stimulatory effects on the receiver plants, followed by four species that gave inhibition levels ranging from 1 – 11 % and only one species that had inhibition level of more than 12 %. The results for the hypocotyl growth (Table 3) showed that some of the tree species have stimulatory effects based on specific amounts of leaf litter leachate. The highest percentage mean of inhibition was found to be that of tree species *Peltophorum pterocaarpum* (12.58 %).

According to the results obtained from this study, the inhibition levels of radicles were higher compared to hypocotyls. Munir et al. (2002) stated that the radicle length parameter tested was more sensitive compared to the hypocotyl length as the roots absorbed a higher level of the substance due to its' high permeability. These results also indicated that the leaching of the allelochemicals by leaf litter leachate samples in the agar and the higher mean inhibition recorded for the radicles might be due to direct contact of the substances with the roots.

The analysis for the data showed that the inhibitory effects of the tested tree species on the radicle length increased in tandem with the increase in concentration of the leaf litter leachate samples (Table 2). This result suggested that the higher amount of leaf litter leachate samples resulted in higher leaching of the allelochemicals into the agar. On the other hand, there were species that have non-toxic effect towards the growth of hypocotyls, instead they were able to stimulate and increase the length of the hypocotyls compared to control with the increasing amount of leaf litter leachate samples. One of them is the tree species *Tabebuia rosea* (Table 3).

Table 2 Growth inhibition of different concentrations of plant leaf litter leachate on lettuce seedling radicles as determined by the Sandwich method.

Species name	Treatment			% Mean inhibition	Rank
	5 mg	10 mg	50 mg		
Mangifera indica	45.79f	61.59d	72.76f	60.05	1
Acacia auriculiformis	31.87de	31.32bc	77.82g	49.34	2
Peltophorum pterocarpum	11.72b	37.81c	87.78i	45.77	3
Manihot esculenta	22.01c	28.73bc	81.56h	44.10	4
Thespesia populnea	27.24cd	40.12c	62.47e	43.28	5
Bucida molineti	11.06b	19.43b	82.83h	37.77	6
Pandanus odoratissimus	34.67e	30.43bc	42.21c	35.77	7
Elaeis guineensis	26.53cd	26.86bc	55.81d	36.40	8
Cocos nucifera	9.85b	27.85bc	52.34d	30.01	9
Tabebuia rosea	20.97c	19.48b	37.53b	25.99	10
Control	0.00a	0.00a	0.00a	0.00	-

Means within the columns followed by the same alphabet were not significantly different ($p \geq 0.05$) according to DMRT. Plants were ranked in order of their inhibitory activity.

Allelopathic Effects as Determined by the Dish pack method

Through the usage of Dish pack method, the percentage of inhibition for radicle and hypocotyl elongation of lettuce seedlings by the ten selected tree species in BRIS soil were shown in Tables 4 and 5. All the tree species have inhibitory effects for radicle length and the allelopathic effects on the radicle length for all the tree species were dependent on the distance of the leaf litter leachate samples with the lettuce seedlings. The nearest distance of the samples with the lettuce seedlings that had been tested was 41 mm and it recorded the maximum percentage of inhibition, whereas, the furthest distance of the samples was 92 mm which recorded the minimum percentage of inhibition when compared to the control. From Table 4, the nearest distance of the samples with the lettuce seedlings at 41 mm and with significant inhibitory effects was observed to be caused by *Manihot esculenta* (89.04 %).

Table 3 Allelopathic effects of different concentrations of tree species leaf litter leachate on lettuce seedling hypocotyls as determined by the Sandwich method.

Species name	Treatment			% Mean inhibition	Rank
	5 mg	10 mg	50 mg		
Peltophorum pterocarpum	-9.22d	12.87e	34.09e	12.58	1
Thespesia populnea	5.48e	6.09de	21.22cde	10.93	2
Acacia auriculiformis	0.00de	-3.04cd	34.87e	10.61	3
Mangifera indica	-6.78d	-0.96cd	29.39de	7.22	4
Cocos nucifera	-2.87de	-1.83cd	25.13cde	6.81	5
Elaeis guineensis	-1.12de	-9.57bc	7.39cd	-1.10	6
Bucida molineti	-21.13c	-13.30b	20.78cde	-4.55	7
Manihot esculenta	-30.87b	-13.91b	29.04de	-5.25	8
Pandanus adoratissimus	-42.70a	-52.70a	-45.74b	-47.05	9
Tabebuia rosea	-40.96a	-48.70a	-70.87a	-53.51	10
Control	0.00de	0.00d	0.00c	0.00	-

Means within the columns followed by the same alphabet were not significantly different ($p \geq 0.05$) according to DMRT. Plants were ranked in order of their inhibitory activity.

The trend of inhibition caused by the tree species differed quite significantly among itself. There were species that showed higher inhibition levels with the decreasing length of the distance such as shown by *Manihot esculenta*, *Pandanus odoratissimus* and *Bucida molineti*, while the others showed higher inhibition level at a certain distance only. Based on the results, the level of inhibition on radicle length caused by the volatilization of the leaf litter leachate samples can be categorized into four levels which are: more than 80 %, 20 – 79 %, 10 – 19 % and less than 10 % as showed in Figure 5.

Table 4 Allelopathic effects (growth inhibition) of different distances of tree species leaf volatilization on lettuce seedling radicles as determined by the Dish pack method.

