Chemical composition and herbicidal effects of *Melaleuca bracteata* F.Muell. essential oil against some weedy species.

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Abstract— The chemical composition of *Melaleuca bracteata* F. Muell. Essential oil isolated by hydrodistillation was analyzed by Gas Chromatography –Mass Spectrophotometer. Twenty – two compounds were identified, representing 92.09 % of the total isolated essential oil. The main constituent of *M. bracteata* was methyl eugenol (81.04%). The herbicidal effect was tested in different doses 1.25, 2,5 ,5 and 10 µL/ml against two grassy weeds *Panicum virgatum* and *Digitaria longiflora* and two broad leaf weeds *Stachytarpheta indica* and *Aster subulatus*. In a laboratory bioassay, the highest concentration of essential oil 10 µL/ml was inhibited seed germination and seedling development in all targeted weeds completely. Chlorophyll content was decreased with increasing concentrations of the essential oil, indicating that essential oil interferes with photosynthetic metabolism. As well as, *M. Bracteate* essential oil causes an electrolyte as a result of membrane disruption and loss of integrity of treated weed leaves. Results showed the possibility of using essential oil of M. *bracteata* as an alternative to synthetic herbicides for future weed control.

Index Terms— *Melaleuca bracteata* F.Muell , essential oil, Herbicidal effects, weeds.

1. INTRODUCTION

Weeds are considered plants that grow where it is not wanted or welcomed and cause a negative impact in agriculture field as a consequence of competition with economical plants (Holzner and Numata, 2013). Losses comes from weeds is the greatest and equal with losses which comes from insects and pathogens together (Bozoglu, 2011). Although the chemical control by synthetic herbicides are considered the best and effective method compared with other methods to be used to control weeds since discovery the first synthetic herbicide (2,4-D) in the 1940s, the risk is very high if it is used indiscriminately. Types, quantity and frequency of applications of the synthetic herbicides can bring about various harmful effects to the environment and its ecosystems and a threat to human health. In the longer term (Qasem, 2011).

Furthermore, continuous application herbicides (Even in case of recommended doses) can also lead to increase weed resistance of these herbicides by producing new genotype Fig. 1 (Heap, 2014). As a consequent, extensive use of herbicides exacerbated the problems further. Like any other chemical compounds, synthetic herbicides are also known to have negative effects on humans. So, replacing synthetic herbicides by alternative ways to be effective to control weeds at the same time environmental friendly become an urgent need.

Allelochemiacl compounds which are known as secondary metabolite compounds produced by some plants to use as a defensive barrier against organisms, including neighboring plants come into the forefront of the available solutions to facilitate reducing the usage of synthetic herbicides (Džamić, *et al.,* 2014)

Furthermore, Allelochemical compounds have a short half-life because they are biodegradable and are therefore regarded as environmentally and toxicologically safer than many of the currently used herbicides (De Almeida *et al.,* 2010). A wide range of allelochemical compounds is synthesized during the shikimate pathway or from the isoprenoid pathway which is responsible for essential oil production (Kruse *et al.,* 2000)

Essential oils are considered the most important natural plant products produced by plants to involved in different operations like, pollination as insects attractant as well as offer protection from predators which are known Allopathic influence (Dayan *et al.,* 2011)

Melaleuca bracteata F.Muell. is an aromatic plant belong to Myrtaceae family and commonly known as the black tea-tree, river tea-tree or mock olive. There are nearly 300 species of Melaleuca genus distributed in Australia and South-East Asia (Brophy *et al.,* 2013).

Essential oil isolated from plants belong to Melaleuca genus showed broad efficacy against bacteria and fungi and used widely as a traditional medicine for a number of conditions including acne, wounds, sores, dandruff, and skin lesions (Carson *et al.,* 2006). A few studies were conducted recently to evaluate the phytotoxicity and herbicidal effects of essential oil of plants belong to Melaleuca genus on weed plants. There is no report about herbicidal effects of essential oils from *Melaleuca bracteata*. Therefore, the aims of this study were to explore the herbicidal effects and physiological mechanisms of essential oil isolated from *M. bracteata* on seed germination and seedling development on some weedy species.

