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Allelopathic screening of Malaysian noxious weeds and several medicinal plants as potential alleloherbicides

M.S. Ishak[1], M.B. Nurul Ain[1], I. Sahid[1] and K. Mardiana-Jansar[1]*

[1]Department of Earth Science and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Malaysia

***Corresponding Author Email :** mardiana@ukm.edu.my

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Abstract

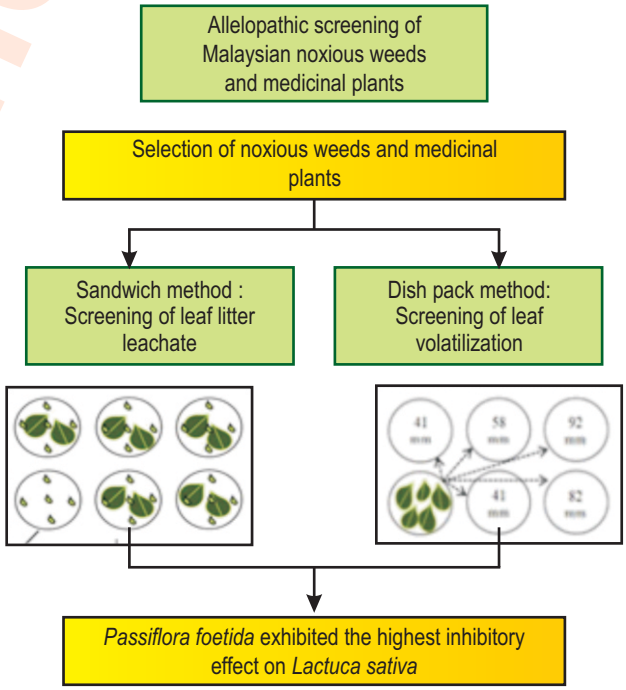
Aim: This research was conducted to assess the allelopathic potential of 100 plants species, including noxious weeds and medicinal plants of Malaysia as donor plant on the growth of *Lactuca sativa* (lettuce) as the recipient plant. This study was also carried out to justify common weeds found in Malaysia to act as a natural herbicide for weed management through allelopathy phenomenon.

Methodology: Weed and medicinal plant species commonly found in Malaysia were identified according to their morphological characteristics. Sandwich method was carried out to determine the allelopathic potential of weed species through leaf litter leachate mechanism. On the other hand, dish pack method was conducted to discover the allelopathic potential of weed species through volatilization pathway.

Results: 100 weed species and medicinal plants commonly found in Malaysia inhibited the radicle and hypocotyl growth of *L. sativa* by both sandwich method and dish pack method. Among 100 weed species, *Passiflora foetida* showed the maximum inhibitory effects on *L. sativa* growth in both sandwich and dish pack method. The highest amount of *P. foetida* leaf litter (50 mg), completely inhibited the growth of radicle and hypocotyl of *L. sativa*. Volatilization of weed and medicinal plants leaf litter inhibited the growth of *L. sativa* with increasing distances of lettuce seed with the weed and medicinal plants leaf litter.

Interpretation: The findings of this study provides a base line for identifying plants with allelopathic properties for controlling weed propagation.

Key words: Allelopathic potential, Allelopathy, Leaf litter leachate, Noxious weed, Volatilization



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Introduction

Interference of weeds in the growth of crops has been one of the contributing factors to low productivity of crops in Malaysia (Dilipkumar *et al*., 2017). The declining pattern of agricultural productivity is due to competition between the weeds and crops grown (Daehler *et al*., 2004). According to Sahid and Chan (2000), the competition between weeds and crops is mainly to obtain available nutrients, moisture, space and resources, which simultaneously reduces the productivity and quality of crops. Various efforts have been made to eradicate the problem of weed propagation, including utilization of more synonymous synthetic herbicides among farmers which reduces time and energy consumption. However, prolonged use of synthetic herbicides is risky as it poses direct or indirect harmful effect on the environment and human health (Aktar *et al*., 2009; Jaishankar *et al*., 2014).

The term allelopathy is defined as a biochemical interaction involving both inhibition and stimulation or one of them through the production of chemicals from one plant including microorganisms on another plant (Asaduzzaman *et al*., 2010). In general, allelopathy is a natural phenomenon that in certain types of plants by releasing allelochemical in their immediate environment through root exudates, litter leachate, volatilization, or decaying of tissue residues (Qin *et al*., 2006). According to de Albuquerque (2010), plants that possess allelopathic properties and release allelochemicals are known as donor plants while plants that are affected by allelochemical compounds secreted by other plants are known as recipient plants. Donor plants involve selected species of noxious weeds consisting of variety of families and morphological features, while *Lactuca sativa* (lettuce) seeds are used as recipient plants.

Volatilization is one way of spreading allelochemicals through allelopathic action that involves the release of volatile chemical compounds in the environment. Naturally, released chemicals are absorbed by the surrounding organisms by entering the soil structure and absorbed by the roots in the form of vapor released during condensation process. Dish pack method developed by Fujii *et al*. (2005) is one of the bioassay method for determining allelochemical volatilization to be carried out in laboratory. Generally, this method is used to analyze volatile allelochemicals where dried leaves of donor plants are placed in one of the holes known as source wells in a six-well multidish to note the effect of donor plant on the growth of bioassay species. This method is used as an approach to conform to the natural volatilization process. Fujii *et al*. (2005) observed that *Cleome spinosa* plant species inhibited 100% elongation of root and shoot of lettuce seedlings representing as bioassay species.

Litter leachate is also one of the pathways of allelopathic dissemination through the action of allelopathy that occurs when dissolved allelopathic compounds from the plants' aerial part (shoots) and released through rainwater, dew or drainage into the soil or to other plant surfaces (Chick and Keilbaso, 1998). Sandwich method was developed by Fujii *et al.* (2004) to determine the effects of leachate in laboratory, which showed the natural process of allelochemical emission from litter leachate to the environment. According to Fujii *et al*. (2004), sandwich method is used to study the inhibition effect of leaf debris placed in two layers of agar as a medium of dispersion. Shiraishi *et al*. (2002) used sandwich method to screen allelopathic activity through leaf litter leachate from 71 plant species and found that at least seven plant species inhibited the growth of lettuce seeds above 50%. In addition, Morikawa *et al*. (2012) evaluated the allelopathic activity of several plant species in Peru and found that families Asteraceae, Anacardiaceae, Fabaceae, Solanaceae and Bombaceae inhibited the root growth of lettuce more than 80%.

