Comparative response of Malaysian weedy rice (*Oryza sativa*) initial growth towards the allelopathic potential of *Leucaena leucocephala* (Lam.) de Wit and *Dicranopteris linearis* (Burm. f.) Underw.

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**Abstract.** The infestation of weedy rice has becoming a threat which leads to competition between weedy rice and commercial rice for nutrient uptake. Weedy rice management is more dependent on the herbicides application that can contribute to various environmental problems. Thus, this study was conducted to assess the allelopathic potential of two invasive plants namely *L. leucocephala* and *D. linearis* in controlling the growth of weedy rice. The allelopathic potential was evaluated through laboratory bioassay namely the dish pack method, sandwich method and plant box method to determine the allelopathic potential on the seedling growth of two weedy rice variants collected in Peninsular Malaysia (designated as WRE and WRN) and MR220 CL2 rice variety.

Highest concentration of *L. leucocephala* leaf litter (50 mg) inhibited the radicle elongation of MR220 CL2, WRN and WRE by 49 %, 51 % and 83 % compared to control, respectively. Through the dish pack method, the volatile compound from *D. linearis* leaf were found to disrupt the radicle growth of MR220 CL2, WRN and WRE by 21 %, 29 % and 25 % of the control at the nearest distance (41 mm) of the bioassay plants with the *D. linearis* leaf. Compared with *D. linearis*, the root exudate from *L. leucocephala* exhibited higher inhibitory effect towards the bioassay species. Overall, the findings from this study showed that the allelopathic potential of *L. leucocephala* was more preferable to be investigated further for sustainable weedy rice management in the future by infusing the biological approach.

**Keywords:** Allelopathy, rice, weedy rice, weedy rice management

**Track Name:** Advanced Technology and Renewable Energy

1. **Introduction**

Malaysian Agricultural Research and Development states that almost 80 % of paddy fields throughout Malaysia faced weedy rice problems [1]. Weedy rice resembles commercial rice in terms of morphology, growth, taxonomy and diversity which makes it difficult to distinguish and to control especially during early stage of its growth [2]. As an initiative to control weedy rice growth, MARDI introduced Clearfield® rice technology in 2010 namely MR220 CL1 and MR220 CL2 varieties that resist towards imidazolinone herbicide [3]. Unfortunately, some farmers did not follow the guidelines

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for the use of Clearfield® rice technology system which affects the effectiveness of this method [1].

Weedy rice seeds have spontaneous shattering characteristic during harvesting process and the ripening period is faster than commercial rice [4]. Moreover, commercial rice has to compete with weedy rice for nutrients and space to grow [5]. Allelopathy is any direct or indirect effect of one plant (including microorganisms) towards other plants through the release of chemicals into the environment (Rice 1984) [6]. This phenomenon involves the inhibition and stimulation of the growth of the recipient plant resulting from the production of chemicals from the donor plant [6]. Chemical compounds or secondary metabolites of the plants possessing allelopathic potential are known as allelochemicals [7]. Allelochemicals are released from the plants by evaporation, leaching, root exudate and decomposition of dead plants [8]. In addition, allelochemical contributes to weed control with the involvement of the allelopathic plants [9]. Hence, the allelopathic plants selected in this study are two invasive plants namely Leucaena leucocephala and Dicranopteris linearis.

Leucaena leucocephala (Lam.) de Wit (family Fabaceae) or commonly known as lead tree is a fast growing and high yielding tree which can be grown in various type of soils especially in the tropical areas [10]. Previous studies discovered that L. leucocephala contains secondary metabolites known as mimosine which responsible to its herbicidal activities towards some plants [11, 12]. A study by Ishak et al. [13] found that the volatilization and leaching from the foliage of L. leucocephala inhibited the radicle and hypocotyl length of Ageratum conyzoides, Tridax procumbens and Emilia sonchifolia. Dicranopteris linearis (Burm. f.) Underw (family Gleicheniaceae) is the most widely distributed ferns in the tropical and temperate regions [14]. According to Russell et al. [15], D. linearis is capable to dominate plant communities and forms enormous pure colonies, frequently. In terms of allelopathic activities, a study by Ismail & Chong [16] discovered that the extract and residues from D. linearis inhibited the seedlings emergence and growth of several common Malaysian weed species.