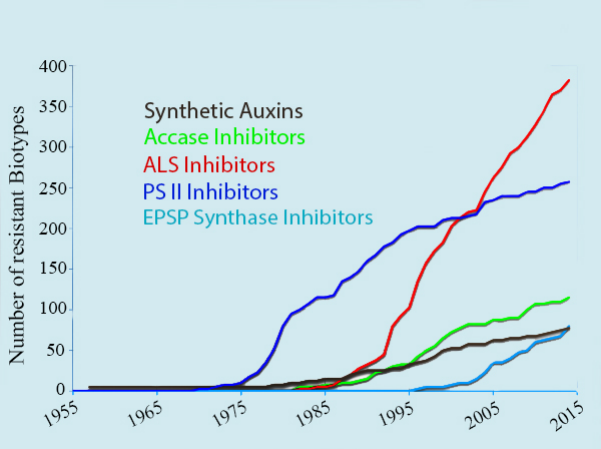

Figure 1 Number of Resistant Species for Several synthetic herbicide Sites of Action (Heap, 2014).

2. MATERIALS AND METHODS

2.1. Plant materials and weed seeds collection

Fresh and healthy leaves of *M. bracteata* were collected during the 2nd week of May 2014 from plants growing in Agrotechnology research station in Sg Chuchuh, which belongs to the University Malaysia Perlis UniMAP, Padang Besar, Perlis, Malaysia. Four samples of Fresh leaves were collected from six plants and washed with tap water, then with distilled water. Samples were dried in the shade for one week in the laboratory, then over a day in an electric oven at 40 °C. Regarding of weed seeds, healthy, mature plants of *Panicum virgatum, Digitaria longiflora, Stachytarpheta indica* and *Aster subulatus* were collected from Agrotechnology research station during March 2015 and dried for two weeks at room temperature. Then, the seeds were extracted and kept at 4C° until the germination test.

2.2. Isolation of the essential oil

The essential oil of *M. bracteata* was isolated by hydrodistillation according to the standard procedure described in the European Pharmacopoeia (2004) using a modified Clevenger-type apparatus (Singh et al., 2009). The final Isolation essential oils were dried over using anhydrous sodium sulfate (Amri *et al.,* 2014). Total Isolated yield was calculated based on the dried weight of the sample (mean of four samples).

2.3. Gas chromatography-Mass Spectrophotometer Analysis

M. Bracteate essential oil was analyzed using a Perkin Elmer-Clarus equipped with Elite-5MS non-polar fused silica capillary column (30 m × 0.25 mm ID, film thickness of 0.25) mm). The Oven temperature was increased from 40 °C to 230 °C at a rate of 8 °C/min; Injector temperature, 250 °C; injection volume, 0.5 µL; transfer temperature, 230 °C. Helium was the carrier gas at a flow rate of 1.0 ml/min, total run time was 25.75min.
Mass spectra were taken over the m/z 40–600 and interface line temperature of 230 °C. The constituents of essential oils were identified based on their Kovats Index, calculated in relation to the retention time of a series of alkanes (C4- C28) as reference products compared with the chemical compounds gathered by Adams table (Adams, 2007). The mass spectra of chemical compounds gathered in the NIST-MS and Wily Library.