Generally, allelopathic plants in the current study in Malaysia involved only certain plant species that were found to have allelopathic potential in controlling weeds. Ishak *et al*. (2016) reported that *Leucaena leucocephala* tree interfered with the germination and growth of *Ageratum conyzoides*, *Tridax procumbens* and *Emilia sonchifolia*. A study by Ismail *et al*. (2015) involving the allelopathic assessment of weed species known as *Pennisetum purpureum* as a donor plant on the germination and growth of *Eleusine indica*. Moreover, Samedani *et al*. (2015) studied the effects of weed species of *Axonopus compressus* to control the growth of important weeds in oil palm plantation. In view of the above, this study aimed to evaluate the allelopathic potential of 100 plant species including noxious weeds and several medicinal plants commonly found in Malaysia through volatilization and litter leachate mode of action towards the growth of *L. sativa*.

Materials and Methods

Plants material: Fresh leaves of 100 species of common weeds and medicinal plants that served as donor plants found in Malaysia were collected from several places around Bangi, Selangor, Klang, Selangor, Sungai Rambai, Melaka , Pekan, Pahang and also around Telok Intan, Perak. Table 1 shows a list of weeds collected and were divided into four categories based on their morphological features namely broad leaves; n=54, grasses; n=24, sedges; n=6, and ferns; n=16. The weed samples were washed and dried in an oven at 60°C for 24 hr. Leaf samples were cut into 3.0 x 3.0 mm pieces with scissors, stored in sealed plastic and labeled with their respective weed species and date of sample collection. Seeds of *Lactuca sativa* (lettuce) purchased from Takii Seed Co. Ltd., Kyoto, Japan were used as a recipient plant in this study due to its easy-to-grow potential and sensitivity to the environmental changes (Fujii *et al*., 2003).

Identification of weed species: The identification of 100 species of noxious weeds and medicinal plants in this study were determined by identifying and comparing the overall morphology

Table 1 : Weed species collected from various location in Malaysia

Morphological features	Location	Family	Weed species
Broad leaves	Bangi	Araceae	*Alocasia macrorrhiza* (L.) G. Don
		Zingiberaceae	*Alpinia galanga* (L.) Willd
		Fabaceae	*Calopogonium mucunoides* Desv.
		Asteraceae	*Chromolaena odorata* (L.) R. M. King & H. Rob.
		Costaceae	*Coctus speciousus* (Koen.) Sm.
		Euphorbiaceae	*Croton hirtus* ¼Hér
		Dilleniaceae	*Dillenia excelsa* (Jack) Gilg.
		Alismatacaea	*Echinodorus palaefolius* (Nees & Mart) J.F. Macbr.
		Asteraceae	*Eliptica alba* (L.) Hassk.
		Euphorbiaceae	*Euphorbia hirta* L.
		Rubiaceae	*Hedyotis verticillata* (L.) Lam.
		Convovulaceae	*Ipomoea cairica* (L.) Sweet.
		Campanulaceae	*Isotoma longiflora* (L.) C. Presl.
		Verbenaceae	*Lantana camara* L.
		Euphorbiaceae	*Manihot esculenta* Crantz.
		Melastomaceae	*Melastoma malabathricum* L.
		Asteraceae	*Mikania micrantha* Kunth
		Cucurbitaceae	*Momordica balsamina* L.
		Fabaceae	*Neptunia oleracea* Lour.
		Solanaceae	*Physalis minima* L.
		Fabaceae	*Senna obtusifolia* (L.) H.S. Irwin
		Spigeliaceae	*Spigelia anthelmia* L.
		Dilleiniaceae	*Tetracera scandens* (L.) Merr.
		Asteraceae	*Vernonia cinerea* (L.) Less.
	Klang	Acanthaceae	*Asystasia gangetica* T. Anders.
		Rubiaceae	*Boereria latifolia* (Aubl.) K. Schum.
		Asteraceae	*Crassocephalum crepidioides* (Benth.) S. Moore
		Asteraceae	*Emillia sonchifolia* L. DC. Ex Wight
		Passifloraceae	*Passiflora foetida* L.
		Malvaceae	*Triumfetta repens* (Blume) Merille & Rolfe
		Malvaceae	*Urena lobata* L.
	Melaka	Acanthaceae	*Andrographis paniculata* Wall.
		Polygonaceae	*Antigonon leptopus* Hook. & Arn.
		Asteraceae	*Biden pilosa* L.
		Asteraceae	*Blumea lacera* (Burm.f) DC.
		Nyctaginaceae	*Boerhavia diffusa* L.
		Fabaceae	*Cassia alata* (L.) Roxb.
		Amaranthaceae	*Centella asiatica* L.
		Fabaceae	*Centrosema pubescens* Benth.
		Acanthaceae	*Clinacanthus nutans* (Burm.f.) Lindau
		Balsaminaceae	*Impatiens balsamina* L.
		Piperaceae	*Piper sarmentosum* Roxb.
		Solanaceae	*Solanum torvum* Sw.
		Cucurbitaceae	*Zehneria marginata* (Blume) Keraudren
		Fabaceae	*Zornia diphylla* (L.) Pers.
	Pahang	Asteraceae	*Ageratum conyzoides* L.
		Araceae	*Pistia stratiotes* L.
	Perak	Amaranthaceae	*Amaranthus viridis* Hook. F.
		Amaranthaceae	*Celosia argentea* L.
		Melastomaceae	*Clidemia hirta* (L.) D. Don.
		Euphorbiaceae	*Phyllanthus niruri* L.
		Malvaceae	*Sida acuta* Burm. F.
		Verbenaceae	*Stachytarpheta jamaicensis* (L.) Vahl
		Asteraceae	*Tridax procumbens* L.
Grasses	Bangi	Poaceae	*Axonopus compresus* Beauv.
		Poaceae	*Brachiaria mutica* (Forssk.) Stapf.

Cont...