Weedy rice cannot be harvested because the seeds fall early to the ground. Although early harvesting can be done to save weedy rice seeds, but the seeds are still immature [17]. Clearfield® rice technology introduced by MARDI which is expected to be able to control the growth of weedy rice is no longer effective as the herbicide-resistant weedy rice were reported emerge from the hybridization of the herbicide-resistant rice variety with the weedy rice [18]. This causes weedy rice becomes more difficult to control and farmers have to continue the use of synthetic herbicides without following the guidelines which in turn contributes to environmental pollution. Therefore, allelopathy can be utilized as an environmentally friendly approach to control the growth of weeds especially weedy rice. Indirectly, the use of synthetic herbicides in rice fields can be reduced and subsequently minimize the impact towards the environment. In particular, this study aims to determine the allelopathic potential of leaf volatilization, foliage leaching and root exudate from L. leucocephala and D. linearis towards the growth of weedy rice.

2. Materials and Methods

In this study, the allelopathic plants (donor plants) were L. leucocephala and D. linearis while the bioassay plants (receiver plants) were weedy rice variants categorized based on its morphological features and MR220 CL2 rice variety.

2.1 Collection of the allelopathic plants and the bioassay plants

L. leucocephala were sampled around KTM UKM Station, Bandar Baru Bangi, Selangor (2.940° N, 101.787° E) while D. linearis were collected around Universiti Kebangsaan Malaysia, Bangi, Selangor (2.921° N, 101.784° E). The bioassay plants were two weedy rice variants and MR220 CL2 variety. The designation, sampling location and morphological features of the bioassay as shown in Table 1.
Table 1. Bioassay plants designations based on the sampling location and morphological features.

| Bioassay designation | Type              | Sampling location            | Coordinate       | Morphological features                   |
|----------------------|-------------------|------------------------------|------------------|------------------------------------------|
| WR1                  | Weedy rice        | Kg. Chermai, Arau, Perlis    | 6.400° N 100.271° E | Awnless; brown-hulled; closed panicle |
| WR2                  | Weedy rice        | Teluk Mesira, Bachok, Kelantan| 6.143° N 102.343° E | Awned; straw-hulled; open/horizontal panicle |
| MR220 CL2            | Modern rice variety | MARDI Seberang Perai        | 5.540° N 100.470° E | -                                        |

2.2 Dish pack method
Developed by Fujii [19], this method was utilized to identify the allelopathic potential of the donor plants towards the growth of the bioassay plants through leaf volatilization. A multi-dish plate (Thermo Scientific Inc.) with six wells (10 cm² each) was used. A total of 2 g of donor plant dried leaf samples were inserted into the first well (source well). The other five wells were lined with Whatman No. 1 filter paper with three replication carried out for each species. By using pipette, each lined-well was then filled with 0.7 ml distilled water. Next, three seeds of each bioassay plant were placed on the surface of the filter paper. The distance between the source well and the other neighboring wells are 41, 58, 82 and 92 mm. For control, the source well was not filled with any sample of the donor plants. To prevent the releasing of the volatile materials, the multi-dish were tightly sealed with adhesive tape. Then, each multi-dish was labeled and incubated in a growth chamber at 28°C for seven days. The radicle and hypocotyl length of the bioassay plants were measured and recorded on the seven days after sowing.

2.3 Sandwich method
This method was developed to evaluate the allelopathic activities of the donor plants towards the growth of the bioassay plants through leaching from its foliage [20]. Two concentrations (10 and 50 mg) of oven-dried leaf of the donor plants were used in this experiment. For each multi-dish (Thermo Scientific Inc.), three wells were filled with 10 mg of oven-dried leaf of the donor plants and the remaining three wells were filled with 50 mg of the respective donor plants. To prevent fungal contamination, the multi-dish were sprayed with 70% ethanol before use. Agar (Nacalai Tesque Inc., Kyoto, Japan) were used as the growth medium (0.75% w/v). The agar mixture were autoclaved for 15 minutes at 121°C and 121 kPa and the agar solution were cooled to 40-45°C. A total of 5 ml of agar solution were poured into each wells containing 10 mg and 50 mg donor plant oven-dried leaves and left to cool until it forms first gelatin layer. After the first layer of agar gelatinized, another 5 ml of agar solution were poured to form two layers of gelatin. Three seeds of the bioassay plant seeds were added to the top layer of the gelatinized agar in each multi-dish. Multi-dish without oven-dried leaf of the donor plants was placed into the multi-dish wells and indicated as control. The multi-dish was then sealed tightly using adhesive tape and incubated in the growth chamber at 28°C for seven days. Each concentration (0, 10, 50 mg) were replicated three times in complete randomized design (CRD). The radicle and hypocotyl length of the bioassay were recorded seven days after sowing.