2.4. Seed germination and Seedling Development.

Isolated essential oil of *M bracteata* was tested for its herbicidal activity in different doses on seed germination and seedling development on four weed species under laboratory conditions.
To prepare an essential oil solution, each dose under current study, 1.25, 2.5,5 and 10ml of *M. bracteata* essential oil were mixed with 100ml of distilled water. Then, volume was completed to 1L by adding distilled water to obtain 1.25, 2.5, 5 and 10 µL/ml. Dimethyl sulfoxide (DMSO) was added with each solution at 1% concentration to dissolve the polar and non-polar compounds and shaken well (Al-Samarrai *et al.,* 2012). The final solutions were shaken over for 1h using a magnetic stirrer to get homogeneous(Almarie *et al.,* 2016) .
Empty and undeveloped weed seeds were removed by floating test. To avoid the possibility causing by fungi and bacteria, healthy weed seeds were sterilized by sodium hypochlorite solution concentration 15% for 15 min.. Then seeds rinsed with water several times.
Twenty Seeds of each weed species were distributed in 9cm diameter Petri dishes on two layers of Whatman No.1. Five ml of each prepared essential oil solution 1.25,2.5,5 and 10µl/ml were added in petri dishes, covered in one layer of Whatman and sealed with Parafilm® tape to prevent escape of volatile compounds and kept in a dark place under laboratory condition at temperature approximately 25±2. Distilled water+DMSO 1% served as controls. After seven days, the number of seeds that germinated were counted. Then, seedlings in all treatments were thinned to five seedling in order to measure seedling length and dry weight after two weeks from sowing.

2.5. Greenhouse studies

In another experiment conducted in greenhouse to study the physiological mechanism of *M. bracteate* essential oil on target weed species. Plastic pots 15cm diameter were filled with agricultural soil. Twenty seeds of each weed species were sown in depth 1cm and watered as needed. When weed seedling arrived to 3-5 true leaves, seedling were thinned to 5 equal-sized healthy plants per pot. Later pots were sprayed with *M. bracteata* essential oil at 1.25, 2.5, 5 and 10% using a hand pressure sprayer adjusted to spray 100ml/m2 (Poonpaiboonpipat *et al.*,2013)

Table 1: Chemical composition of *Melaleuca bracteata* essential oil

Peaks	Compounds	RI	Means± S.E.)
1	α- pinene	9.38	0.14 ± 0.01
2	β-pinene	9.78	tr
3	α- Terpinene	10.12	tr
4	*p*-Cymene	10.20	0.63 ± 0.23
5	Limonene	10.30	0.99 ± 0.25
6	1,8-cinole	10.34	0.16 ± 0.02
7	Linalool	10.97	0.33 ± 0.04
8	Camphor	11.45	0.76 ± 0.18
9	Terpinol-4-ol	11.76	1.8 ± 0.31
10	α-Terpineol	11.89	0.70 ± 0.09
11	Carvacrol	12.97	1.2 ± 0.14
12	Undecanal	13.09	1.07 ± 0.12
13	Eugenol	13.53	2.97 ± 0.41
14	Methyl eugenol	13.69	81.4 ± 7.34
15	Caryophyllene	14.18	1.39 ± 0.63
16	Germacrene- D	14.80	0.85 ± 0.12
17	Nopinone	15.62	0.14 ± 0.07
18	γ-Eudesmol	16.13	1.26 ± 0.28
19	β-Atlantol	16.56	0.15 ± 0.07
20	Genipin	16.68	3.17 ± 0.28
21	Palmitic acid	19.84	0.29 ± 0.09
22	Spathulenol	21.19	0.36 ± 0.16
Total Monoterpenes		6.72 ± 0.87	
Total sesquiterpenes		3.48 ± 0.93	
Aromatic Compounds		84.2 ± 4.76	
Others		5.38 ± 0.54	
Total identified		92.09 ± 1.85	

RI: Retention Index on non-polar Elite-5MS column. tr: trace amounts <0.05. Values are means ± standard error of Four samples.

Sodium Dodecyl Sulfate was added as surfactant at 0.5% concentration. Four pots were treated at each concentration and weedy species. Distilled water also sprayed as controls. Visual observation of phytotoxic, chlorophyll content and relative electrolyte leakage were determined at 24 hours after spraying.

2.6.1 Estimation of total chlorophyll content.

To measure total chlorophyll, one hundred mg of fresh leaves from all treatments (Treated and control) was extracted in 20 ml of Dimethyl sulphoxide (DMSO) according to the method of Hiscox and Israesltam (1979). Total chlorophyll content was measured at dual wave length of 645 and 663 nm following the equation of (Arnon 1949) (Kaur *et al.,* 2010) .