		Poaceae	*Chrysopogon aciculatus* (Retz.) Trin.
		Poaceae	*Dactylotenium aegyptium* (L.) Willd.
		Poaceae	*Ischaemum muticum* L.
		Poaceae	*Paspalum conjugatum* P.J. Bergius
		Poaceae	*Rhynchelytrum repens* (Willd.) C.E. Hubb.
		Poaceae	*Sporobolus indicus* (L.) R.Br.
	Klang	Poaceae	*Cynodon dactylon* (L.) Pers.
		Poaceae	*Eleusine indica* (L.) Gaertn.
		Poaceae	*Imperata cylindrica* (L.) Raeusch.
		Poaceae	*Pennisetum purpureum* Schumach.
		Polygonaceae	*Polygonum barbatum* L.
	Melaka	Poaceae	*Bothriochloa bladhii* (Retz.) S. T. Blake
		Poaceae	*Chloris barbata* Sw.
		Poaceae	*Digitaria setigera* Roth.ex Roem. & Schult.
		Poaceae	*Echinochloa colona* (L.) Link.
		Poaceae	*Eragrostis atrovirens* (Desv.) Trin. Ex. Steud.
		Poaceae	*Eragrostis tenella* (L.) P. Beauv.
		Poaceae	*Leptochloa chinensis* (L.) Nees.
		Poaceae	*Ottochloa nodosa* (Kunth) Dandy
		Poaceae	*Panicum maximum* Jacq.
		Polygonaceae	*Polygonum barbatum* L.
		Poaceae	*Sporobolus diander* (L.) R.Br.
Sedges	Melaka	Cyperaceae	*Cyperus iria* L.
		Cyperaceae	*Cyperus kyllingia* L.
		Cyperaceae	*Cyperus rotundus* L.
		Cyperaceae	*Fimbristylis globulosa* (Retz.) Kunth.
		Cyperaceae	*Fimbristylis miliacea* L. Vahl.
		CyperaceaeCyperaceae	*Rhynchospora corymbosa* (L.) Britton*Scirpus grossus* L.
Ferns	Bangi	Pteridaceae	*Adiantum latifolium* Lam.
		Aspleniaceae	*Asplenium nidus* L.
		Blechnaceae	*Blechnum finlaysonianum* Wall.
		Adiantaceae	*Cheilanthes tenuifolia* (Burm.f.) Sw.
		Thelyepteridaceae	*Christella dentate* (Forssk.) Brownsey & Jermy
		Davalliaceae	*Davallia denticulate* (Burm.) Mett.
		Gleicheniaceae	*Dicranopteris linearis* (Burm.f.) Underw.
		Lycopodiaceae	*Lycopediella cernua* (L.) Pic. Serm.
		Schizaeaceae	*Lygodium flexuosum* (L.) Sw.
		Polypodiaceae	*Microsorum pustulatum* (G. Forst.) Copel.
		Dryopteridaceae	*Nephrolepis biserrata* (Sw.) Schott
		Pteridaceae	*Pteris vittata* L.
		Polypodiaceae	*Pyrossia lanceolata* L. Farw.
		Blechnaceae	*Staechnoclaena palustris* (Burm.f.) Bedd.
		Pteridaceae	*Taenitis blechnoides* (Willd.) Sw.
		Vittariaceae	*Vittaria ensiformis* Sw.

aspects of the plant such as flower, leaves, fruits, roots, stems and the nature of plants in their habitat. The species were then divided into four categories based on their morphological features namely broad leaves, grasses, sedges and ferns.

Sandwich method: A 0 mg, 10 mg and 50 mg of different leaf litters were used in sandwich method in three replicates (n=3). Leaf samples placed in 35 mm x 18 mm multidish (Nalga Nunc. International, Tokyo). A 5 ml of autoclaved agar growth medium (Nacalai Tesque, Kyoto, Japan) (for 15 min at 121°C at 121 kPa) was then added to each leaf litter sample as first agar layer and left until the agar solidified at room temperature. Subsequently, another 5 ml of growth medium was added to each multidish as a second layer and left to solid by for 5 min. A total of 10 ml of agar media for growth was poured into a multidish with no testable leaf samples being used as control. After the agar medium layer solidified, five seeds of lettuce are added upright onto the surface of agar. Each multidish was labeled with the name of respective weed species and sealed with adhesive tape. Each of the multi-well plates were then incubated at 20°C (24 hr photoperiod) for four days. The length of hypocotyl and radicle of lettuce seeds were measured and recorded after four days. The collected data were used to calculate the percentage of growth of lettuce seeds at both leaf volume levels (10 mg and 50 mg) compared to the

percentage of growth from control.

Dish pack method: Six well multidish plates 35 mm x 18 mm were used for dish pack method where the first hole of the multi-plate was filled with 200 mg of leaf sample and named as source well. Meanwhile, the remaining five mutildish holes were dispatched with Whatman No. 6 filter paper and three replicates (n=3) were used for each weed species. Approximately, 0.7 ml of distilled water was added onto the filter paper. Based on six well multidish plates used, the distances between the source well and other wells were 41, 58, 82 and 92 mm, respectively. A total of five lettuce seeds as a bioassay plant were added to the surface of filter paper at a uniform distance. For control treatment, no source well was employed; the first well of the multidish did not contain any weed leaf samples. Next, each of the multidish plates were labeled with their respective weed species and tightly sealed with plastic tape to prevent the emission of volatile matter from the leaf samples. Then, each multidish plate was stored at 20°C (24 hr photoperiod) for 4 days. Hypocotyl and radicle length of lettuce seedlings were measured and recorded after 4 days. The data collected were used to calculate the growth percentage of lettuce seeds at four different distances and compared with the growth percentage of control plants.

Statistical analysis: The elongation of hypocotyl and radicle length of lettuce seedlings were measured and the percentage was calculated by comparison to the control readings. The experimental design was carried out using a complete randomized design with each concentration and species involving three replicates. The mean and standard deviation were calculated using Microsoft Excel 2013. To assess the activity and allelopathic effects of each weed species studied, the criterion was translated into SD (SDV) value using the formula [(100% - Min) - (0.5/1.0/1.5 (SD)] (Fujii *et al*., 2003, 2004). SDV value was used to identify the potential of 100 weed species studied as the best allelopathic plant that can inhibition the growth of lettuce.

Results and Discussion

Allelopathic potential screening using sandwich method: Sandwich method presents preliminary information as a benchmark in which it is closely related to the release of chemical compounds involved in natural allelopathy phenomena through leachate mechanism (Fujii *et al*., 2003; Shinwari *et al*., 2013). Table 2 shows the percent inhibition on radicle and hypocotyl growth of lettuce as a bioassay species when treated with two different mass of leaf debris, *i.e.*, 10 mg and 50 mg of 100 dried samples of weed species having different morphological characteristics. Weed species listed in Table 2 were divided by the level of allelopathic activity ranging from the highest to the lowest percentage and classified into five categories (> 80%, 60-79%, 40-59%, 20-39% and 0-19%) by following the method of Fujii *et al*. (2003; 2004).