2.4 Plant box method
This method was developed by Fujii et al. [21] to assess the allelopathic activities of the root exudates from the donor plants towards the growth of the bioassay plants. Two-month-old donor plant seedlings were harvested and the root part was cleaned using distilled water. Next, an individual donor plant was then placed in a PVC tube equipped with nylon net (root zone separating tube) located at the corner of the magenta box (6 x 6 x 10 cm; Magenta Corporation, Chicago, USA). Each magenta box was sprayed with 70% ethanol to prevent any fungal contamination. Autoclaved agar (Nacalai Tesque Inc.,
Kyoto, Japan) used as growth medium (0.75 % w/v; 40 °C) was poured into the magenta box and left to form gelatin. Thirty three bioassay seeds were placed on the surface of the gelatinized agar following the distances of each seed with the donor plant. Prior to root phototropism, the base of the plant box then covered with aluminium foil. The top of the plant box were sealed with transparent plastic to avoid fungal contamination. The plant boxes without the donor plants were indicated as untreated control. All plant boxes were then incubated for seven days in the growth chamber at 28 °C. Each treatments were replicated three times in complete randomized design (CRD). The radicle and hypocotyl length were recorded at seven days after sowing.

2.5 Statistical analysis
The mean ± standard error and analysis of variance from the dish pack and sandwich method were determined by using one-way ANOVA with post-hoc test using Duncan's Multiple Range Test (DMRT) at ρ<0.05. Data from the plant box method was analyzed by using correlation bivariate analysis (Pearson) at ρ<0.01 and linear regression analysis. All analysis were determined and generated using SPSS software version 23.

3. Results and Discussions
Table 2 shows the allelopathic effects of the leaf volatilization of *L. leucocephala* and *D. linearis* on root growth of the bioassay plants. The inhibitory effect was tested based on the distance of bioassay (41 mm, 58 mm, 82 mm and 92 mm) from the dry leaves of the allelopathic plants in the multi-dish. The results showed that the volatilization from the leaves of *L. leucocephala* and *D. linearis* hold allelopathic activity on the growth of the bioassay plants. The radicle growth of WR1 experienced insignificant stimulation with the presence of volatilization from *L. leucocephala* foliage at all distances while the leaf volatilization of *D. linearis* was found to inhibit radicle growth of WR1 significantly by 29 % (41 mm), 0.4 % (58 mm), 21 % (82 mm) and 19 % (92 mm) compared to control. The leaf volatilization activity of *L. leucocephala* and *D. linearis* displayed inhibitory effect on WR2 radicle growth at all distances. However, there was a growth stimulation of 3 % by the volatiles from *L. leucocephala* at distance of 82 mm. Furthermore, the volatiles from *L. leucocephala* leaves were found to inhibit the radicle growth of MR220 CL2 at all distances.

The nearest distance (41 mm) from *D. linearis* had a higher percentage of inhibition than the farthest distance (92 mm). This indicates that the presence of volatile allelochemical compounds decreases as the recipient plant moves further away from the donor plant. This study was supported by Kato-Naguchi et al. [14] who found that *D. linearis* extract had an inhibitory effect on *Echinochloa colonum* and *Avena fatua* which are two plant species that grow near *D. linearis* colonies. In addition, cinnamtannin B-1 which is a secondary metabolite of *D. linearis* was not detected in soil at a distance of 100 cm of the colony [14]. The radicle growth of MR220 CL2 were found to be inhibited at all distances by the volatiles from the leaves of both allelopathic plants. However, all bioassay plants did not display the same inhibition pattern. This is happened because the recipient plants are not sensitive enough to fully respond to the distance differences but there is still inhibitory activity by the allelopathic plant through volatilization from its foliage [22]. When comparing the efficacy between the two allelopathic plant species, volatiles from *D. linearis* leaves were found to have a significantly higher inhibitory effect rather than the volatiles from *L. leucocephala* leaves.
Table 2. Allelopathic effects of leaf volatilization from the donor plants on the radicle elongation of the bioassay by using dish pack method.