2.6.2 Relative electrolyte leakage.

To elucidate the effect of essential oil under study on solute leakage and thus membrane integrity, relative electrolyte leakage was determined in seedling leaves of all weed species integrity according to Pal et al., (2008).

Five leaf discs (0.5cm diameter) were cut and soaked in 5mL of distilled water for 30 minutes and the conductivity of the medium was measured (C1). Then, test tube which contains leaf tissue was boiled for 15 min and the conductivity was measured again (C2). The relative electrolyte leakage (% REL) was calculated using following formula:

REL% = (C1/C2) ×100 (Kaur *et al.,* 2010).

2.6. Statistical analysis.

The experiments were conducted using a complete randomized design. All treatments were replicated four times. Data were subjected to one-way analysis of variance (ANOVA). The significant differences between mean values were determined using Duncan's multiple range test (P≤ 0.05). The ANOVA statistical analysis was performed using SASS version 9.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of the essential oil.

The chemical composition of the hydrodistilled essential oil was obtained from the leaves of *M. bracteate* was analyzed by GC/MS are shown in Table 1. The Final Isolated oil gave yield 0.42±0.32% basis of the dry weight of Four Samples. Twenty - two compounds accounting for 92.09% of the total oil were identified.

The chromatogram of *M. bracteate* essential oil obtained by gas chromatography coupled with mass spectrometry indicates that methyl eugenol was the dominant compound (81.04%) followed by Genipin (3.17%) and Eugenol (2.97%).

Monoterpenes and sesquterepens were represented by a small quantity (7.72%) and (3.48%) respectively. The major monoterpenes component was Terprnol-4-oil (1.8%), while caryophyllene was the major sesquterepens component (1.39%).

As can be seen from Table 1, the essential oil of *M. bracteata* showed different chemical compositions compared with the other species of Melaleuca genus. In study conducted by Amri *et al.* (2012) on three Melaleuca species,

Table .2 Herbicidal effects of *M. bracteata* essential oil on seed germination and seedling development in some weedy species.

Weeds	Dose (µl/mL)	Germination %	Seedling development	
			Seedling length (cm)	Dry weight (g)
P. virgatum	0	25.5 ± 1.3 a	9.12 ± 0.8 a	3.32 ± 0.9 a
	1.25	21.3 ± 0.9 b	8.15 ± 0.3 ab	3.02 ± 0.8 ab
	2.5	20.2 ± 1.1 b	7.33 ± 1.3 b	1.54 ± 0.5 b
	5	12.9 ± 0.7 c	3.00 ± 0.3 c	0.79 ± 0.5 c
	10	0.0 ± 0.0 d	0.00 ± 0.0 d	0.00 ± 0.0 d
D. longiflora	0	72.5 ± 7.1 a	10.05 ± 1.0 a	6.43 ± 0.8 a
	1.25	51.8 ± 4.6 b	8.94 ± 0.5 ab	6.17 ± 0.8 ab
	2.5	48.6 ± 3.9 b	8.20 ± 0.6 b	5.61 ± 0.8 b
	5	21.5 ± 1.1 c	2.01 ± 0.3 c	1.01 ± 0.8 c
	10	0.0 ± 0.0 d	0.00 ± 0.0 d	0.00 ± 0.0 d
S. indica	0	60.0 ± 6.0 a	6.53 ± 1.0 a	4.28 ± 1.3 a
	1.25	31.6 ± 5.3 b	5.70 ± 1.1 a	4.30 ± 0.9 a
	2.5	28.5 ± 2.5 b	3.36 ± 0.7 b	4.32 ± 1.1 a
	5	18.0 ± 0.8 c	1.87 ± 0.3 c	1.16 ± 0.5 b
	10	0.0 ± 0.0 d	0.00 ± 0.0 d	0.00 ± 0.0 c
A. subulatus	0	43.1 ± 1.8 a	8.37 ± 1.0 a	3.12 ± 0.8 a
	1.25	41.2 ± 1.1 a	7.96 ± 1.0 a	2.35 ± 0.6 ab
	2.5	39.9 ± 1.2 a	6.16 ± 0.3 b	1.78 ± 0.8 b
	5	11.8 ± 0.5 b	2.24 ± 0.5 c	1.57 ± 0.7 b
	10	0.0 ± 0.0 c	0.00 ± 0.0 d	0.00 ± 0.0 c