The perusal of data showed that leaf extract of 100 weed and medicinal plant species inhibited the growth of radicle and hypocotyl of lettuce with the value ranging from 19.26 to 85.41% when tested with 10 mg of dried sample for each weed species. Based on the screening through sandwich method, four species of weeds including *Passiflora foetida*, *Piper sarmentosum*, *Clidemia hirta* and *Dicranopteris linearis* strongly inhibited the root of lettuce seedlings by more than 80%. On the contrary, only three weed species (*Lygodium flexuosum*, *Christella dentata* and *Stachytarpheta jamaicensis*) caused less inhibition of root seedlings of lettuce when sown with 10 mg of weed leaf debris. Higher amount of leaf litter leachate (50 mg) applied to all test weeds caused 34-100% higher inhibition on the root growth of lettuce compared to the application of 10 mg weed leaf extract. These results corroborate with study from Fujii *et al.* (2003) who demonstrated that inhibition percentage of lettuce shoots increased with the increasing amount of leaf extract from several medicinal plants species such as *Eucharis grandifolia*, *Annona cherimola* and *Emblica pectinata*. Morikawa *et al*. (2012) had screened 170 plant species in Peru using sandwich method and found that 50 mg of leaf debris from *Aristeguieta ballii*, *Diplostephium foliosissimum* and *Spondias mombin* resulted in 90% inhibition of root and shoot growth of lettuce seedlings. Similarly, Ali *et al*. (2019) found that the radicle growth of lettuce seedlings were inhibited when treated with 50 mg of leaf debris of 196 medicinal plants species in Pakistan. From this study, it can be concluded that the leaf litter leachate of *P. foetida* showed the highest allelopathic effect among 100 weed species tested. Allelochemicals accumulated in *P. foetida* leaves can inhibit the growth and production of target plants by interfering with the plant physiology, directly or indirectly (Chou, 1999; Harper and Balke, 1981; Reigosa *et al*., 1999).

Allelopathic potential screening using dish pack method: Tables 3 and 4 shows the percent growth inhibition of root and shoots of lettuce treated with 200 mg leaf litter from 100 weed species of different families having different morphological characteristics. This study was conducted by following the method of Fujii *et al*. (2005) to evaluate the effect of allelopathy on the growth of bioassay species by releasing chemical compounds through volatilization. In dish pack method, the position between the source well (contain weed leaf litter) and the position of lettuce seed (as the recipient plant) was tested at four different distances of 41 mm, 58 mm, 82 mm and 92 mm. The control was multidish without weed and leaf litter of medicinal plants.

Inhibition of radicle and hypocotyl growth of lettuce were significant ($p < 0.05$) by leaf volatilization of different weed species at different distances. Volatilization of leaf litter for 100 weed species studied at the nearest distance of 41 mm inhibited the growth of lettuce radicle by 8.6 to 100%. Percentage inhibition of radicle and hypocotyl of lettuce seed showed positive (inhibition) or negative value (growth promotion). After treating

Table 2 : Allelopathic activity (% inhibition of lettuce seed growth) based on 100 common weed and medicinal plant species found in Malaysia using sandwich method

Species name	Morphological characteristics	Debris mass10 mg		Debris mass50 mg	
		Inhibition percentage (%)			
		Radicle(±)	Hypocotyl(±)	Radicle(±)	Hypocotyl(±)
Passiflora foetida	Broad leaves	85.41	23.92	100.00	100.00
Piper sarmentosum	Broad leaves	87.93	77.65	96.59	90.20
Ageratum conyzoides	Broad leaves	76.81	46.67	94.44	54.64
Zehneria marginata	Broad leaves	68.59	44.84	93.93	70.46
Asplenium nidus	Fern	56.37	13.46	93.93	42.09
Melastoma malabathricum	Broad leaves	64.81	38.43	93.11	77.78
Clidemia hirta	Broad leaves	81.56	52.29	93.04	86.14
Momordica balsamina	Broad leaves	78.37	52.29	91.78	82.75
Centrosema pubescens	Broad leaves	77.04	39.08	91.26	70.07
Rhynchelytrum repens	Grass	65.19	25.62	91.11	61.31
Croton hirtus	Broad leaves	75.11	43.79	91.04	72.81
Eliptica alba	Broad leaves	71.93	29.41	90.81	57.91
Digitaria setigera	Grass	59.11	29.28	88.67	72.03
Tridax procumbens	Broad leaves	56.59	14.89	88	64.97
Clinacanthus nutans	Broad leaves	62.74	30.33	87.85	63.79
Neptunia oleracea	Broad leaves	66.96	41.96	87.70	68.63
Amaranthus viridis	Broad leaves	66.96	4.84	87.33	67.58
Zornia diphylla	Broad leaves	73.41	43.01	87.11	58.56
Cynodon dactylon	Grass	73.63	20.78	86.44	48.37
Adiantum latifolium	Fern	71.11	0.26	86.33	50.39
Fimbristylis miliacea	Sedge	56.59	37.39	86.22	58.82
Dicranopteris linearis	Fern	81.70	53.73	86.07	59.08
Solanum torvum	Broad leaves	56.89	23.53	85.70	72.29
Alocasia macrorrhiza	Broad leaves	59.78	22.35	84.96	47.71
Hedyotis verticillata	Broad leaves	63.63	34.51	84.52	53.13
Ipomoea cairica	Broad leaves	64.22	25.88	84.37	64.84
Pistia stratiotes	Broad leaves	56.30	38.95	84.15	63.53
Echinochloa colona	Grass	64.96	3.92	84.00	23.92
Asystasia gangentica	Broad leaves	78.07	41.44	83.48	47.06
Polygonum barbatum	Grass	64.30	28.10	83.26	58.69
Andrographis paniculata	Broad leaves	64.37	56.21	83.11	72.55
Calopogonium mucunoides	Broad leaves	63.11	5.49	83.04	29.80
Chromolaena odorata	Broad leaves	79.41	51.90	82.89	66.27
Blechnum finlaysonianum	Fern	22.81	2.88	82.59	25.49
Triumfetta repens	Broad leaves	66.52	-15.56	82.44	31.76
Sporobolus indicus	Grass	61.33	31.24	81.93	37.12
Isotoma longiflora	Broad leaves	58.52	26.14	81.85	52.68
Microsorum pustulatum	Fern	41.26	18.30	81.78	64.18
Chloris barbata	Grass	63.93	20.26	81.78	52.16
Eragrostis atrovirens	Grass	47.93	24.71	81.41	58.95
Dactylotenium aegyptium	Grass	56.81	33.45	81.04	59.74
Senna obtusifolia	Broad leaves	73.63	54.90	80.89	83.46
Phyllanthus niruri	Broad leaves	37.48	37.48	80.74	29.54
Boereria latifolia	Broad leaves	66.22	20.39	80.52	42.88
Sporobolus diander	Grass	54.30	26.93	80.22	48.24
Eragrostis tenella	Grass	66.22	21.70	80.15	32.68
Biden pilosa	Broad leaves	47.33	24.31	80.15	20.52
Urena lobata	Broad leaves	48.44	27.84	79.93	16.60
Vernonia cinerea	Broad leaves	58.44	29.67	79.33	59.61
Sida acuta	Broad leaves	69.63	26.54	79.19	38.43
Stachytarpheta jamaicensis	Broad leaves	3.41	28.76	78.96	46.93