| Bioassay      | Distances (mm) | Radicle elongation (mm) | L. leucocephala | IP (%) | D. linearis | IP (%) |
|---------------|----------------|-------------------------|-----------------|--------|-------------|--------|
|               |                |                         | WR1             |        |             |        |
| 0 (control)   | 62.67 a        | 0                       | 62.67 a         | 0      |             | 0      |
| 41            | 65.50 a        | +4.52                   | 44.56 b         | -28.90 |
| 58            | 62.89 a        | +0.35                   | 62.44 a         | -0.37  |
| 82            | 64.11 a        | +2.30                   | 49.78 ab        | -20.57 |
| 92            | 65.44 a        | +4.42                   | 51.00 ab        | -18.62 |
|               |                |                         | WR2             |        |             |        |
| 0 (control)   | 67.00 a        | 0                       | 67.00 a         | 0      |             | 0      |
| 41            | 61.56 a        | -8.12                   | 49.94 b         | -25.46 |
| 58            | 58.67 a        | -12.43                  | 47.22 b         | -29.52 |
| 82            | 69.22 a        | +3.31                   | 53.11 b         | -20.73 |
| 92            | 64.78 a        | -3.31                   | 54.33 b         | -18.91 |
|               |                |                         | MR220 CL2       |        |             |        |
| 0 (control)   | 61.11 a        | 0                       | 61.11 a         | 0      |             | 0      |
| 41            | 59.17 a        | -3.17                   | 48.11 b         | -21.27 |
| 58            | 54.44 ab       | -10.92                  | 45.89 b         | -24.90 |
| 82            | 47.33 b        | -22.55                  | 53.89 ab        | -11.81 |
| 92            | 57.56 a        | -5.81                   | 51.11 b         | -16.36 |

* Values within columns followed by different letter are significantly different at p<0.05 according to Duncan's multiple range test (DMRT). The symbol “-” indicates inhibitory effect, whilst the symbol “+” indicates stimulatory effect.

The sandwich method was carried out using two different total weights of allelopathic plants dry leaves namely 10 mg and 50 mg. Table 3 shows the leaching of leaves L. leucocephala and D. linearis exert an inhibitory effect on the radicle elongation of both weedy rice variants and the MR220 CL2 variety. The percentage of radicle growth inhibition of the weedy rice variants increased significantly when the total weight of the dry leaves increased from 10 mg to 50 mg. For instance, the radicle elongation of WR2 were significantly reduce by 83 % with the presence of leaching from the L. leucocephala foliage at highest concentration (50 mg). According to Sahid et al. [12], mimosine which is the allelochemical of L. leucocephala was found mostly in the young shoots and seeds of the plant. In the same study, it was found that the growth of Ageratum conyzoides, Tridax procumbens and Emilia sonchifolia was further inhibited as the mimosine concentration increased. Overall, the leaching from the foliage of L. leucocephala was found to significantly inhibit the radicle growth of both weedy rice variants compared to D. linearis especially at the highest total dry leaves weight (50 mg). Moreover, lower total dry leaves weight (10 mg) of L. leucocephala was found significantly promoted the radicle growth of MR220 CL2 rice variety and depicts that L. leucocephala is suitable to be investigated further for future weedy rice management.
Table 3. Allelopathic effects of foliage leaching from the donor plants on the radicle elongation of bioassay by using sandwich method.

| Bioassay  | Total dry leaves weight (mg) | Radicle elongation (mm) | L. leucocephala IP (%) | D. linearis IP (%) |
|-----------|-----------------------------|-------------------------|------------------------|--------------------|
| WR1       | 0 (control)                 | 58.67 a                 | 0                      | 58.67 a            |
|           | 10                          | 45.93 b                 | -21.72                 | 41.19 b            |
|           | 50                          | 28.93 c                 | -50.69                 | 34.96 c            |
| WR2       | 0 (control)                 | 53.72 a                 | 0                      | 53.72 a            |
|           | 10                          | 47.56 b                 | -11.47                 | 51.85 a            |
|           | 50                          | 9.19 c                  | -82.89                 | 31.41 b            |
| MR220 CL2 | 0 (control)                 | 58.72 b                 | 0                      | 58.72 a            |
|           | 10                          | 67.30 a                 | +14.60                 | 50.63 b            |
|           | 50                          | 29.93 c                 | -49.03                 | 24.22 c            |

* Values within columns followed by different letter are significantly different at p<0.05 according to Duncan's multiple range test (DMRT). The symbol “-” indicates inhibitory effect, whilst the symbol “+” indicates stimulatory effect.

Table 4 shows the allelopathic effects of the root exudates from the donor plants on the radicle growth of bioassay plants. The results discovered that the radicle growth of all bioassay plants was inhibited with the presence of exudation from the root of both donor plants. The radicle growth of WR1 was reduced by 38 % when sown with L. leucocephala and significantly influenced by its distance with the L. leucocephala plant (R² = 0.154). The radicle of MR220 CL2 showed less sensitivity towards the root exudate from L. leucocephala and only inhibited by 17 % compared to control with low R² value (0.006). On the other hand, the root exudates from D. linearis were greatly inhibited (reduced by 85 % compared to control) the radicle growth of MR220 CL2 with. According to a study conducted by Ishak et al. [13], root exudates of L. leucocephala cause inhibition of root growth of T. procumbens, E. sonchifolia and A. conyzoides. The great inhibitory effects from D. linearis roots were in line with the findings by Kato-Noguchi et al. [14] in turn stated that cinnamantannin B-1 may be released either by the exudate from living plants or through the decomposition of the plant residues. Some bioassay such as WR1 and WR2 were significantly influenced by its distance with the donor plant (L. leucocephala). This indicates that there are relationships between the distance and radicle length. The findings also suggested that distance is one of the contributing factors in the efficiency of the allelopathic plants to control other plant's growth [23]. In addition, the roots of L. leucocephala may secrete allelochemicals that probably high at the nearest distance and become low at the farthest distance because of the diffusion.