- Means in the same column by the same letter are not significantly different of the Duncan ($p \leq 0.05$). (Mean of four replicates) - Seedling length was taken by an average of five seedlings. - Dry weight was taken as the sum of five seedlings.

found that the essential oil isolated from *M. acuminata* oil was rich in oxygenated monoterpenoids (95.6%) and *M. armillaris* essential oil showed high content of sesquterepens (52.2%), while methyl eugenol was the major compound in essential oil of *M. styphelioides* (91.1%). In this regards, Chabir *et al.* (2011) reported that 1,8cineole (oxygenated monoterpene compound) was the main component of the *M. armillaris* essential oil (85.8%) and the essential oil of *M. styphelioides* contained mainly caryophyllene (50.0%) and methyl eugenol (26.6%). In another study conducted by Padalia *et al.* (2015) found the essential oil of *M. linarrifolia* was characterized by a higher content of oxygenated monoterpenes (86.63%) mainly represented by 1,8-cineole (77.40%) followed by α-terpineol (7.72%).

This finding comes in a lane with finding as mentioned by (Ismail et *al., 2013*; Padalia *et al.,* 2015) that variation of Essential oil components depends on geographical variation and genetic background in isolated plant.

In the previous literature, there is only one study reported the chemical composition of the essential oil *M. bracteata*, and found that methyl eugenol was the main component 97.7% (Aboutabl *et al.,* 1991).

3.2. Seed germination and seedling development

The herbicidal effects of *M. Bracteate* essential oil was tested on seed germination and seedling development of two grassy weeds *Panicum virgatuma* and *Digitaria longiflora* and broad leaf weeds, *Stachytarpheta indica* and *Aster subulatus.*

As can be seen from the data presented in Table.2, the seed germination of all targeted weeds was significantly inhibited when treated with *M. bracteata* essential oil. In general, complete inhibition of seed germination was observed of all targeted weeds at higher Dose of *M. bracteata* essential oil 10µL/ml. Whereas at lower doses of the essential oil decreased the germination and seedling development partially.

Our results agree with most studies have been conducted previously to evaluate herbicidal effects of essential oils which found that the essential oils were isolated

from plants belong to Myrtaceae, Lamiaceae, Cupressaceae and Rutaceae have been shown a high efficacy agonist weed seed germination and seedling growth, (Kaur *et al.,* 2012; Pal Singh *et al.,* 2008 ; Amri *et al.,* 2013 ; Verdeguer *et al.,* 2011).

Based on date obtained in the current study, Methyl eugenol was characterized by a good efficacy of suppressing weed seed germination and seedling development as the dominant component in *M. bracteata* essential oil.

This result has come in the lane with the literature on the herbicidal effects of essential oils against weed seed germination and seedling development were generally attributed to some compounds (Andrianjafinandrasana et *al.,* 2013; Pal Singh *et al.,* 2008; De Almeida *et al.,* 2010).