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Coctus speciousus	Broad leaves	48.89	23.53	78.81	48.37
Lantana camara	Broad leaves	48.07	25.10	78.74	46.93
Panicum maximum	Grass	56.81	25.49	78.74	32.29
Pennisetum purpureum	Grass	56.67	18.95	78.00	38.04
Manihot esculenta	Broad leaves	53.63	3.40	76.89	36.73
Impatiens balsamina	Broad leaves	54.52	20.65	76.59	40.26
Ottochloa nodosa	Grass	62.30	28.10	76.00	32.55
Crassocephalum crepidioides	Broad leaves	44.37	-10.98	75.78	30.59
Imperata cylindrica	Grass	47.70	13.07	75.63	38.17
Lycopediella cernua	Fern	60.37	49.15	75.56	67.45
Antigonon leptopus	Broad leaves	49.85	18.95	75.48	44.44
Leptochloa chinensis	Grass	53.04	15.56	75.11	36.21
Pyrossia lanceolata	Fern	26.59	-2.48	75.04	42.61
Eleusine indica	Grass	42.37	-8.63	74.22	32.55
Nephrolepis biserrata	Pakis	50.37	39.61	73.48	45.75
Boerhavia diffusa	Broad leaves	56.00	-43.53	73.33	-13.33
Blumea lacera	Broad leaves	36.81	3.14	73.19	37.91
Pteris vittata	Fern	46.89	8.24	72.81	27.71
Ischaemum muticum	Grass	48.37	12.03	72.15	28.63
Echinodorus palaefolius	Broad leaves	58.81	33.86	72.00	44.71
Chrysopogon aciculatus	Grass	54.96	28.37	71.78	44.31
Emilia sonchifolia	Broad leaves	48.44	6.54	71.70	43.27
Stenochlaena palustris	Fern	33.70	-14.38	71.63	15.50
Cyperus iria	Sedge	43.04	20.65	71.48	36.21
Euphorbia hirta	Broad leaves	37.93	-33.86	71.19	-5.10
Tatracera scandens	Broad leaves	39.27	20.29	70.37	40.78
Davallia denticulata	Fern	40.22	0.52	70.37	22.09
Brachiaria mutica	Grass	39.27	20.59	69.93	29.67
Mikania micrantha	Broad leaves	39.26	0.00	69.63	38.30
Spigelia anthelmia	Fern	45.48	-4.58	69.26	17.12
Cheilanthes tenuifolia	Fern	34.74	30.07	69.19	46.93
Celosia argentea	Broad leaves	38.07	-15.95	68.37	14.64
Cyperus kyllingia	Sedge	47.11	13.07	68.37	37.91
Physalis minima	Broad leaves	39.26	0.00	67.85	35.29
Axonopus compresus	Grass	47.56	3.14	66.67	18.95
Taenitis blechnoides	Fern	34.37	9.02	66.22	25.23
Cyperus rotundus	Sedge	46.67	12.68	64.96	13.33
Rhynchospora corymbosa	Sedge	42.67	17.39	64.81	26.93
Scirpus grossus	Sedge	29.93	5.62	63.78	30.94
Bothriochloa bladhii	Grass	36.07	1.24	63.78	44.97
Cassia alata	Broad leaves	41.93	25.88	62.37	36.21
Alpina galanga	Broad leaves	37.11	17.65	60.89	32.81
Centella asiatica	Broad leaves	40.22	0.52	60.59	9.84
Dillenia excelsa	Broad leaves	56.52	19.74	59.63	16.37
Paspalum conjugatum	Grass	35.78	1.96	53.85	7.54
Fimbristylis globulosa	Sedge	37.48	18.43	51.27	12.37
Vittaria ensiformis	Fern	34.00	-1.96	42.89	23.40
Christella dentate	Fern	15.33	-11.50	41.04	19.61
Lygodium flexuosum	Fern	19.26	4.58	34.00	0.39
Min (M)		53.55	20.04	77.17	43.54
Standard deviation (¡)		15.93	19.74	11.59	21.34
*** 100- ((M-1.5(¡))				94.55	75.55
** 100- ((M-1.0(¡))				88.75	64.88
* 100 - ((M-0.5(¡))				82.96	54.21

* Criterion (*), (**) and (***) show a higher percentage of growth inhibition on radicle and hypocotyl of lettuce seedlings compared to controls, 50mg leaf litter debris was used in the sandwich method for criterion assessment due to its high inhibition percentage on the radicle and hypocotyl growth of *L. sativa* rather than 10 mg leaf litter debris

Table 3: Allelopathic activity (% inhibition of lettuce radicle growth) based on 100 common weed species found in Malaysia using dish pack method