Species name	Distance from donor plant				% Mean inhibition	Rank
	41 mm	58 mm	82 mm	92 mm		
Manihot esculenta	89.04f	86.49e	86.20f	84.26g	86.50	1
Cocos nucifera	50.87e	46.30d	64.72e	62.69f	56.14	2
Tabebuia rosea	39.92de	32.18dc	40.17d	27.68cde	34.99	3
Pandanus odoratissimus	41.15de	40.19d	21.78c	15.46bc	29.65	4
Acacia auriculiformis	34.28cd	19.62bc	31.58cd	30.31de	28.95	5
Peltophorum pterocarpum	21.92bc	22.83bc	21.48c	31.20e	24.36	6
Elaies guineensis	17.69b	19.62bc	18.72bc	24.77cde	20.20	7
Thespesia populnea	18.48b	10.30ab	21.78c	18.08bcd	17.16	8
Mangifera indica	22.23bc	2.89a	6.13ab	6.11ab	9.34	9
Bucida molineti	22.54bc	2.26a	6.43ab	0.29a	7.88	10
Control	0.00a	0.00a	0.00a	0.00a	0.00	-

Means within the rows followed by the same alphabet were not significantly different ($p \geq 0.05$) according to DMRT. Plants were ranked in order of their inhibitory activity

From Table 5 and Figure 6, five among the ten tree species were recorded to have negative values of mean inhibition towards hypocotyl growth. This means that they were able to stimulate the growth of hypocotyls of *Peltophorum pterocarpum* (-3.30 %), *Elaeis guineensis* (-5.75 %), *Bucida molineti* (-6.64 %) and *Mangifera indica* (-7.36 %). The other five species were recorded to give inhibitory effects to the hypocotyl length and they can be categorized into the ranges of more than 70 % (*Manihot esculenta* 76.63 %), 10 – 69 % (*Cocos nucifera* 18.03 %), 0 – 9 % (*Acacia auriculiformis* 7.21 %), (*Tabebuia rosea* 6.90 %) and (*Pandanus odoratissimus* 2.27 %).

According to the percentage mean growth inhibition, only some of the tree species have good inhibitory effects compared to the other species. The highest percentage mean inhibition was by *Manihot esculenta* (86.50 %) where *Manihot esculenta* was also found to inhibit the seed growth of the receptor plant by decreasing the root vigour and chlorophyll content (Huang et al., 2010). Burns et al. (2012) reported in their study that most of the *Manihot eculenta* cultivars contained a high level of cyanide which is more than 100 ppm and is also considered to be dangerous.The higher mean inhibition that was recorded for the radicles may be due to the sensitivity towards the volatile substances released by the leaf samples. The analysis of the data showed that the readings for inhibitory effects of the leaf litter leachate samples on the radicles are dependent on the tree species and also on the distances. Different tree species showed different inhibitory and stimulatory effects for the leaf volatilization (Tables 4 and 5).

Table 5 Allelopathic effects (growth inhibition) of different distances of tree species leaf volatilization on lettuce seedling hypocotyls as determined by the Dish pack method.

Species name	Distance from donor plant				% Mean inhibition	Rank
	41 mm	58 mm	82 mm	92 mm		
Manihot esculenta	77.15d	73.75d	77.79f	77.83d	76.63	1
Cocos nucifera	19.15c	15.33c	23.17e	14.47c	18.03	2
Acacia auriculiformis	13.69bc	9.89bc	-0.46abc	5.71c	7.21	3
Tabebuia rosea	5.47abc	6.91bc	6.96bcd	8.26c	6.90	4
Pandanus odoratissimus	10.48abc	-3.97ab	13.88de	-11.32ab	2.27	5
Peltophorum pterocarpum	9.12abc	-10.92a	9.71cd	-21.10a	-3.30	6
Elaies guineensis	4.56ab	-11.41a	1.83abcd	-18.00a	-5.75	7
Bucida molineti	6.37abc	-9.94a	- 6.00ab	-16.98ab	-6.64	8
Mangifera indica	3.16ab	-8.42a	-9.25a	-14.94ab	-7.36	9
Thespesia populnea	-1.40a	-15.37a	-3.71ab	-12.34ab	-8.20	10
Control	0.00ab	0.00ab	0.00abc	0.00bc	0.00	-

Means within the rows followed by the same alphabet were not significantly different ($p \geq 0.05$) according to DMRT. Plants were ranked in order of their inhibitory activity.

CONCLUSION

The evaluation for the leaf litter leachate and leaf volatilization of the ten selected different tree species in BRIS soil in this study showed that there were significant allelopathic activities among the tree species and provided evidence for the presence of allelochemicals in the plants. The leaf litter leachate of *Mangifera indica* showed the highest allelopathic activity whilst, *Manihot esculenta* showed the highest allelopathic activity for the leaf volatilization. From this study, it was found that the leaf volatilization showed higher allelopathic activity compared to the leaf litter leachate. The leaf volatilization form *Manihot esculenta* showed 86.50 % of radicle inhibition compared to the leaf litter leachate of *Mangifera indica* which showed radicle inhibition of 60.05 % compared to control. Further study needs to be carried out to determine the presence of allelochemicals on selected tree species which showed the highest allelopathic activity.

ACKNOWLEDGEMENTS

This research was supported by Research Grant No UniSZA/2017/DPU/06 from Universiti Sultan Zainal Abidin. The authors wish to express their gratitude to laboratory staff at School of Agricultural Sciences and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin for their invaluable assistance throughout the study.

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