Table 4. Allelopathic effects of root exudates from the donor plants on the radicle growth of bioassay plants by using plant box method.

| Bioassay     | L. leucocephala Radicle inhibition (%) | D. linearis Radicle inhibition (%) | R² | R² |
|--------------|---------------------------------------|-----------------------------------|----|----|
| WR1          | 38**                                  | 0.154                             | 32**| 0.103|
| WR2          | 21**                                  | 0.116                             | 74 | 0.005|
| MR220 CL2    | 17                                    | 0.006                             | 85 | 0.001|
4. Conclusions

Based on the present study, it was found that allelopathic potential was present in both *L. leucocephala* and *D. linearis* which affected the growth of both weedy rice variants. The foliage leaching and root exudates from *L. leucocephala* displayed higher inhibitory effects on the growth of weedy rice variants rather than *D. linearis*, whilst the volatiles from the foliage of *D. linearis* showed higher inhibitory effects on the bioassay plants compared with *L. leucocephala*. This study also revealed that *L. leucocephala* is the most promising candidate to be infused as a biological control of weedy rice and its allelopathic potential can be explored further in order to manage weedy rice or other weeds in various agricultural activities.

References

[1] MARDI 2013 80% Sawah Padi Diancam Padi Angin. [https://blogmardi.wordpress.com/2013/08/01/80-sawah-diancam-padi-angin/](https://blogmardi.wordpress.com/2013/08/01/80-sawah-diancam-padi-angin/)
[2] Chauhan B S 2013 *Crop Protection* **48** 51-56
[3] Harun R and al 2018 *Economic and Technology Management Review* **13** 63-73
[4] Karim S R and al 2006 *Plant Protection Quarterly* **21**(1) 13.
[5] Olajumoke B and al 2016 *Chilean Journal of Agricultural Research* **76**(2) 243-252.
[6] Rice E L 1984 *Allelopathy* (Orlando, Florida: Academic Press)
[7] Kong C H and al 2019 *Molecules* **24**(15) 2737
[8] Dahiya S and al 2017 *Journal of Pharmacognosy and Phytochemistry* **6** 832-837
[9] Putnam A R 1988 *Weed Technology* **2**(4) 510-518
[10] Pandey V C and Kumar A 2013 *Genetic Resources and Crop Evolution* **60**(3) 1165-1171
[11] Xuan T D and al 2013 *Herbicidal activity of mimosine and its derivatives* *Herbicides: Advances in Research* (Rijeka, Croatia: Intech) chapter 15 pp 299-312
[12] Sahid I and al 2017 *Transactions on Science and Technology* **4**(2) 62-67
[13] Ishak M S and al 2016 *Allelopathy Journal* **37**(1) 109-122
[14] Kato-Noguchi H and al 2012 *Plant Ecology* **213**(12) 1937-1944
[15] Russell A E and al 1998 *Journal of Ecology* **86**(5) 765-779
[16] Ismail B S and Chong T V 2009 *Allelopathy Journal* **23**(2) 277-286
[17] Azmi M and al 2001 *Pengendalian Dan Pengawalan Padi Angin Di Malaysia* (Serdang: MARDI)
[18] Dilipkumar M and al 2018 *Planta Daninha* **36** 018182239
[19] Fuji Y and al 2005 *Thymus* **2**(5) 493-497
[20] Fujii Y and al 2003 *Weed Biology and Management* **3**(4) 233-241
[21] Fuji Y and al 2007 *Plant Box Method: A specific bioassay to evaluate allelopathy through root exudates* *Allelopathy: New Concepts and Methodology* eds Y Fujii and S Hiradate (Enfield: Science Publishers) chapter 3 pp 39-56
[22] Yusoff N and al 2018 *International Journal of Science and Applied Technology* **2**(2) 1-9
[23] Syed S and al 2014 *Pakistan Journal of Botany* **46**(5) 1693-1701

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