Species name	Morphological characteristics	Distances			
		41 mm	58 mm	82 mm	92 mm
		Radicle inhibition percentage (% ±)			
Passiflora foetida	Broad leaves	100.00	100.00	100.00	100.00
Clinacanthus nutans	Broad leaves	94.06	89.66	76.63	71.26
Tetracera scandens	Grass	89.85	44.06	27.59	26.82
Ischaemum muticum	Grass	84.87	40.61	35.25	36.78
Digitaria setigera	Grass	78.16	80.84	75.86	75.86
Stenochlaena palustris	Fern	68.20	45.59	57.09	45.21
Pyrossia lanceolata	Fern	68.01	35.63	55.56	47.51
Blechnum finlaysonianum	Fern	67.05	42.53	35.25	38.70
Vittaria ensiformis	Fern	65.90	56.70	46.74	47.51
Manihot esculenta	Broad leaves	62.84	59.00	60.92	61.30
Cheilanthes tenuifolia	Fern	62.24	60.15	31.42	30.65
Adiantum latifolium	Fern	61.11	42.91	49.43	41.38
Lygodium flexuosum	Fern	59.77	47.89	49.43	55.94
Microsorum pustulatum	Fern	59.77	46.74	40.61	34.48
Christella dentata	Fern	58.81	49.81	48.28	41.00
Sporobolus diander	Grass	56.32	46.74	46.74	45.21
Amaranthus viridis	Broad leaves	55.94	52.11	52.11	49.81
Pteris vittata	Fern	55.94	43.30	27.20	8.81
Ipomoea cairica	Broad leaves	55.17	52.87	27.97	47.13
Triumfetta repens	Broad leaves	54.79	53.64	54.79	47.89
Senna obtusifolia	Broad leaves	53.26	48.28	51.72	50.57
Echinochloa colona	Grass	52.68	48.28	43.68	40.61
Imperata cylindrica	Grass	52.30	48.66	55.56	47.51
Lycopediella cernua	Fern	51.92	41.00	25.29	32.18
Alpina galanga	Broad leaves	51.72	52.49	51.72	54.41
Celosia argentea	Broad leaves	51.34	48.28	48.28	55.17
Vernonia cinerea	Broad leaves	50.77	51.34	43.68	57.85
Davallia denticulata	Fern	50.77	38.70	47.89	32.95
Pistia stratiotes	Broad leaves	50.38	47.89	43.30	51.34
Axonopus compresus	Grass	49.43	37.16	39.46	37.16
Rhynchelytrum repens	Grass	47.51	42.53	38.70	40.23
Crassocephalum crepidioides	Broad leaves	47.13	42.91	50.57	51.34
Isotoma longiflora	Broad leaves	46.74	50.57	49.04	41.00
Spigelia anthelmia	Broad leaves	46.17	53.26	46.74	50.96
Nephrolepis biserrata	Fern	45.02	33.33	41.76	26.05
Asplenium nidus	Fern	43.68	34.10	42.15	26.44
Tridax procumbens	Broad leaves	41.76	44.44	45.21	45.59
Alocasia macrorrhiza	Broad leaves	39.85	24.90	45.98	52.87
Taenitis blechnoides	Fern	39.46	45.21	37.93	21.46
Asystasia gangentica	Broad leaves	38.89	21.46	24.52	36.78
Clidemia hirta	Broad leaves	38.70	17.24	22.22	18.01
Brachiaria mutica	Grass	37.93	33.33	27.59	32.18
Sporobolus indicus	Grass	37.93	32.95	32.57	35.63
Dicranopteris linearis	Fern	37.16	34.87	36.02	29.50
Neptunia oleracea	Broad leaves	36.97	16.86	27.97	22.22
Boereria latifolia	Broad leaves	36.59	40.23	32.57	33.72
Blumea lacera	Broad leaves	35.82	24.14	20.69	18.01
Leptochloa chinensis	Grass	34.87	33.33	21.84	26.44
Stachytarpheta jamaicensis	Broad leaves	34.67	39.08	26.82	32.57
Ottochloa nodosa	Grass	34.29	21.46	34.48	36.02
Chromolaena odorata	Broad leaves	32.38	19.54	29.50	28.74

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Mikania micrantha	Broad leaves	32.38	21.46	30.27	39.85
Zehneria marginata	Broad leaves	31.99	25.29	52.49	50.19
Polygonum barbatum	Grass	31.99	34.48	30.65	29.89
Centrosema pubescens	Broad leaves	31.80	29.89	32.57	38.31
Scirpus grossus	Sedge	31.42	23.37	10.73	19.54
Emilia sonchifolia	Broad leaves	31.23	19.54	26.44	24.57
Eragrostis tenella	Grass	31.23	27.59	29.12	34.10
Impatiens balsamina	Broad leaves	30.84	22.99	24.90	27.20
Eleusine indica	Grass	30.84	28.74	22.99	28.74
Fimbristylis globulosa	Sedge	30.84	25.67	30.27	31.42
Urena lobata	Broad leaves	30.08	34.10	45.59	53.64
Cyperus kyllingia	Sedge	30.08	24.14	22.22	18.01
Eragrostis atrovirens	Grass	29.89	25.67	20.69	29.12
Boerhavia diffusa	Broad leaves	29.69	27.59	49.81	39.85
Calopogonium mucunoides	Broad leaves	29.31	33.33	36.78	24.14
Solanum torvum	Broad leaves	28.16	18.01	29.12	32.95
Cassia alata	Broad leaves	27.97	27.20	31.42	40.23
Antigonon leptopus	Broad leaves	27.39	24.90	31.42	28.74
Cyperus rotundus	Sedge	27.20	24.90	21.84	32.57
Eliptica alba	Broad leaves	26.82	27.97	18.39	18.39
Coctus speciousus	Broad leaves	26.25	22.61	40.23	22.61
Andrographis paniculata	Broad leaves	25.86	26.05	26.05	17.24
Panicum maximum	Grass	25.48	14.94	23.37	22.61
Cyperus iria	Sedge	25.29	25.29	21.07	14.56
Rhynchospora corymbosa	Sedge	25.29	26.44	31.80	17.62
Zornia diphylla	Broad leaves	25.19	13.79	14.56	23.37
Lantana camara	Broad leaves	25.10	22.22	23.37	15.71
Fimbristylis miliacea	Sedge	24.90	20.31	22.22	22.61
Sida acuta	Broad leaves	24.71	20.31	11.88	5.75
Melastoma malabathricum	Broad leaves	24.33	27.59	34.87	31.80
Dactylotenium aegyptium	Grass	24.14	22.99	20.69	30.27
Chloris barbata	Grass	24.14	19.92	29.50	32.18
Croton hirtus	Broad leaves	23.95	15.33	22.22	14.94
Bothriochloa bladhii	Grass	23.75	17.24	26.44	19.92
Phyllanthus niruri	Broad leaves	23.56	5.75	21.46	17.24
Ageratum conyzoides	Broad leaves	23.18	13.03	27.97	19.16
Echinodorus palaefolius	Broad leaves	22.03	18.01	22.99	24.52
Pennisetum purpereum	Grass	21.46	21.84	16.09	22.61
Cynodon dactylon	Grass	21.46	26.05	13.03	24.14
Centella asiatica	Broad leaves	21.07	16.86	23.75	14.18
Physalis minima	Broad leaves	20.11	23.37	13.79	19.16
Euphorbia hirta	Broad leaves	20.11	18.77	15.33	11.11
Paspalum conjugatum	Grass	19.92	12.26	22.99	16.48
Hedyotis verticillata	Broad leaves	18.58	25.29	11.49	4.98
Piper sarmentosum	Broad leaves	17.24	16.09	15.33	8.81
Dillenia excelsa	Broad leaves	17.05	19.16	16.86	16.48
Biden pilosa	Broad leaves	16.48	13.03	14.94	11.49
Chrysopogon aciculatus	Grass	16.09	18.01	29.12	42.53
Momordica balsamina	Broad leaves	8.62	19.16	8.81	15.71
Min (M)		39.43	33.47	34.04	33.20
Standard deviation (¡)		18.16	16.25	15.41	15.85
* 100- ((M-0.5(¡))					
** 100- ((M-1.0(¡))					
*** 100- ((M-1.5(¡))					