Eugenol (The main compound in essential oil of clove plant *Syzygium aromaticum*) also showed high phytotoxicity against weed and used previously as a natural herbicide (Vaid *et al.,* 2010 ; Ahuja *et al.,* 2014; Stoklosa *et al.,* 2012). Individual monoterpene compounds also showed a good effect on seed germination and suppressing seedling growth of weed plants, such as camphor, 1,8-cineole, thymol, and carvacrol (Amri *et al .,* 2013)

3.3. Chlorophyll content

The results presented in Fig. 2. Showed that the chlorophyll content reduces with increasing of *M. bracteata* essential oil concentration in all targeted weed species. No significant difference was observed with lower concentration 1.25% and 2.5% compared with control treatment. Furthermore, broad leaf weeds more affected than grassy weeds. The decreasing in total chlorophyll percentage reached to 79.58% and 81.51% in *S.indica* and *A. subulatus* respectively at 10% concentration compared with control.

Chlorophyll plays an important role in plant photosynthesis. So any threat to this material means that the life of the plant is at risk and lead to death. Recent studies suggest that in the destruction of chlorophyll pigment is one of the physiological mechanisms of allelochemical compounds in essential oils, particularly volatile compounds hence, the food mechanism of the plants broke down (Ahuja *et al.,* 2014; Almarie *et al.,* 2016).

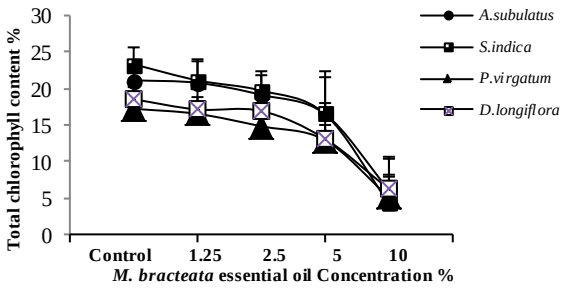

Figure 2 Herbicidal effects of *M. bracteata* essential oil on total chlorophyll content of targeted weedy species.

3.4. Relative electrolyte leakage

Changes in electrical conductivity values of different concentrations in targeted weeds are shown in Fig. 3. The electrolyte leakage showed a different response depending on the concentration of *M. bracteata* essential oil. Grassy weed were more affected by treating with *M. bracteata* compared with broad leaf weeds. However, there was no significant difference in lower concentration 1.25% and 2.5% compared with the control. The electrolyte leakage was obviously increased in all targeted weeds at the highest concentration 10%.

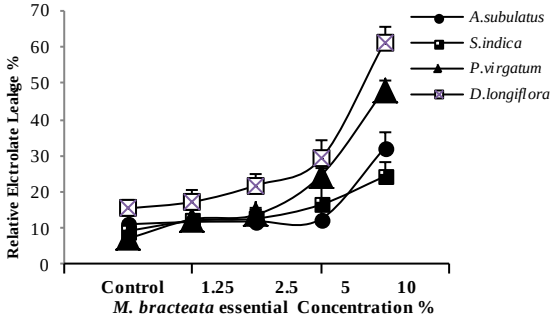

Figure 3 Herbicidal effects of *M. bracteata* essential oil on Relative electrolyte leakage of targeted weedy species.

The results in our study in agreement with the latest studies in this field (Ismail et al., 2012; Koul et al., 2008; Poonpaiboonpipat et al., 2013; Almarie et al., 2016) which reported that the essential oils inhibit plant growth through degradation of cell membranes. Therefore, these characteristics can be considered a good indicator in evaluating the herbicidal effects of essential oils. Based on our study, this is shown clearly that the lower concentration, especially 5%, although not highly effective in suppressing the Seed germination, they succeeded in influencing the seedling that does not have the ability to grow healthy through effecting of decrease seedling growth.

4. CONCLUSION

This study is considered the first study regarding of herbicidal effects of *Melaleuca bracteata* F.Muell., according to our knowledge. The results of the current study showed that *M. bracteata* essential oil was effective to inhibit germination of seed and suppressing seedling development from targeted weeds. Furthermore, the present study showed the targeted weed plant injured strongly by *M. bracteate* essential due to effecting on the destruction of chlorophyll pigment and cell membrane and could have value as potential bioherbicides if used as alternatives to synthetic herbicides. However, further studies are required to determine the feasibility of using this essential oil under open field as a postemergence

bioherbicide.

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