*Criterion (*), (**) and (***) show a higher percentage of radicle growth inhibition of lettuce seedlings compared with controls, 41 mm position distance used in the dish pack method for criterion assessment due to its higher inhibitory activity on the radicle growth of *L. sativa*

Table 4 : Allelopathic activity (% inhibition of lettuce hypocotyl growth) based on 100 common weed species found in Malaysia using dish pack method

Species name	Morphological characteristics	Distances			
		41 mm	58 mm	82 mm	92 mm
		Hypocotyl inhibition percentage (% ±)			
Passiflora foetida	Broad leaves	100.00	100.00	100.00	100.00
Digitaria setigera	Grass	72.55	77.78	73.20	69.93
Clinacanthus nutans	Broad leaves	71.57	67.32	43.14	41.18
Tetracera scandens	Broad leaves	69.93	7.84	4.58	0.00
Vittaria ensiformis	Fern	69.61	49.02	43.14	35.95
Christella dentata	Fern	60.78	49.67	41.83	41.18
Staechnoclaena palustris	Fern	60.46	50.33	30.72	41.18
Blechnum finlaysonianum	Fern	55.56	23.53	30.72	7.84
Manihot esculenta	Broad leaves	53.27	55.56	54.25	52.94
Lygodium flexuosum	Fern	52.61	40.52	30.07	44.44
Microsorum pustulatum	Fern	51.63	42.48	33.33	37.25
Ischaemum muticum	Grass	50.98	7.84	-3.92	-3.27
Pyrossia lanceolata	Fern	50.65	35.95	41.18	44.44
Cheilanthes tenuifolia	Fern	36.93	32.03	32.68	26.14
Ipomoea cairica	Broad leaves	35.95	33.99	32.68	34.64
Adiantum latifolium	Fern	32.68	26.14	31.37	24.18
Senna obtusifolia	Broad leaves	31.37	20.26	24.84	16.99
Piper sarmentosum	Broad leaves	31.05	28.76	18.30	5.88
Sporobolus diander	Grass	31.05	30.07	30.07	22.88
Imperata cylindrica	Grass	30.16	27.45	29.41	22.22
Amaranthus viridis	Broad leaves	29.74	33.33	26.80	22.22
Boerhavia diffusa	Broad leaves	28.76	23.53	23.53	20.26
Urena lobata	Broad leaves	25.82	25.49	20.26	20.92
Pteris vittata	Fern	25.49	15.69	9.80	5.23
Triumfetta repens	Broad leaves	25.16	27.45	28.10	20.92
Zehneria marginata	Broad leaves	23.86	26.80	24.84	22.22
Pistia stratiotes	Broad leaves	23.86	18.95	18.95	27.45
Spigelia anthelmia	Broad leaves	23.53	27.45	22.88	23.53
Asplenium nidus	Fern	23.53	39.22	11.11	12,42
Melastoma malabathricum	Broad leaves	23.20	16.99	21.57	17.65
Neptunia oleracea	Broad leaves	22.22	14.38	14.38	16.34
Rhynchelytrum repens	Grass	22.22	15.69	17.65	16.99
Emilia sonchifolia	Broad leaves	20.26	11.11	11.76	16.99
Lycopediella cernua	Fern	20.26	20.26	15.03	5.88
Vernonia cinerea	Broad leaves	19.85	16.18	15.44	13.97
Taenitis blechnoides	Fern	19.28	18.95	-13.73	-3.27
Mikania micrantha	Broad leaves	18.63	22.22	18.95	17.65
Crassocephalum crepidioides	Broad leaves	18.30	18.95	16.34	16.99
Isotoma longiflora	Broad leaves	17.94	17.06	12.35	13.53
Leptochloa chinensis	Grass	17.65	11.11	0.00	3.27
Alpina galanga	Broad leaves	17.32	21.57	19.61	25.49
Clidemia hirta	Broad leaves	16.67	25.49	10.46	11.76
Echinochloa colona	Grass	15.36	13.73	22.22	14.38
Asystasia gangentica	Broad leaves	14.05	22.22	11.76	11.76
Dicranopteris linearis	Fern	13.73	7.84	2.61	-6.54
Ageratum conyzoides	Broad leaves	13.07	21.57	21.57	15.03
Tridax procumbens	Broad leaves	13.07	9.8	16.99	8.50
Celosia argentea	Broad leaves	13.04	16.49	16.27	10.97
Alocasia macrorrhiza	Broad leaves	12.92	16.43	12.92	11.24
Eragrostis atrovirens	Grass	12.09	12.42	8.5	20.26

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Chromolaena odorata	Broad leaves	11.44	3.27	14.38	-1.96
Cyperus kyllingia	Sedge	11.44	4.58	10.46	10.46
Ottochloa nodosa	Grass	11.44	7.19	5.88	13.07
Euphorbia hirta	Broad leaves	10.78	1.31	-3.92	-10.46
Lantana camara	Broad leaves	9.48	9.8	5.23	-0.65
Eragrostis tenella	Grass	9.48	-2.61	0.65	-1.31
Rhynchospora corymbosa	Sedge	9.15	11.11	6.54	-3.27
Brachiaria mutica	Grass	8.82	7.19	7.19	10.46
Cyperus rotundus	Sedge	7.84	7.19	15.69	13.07
Panicum maximum	Grass	7.52	3.27	14.38	13.73
Boereria latifolia	Broad leaves	7.19	16.34	7.19	9.8
Fimbristylis globulosa	Sedge	6.86	5.23	10.46	-0.65
Eliptica alba	Broad leaves	6.21	-4.58	-5.88	-3.27
Coctus speciousus	Broad leaves	5.56	3.92	11.11	4.58
Axonopus compresus	Grass	5.56	4.58	9.15	3.92
Antigonon leptopus	Broad leaves	4.90	5.88	7.19	5.23
Blumea lacera	Broad leaves	4.58	4.88	3.27	-4.58
Cassia alata	Broad leaves	4.25	9.8	7.19	7.84
Scirpus grossus	Sedge	3.59	-1.96	-19.61	-1.31
Croton hirtus	Broad leaves	2.61	-2.61	14.38	0.00
Polygonum barbatum	Grass	2.61	8.50	2.61	8.5
Nephrolepis biserrata	Fern	2.29	1.31	10.46	-3.27
Fimbristylis miliacea	Sedge	2.21	4.41	11.03	3.68
Impatiens balsamina	Broad leaves	1.96	1.96	1.31	-2.61
Chrysopogon aciculatus	Grass	1.96	1.96	3.92	3.27
Sporobolus indicus	Grass	1.31	6.54	0.65	1.96
Cyperus iria	Sedge	0.65	7.19	-0.65	3.92
Phyllanthus niruri	Broad leaves	0.33	-13.07	-3.27	-9.15
Andrographis paniculata	Broad leaves	0.33	-1.96	8.5	-5.23
Centella asiatica	Broad leaves	0.00	1.96	9.8	5.88
Chloris barbata	Grass	-0.33	0.00	-0.65	1.31
Calopogonium mucunoides	Broad leaves	-0.65	-4.58	13.07	5.88
Cynodon dactylon	Grass	-0.98	1.96	-5.88	-5.88
Bothriochloa bladhii	Grass	-1.31	-0.65	-3.27	-5.23
Eleusine indica	Grass	-1.63	7.19	0.65	3.92
Biden pilosa	Broad leaves	-2.61	-7.19	-9.15	-458
Davallia denticulata	Fern	-2.61	9.15	5.88	-1.31
Physalis minima	Broad leaves	-4.25	-1.96	-3.23	-3.78
Echinodorus palaefolius	Broad leaves	-4.58	-2.61	-8.50	-3.27
Pennisetum purpereum	Grass	-4.90	-4.58	-10.46	-2.61
Dactylotenium aegyptium	Grass	-5.23	2.61	4.48	3.92
Stachytarpheta jamaicensis	Broad leaves	-6.54	5.23	-6.54	1.96
Paspalum conjugatum	Grass	-7.84	-6.54	-3.27	-12.42
Solanum torvum	Broad leaves	-10.13	-13.07	-8.50	-7.84
Zornia diphylla	Broad leaves	-12.09	-11.76	-10.46	-8.50
Sida acuta	Broad leaves	-13.73	-9.80	-16.34	-18.30
Dillenia excelsa	Broad leaves	-15.36	-19.61	-11.11	-11.11
Hedyotis verticillata	Broad leaves	-21.24	-19.61	-24.18	-24.18
Momordica balsamina	Broad leaves	-21.57	-32.68	-13.07	-16.34
Min (M)		16.81			
Standard deviation (¡)		22.25			
* 100- ((M-0.5(¡))					
** 100- ((M-1.0(¡))					
*** 100- ((M-1.5(¡))					

*Criterion (*), (**) and (***) show a higher percentage of hypocotyl growth inhibition of lettuce seedlings compared with controls, 41 mm position distance used in the dish pack method for criterion assessment due to its higher inhibitory activity on the hypocotyl growth of *L. sativa*

with 200 mg of leaf litter, *P. foetida*, *Clinacanthus nutans*, *Tetracera scandens* and *Ischaemum muticum* showed 80% inhibition in lettuce radicle. It is noteworthy that some weed species (88% of species) decreased the growth of lettuce seedlings with increasing distances from the source well (from 92 mm, 82 mm, 58 mm to 41 mm). The results of this study are in line with the study by Appiah *et al*. (2015) who found that several plant species in Sino-Japanese region like *Photinia glabra*, *Liquidambar styraciflua* and *Cinnamomum camphora* inhibited the growth of lettuce radicle by dish pack method. Similarly, Nurul Ain *et al*. (2017) found that 15 common weed species in Malaysia inhibited the radicle growth of lettuce seedlings by dish pack method at the nearest distance of 41 mm from the leaf litter of common weed. Based on the effects of weeds leaf volatilization studied, *P. foetida* showed the highest percent inhibition on lettuce seedling with 100% inhibition at all distances.

On the basis of results it can be concluded that *P. foetida* weed possessed the highest allelopathic effect than other 99 weed and medicinal plants species studied by sandwich and dish pack method. The ability of *P. foetida* to completely inhibit the radicle and hypoctyl growth of lettuce at all distances tested using dish pack method proved the presence of volatile compound in *P. foetida* leaf extract release of their allelochemical by volatilization. On the other hand, inhibition of radicle and hypocotyl of lettuce was cent percent achieved when 50 mg of *P. foetida* leaf litter was treated by sandwich method and showed that leachate from the leaf extract contributed to the highest allelopathic effect compared to other weed species. This study is a preliminary work of screening some common weeds found in Malaysia that have allelopathic potentials. Subsequently, further studies should be conducted on the identification and effects of allelochemicals secreted by the plants. This effort is important for synthesizing bioactive compounds from weed species as a natural product for controlling weed propagation.

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Add-on Information

Authors' contribution: M.S. Ishak: Conceived the presented idea, wrote the first draft; **M.B. Nurul Ain:** Conducted experiment, conceived the presented idea; **I. Sahid:** supervised, contributed to final version; **K. Mardiana-Jansar:** supervised, contributed to final version.

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