

Assessment of Allelopathic Potential of *Melastoma malabathricum* L. on *Radish raphanus sativus* L. and Barnyard Grass (*Echinochloa crus-galli*)

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Abstract

Melastoma malabathricum L. is a weedy invasive shrub in arable lands, abandoned farmlands, secondary forest openings and derelict areas in Malaysia. Some allelochemicals present in this plant extracts may, directly, prevent or promote germination when environmental conditions are conducive to growth and establishment. It may have an important role, indirectly, in determining plant community structures. The aqueous extract and methanol extracts, were assayed for the aqueous extract of fresh materials with concentrations of 0, 50, 100, 150 and 200 g l⁻¹ and at aqueous of oven dried materials extract with concentrations of 40, 80, 120, 160, and 200 g l⁻¹. The crude methanol extracts were prepared using extract concentrations of 10.8, 14.28, 18 and 30 g l⁻¹ of shoot and root materials. The extracts were tested with the widely used radish seed barnyard grass seed. Radish seed germination was inhibited at concentrations ranging from 200 g l⁻¹ in the extract aqueous of dried materials and in the methanol extract concentrations of 14.28 and 30 g l⁻¹. The inhibition of root and shoot growth was also observed in the Barnyard grass seed. Both species were susceptible to allelopathy by extracts isolated from shoot and root of *M. malabathricum* and also their rate of germination, root length and shoot length in were decreased upon the application of both type of extractions. The results from this study strongly suggest that allelopathy may be a possible mechanism controlling the timing of barnyard grass germination and seedling establishment.

Keywords: allelopathy, *Melastoma malabathricum*, germination, barnyard grass, radish

Introduction

Malaysia, with a tropical climate, is home to a very large number of plant species, many of which are used by natives in folk medicine. Malaysia is among the world's mega biodiversity – rich countries in terms of number of plant species. So far only a small number of these plants have been examined biochemically.

Chemical compounds

One of the herbal remedies found in Malaysia is *Melastoma malabathricum*. It is an important source of chemicals such as carbohydrates, proteins and amino acids, are classified as primary metabolites, vital for the maintenance of life processes. Others are classified as secondary metabolites, for examples, terpenoids and phenolics. In the last few years, chemical studies on *Melastoma malabathricum* have been intensified. Generally phytochemical constituents of *M. malabathricum* are flavonoids, triterpene, tannins, saponins and steroids. The interesting isolated compounds from *Melastoma malabathricum*: a dyestuff, β -sitosterol and a triterpenoid designated as melastomic acid, were isolated from the ethanolic extract of the roots (Manzoor-I-Khuda et al., 1981, cited by Dafaalla, 2004). *Melastoma*

malabathricum may have a potential of allelopathy as some allelochemicals like terpenoid, flavonoid and phenolic compounds were found in its isolated compounds.

Evaluation of allelopathic activity

Allelopathy is an interference mechanism by which plants release chemicals which affect other plants; while it has often been proposed as a mechanism for influencing plant populations and communities (Khanh et al., 2007). Increasing attention has been given to the role and potential of allelopathy as a management strategy for crop protection against weeds and other pests. Incorporating allelopathy into natural and agricultural management systems may reduce the use of herbicides, insecticides, and other pesticides, reducing environment/soil pollution and diminish autotoxicity hazards. There is a great demand for compounds with selective toxicity that can be readily degraded by either the plant or by the soil microorganisms which provide new strategies for maintaining and increasing agricultural production in the future (Inderjit and Mukerji, 2006).

Interactions occurring between plants that are biochemically facilitated by secondary compounds were first referred to as allelopathic by Hans Molisch (1937).

Though the study of allelopathy can encompass both positive and negative interactions among plants; negative interactions are generally the focus of most research studies. Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone. More recently, allelopathy has come to also include the microbial breakdown of secondary plant compounds that result in chemicals with suppressive properties (Weston and Duke, 2003). Monoterpenes and diterpenes, which are the main components of essential oils, act as allelopathic agents, attractants in plant-plant or plant-pathogen/herbivore interactions or repellants (Grassmann, 2005). These chemicals are referred to as allelochemicals and can be found in the tissues of nearly all plants. However, not all of these compounds with allelopathic potential will be released into the environment (Putnam, 1988; Wardle et al., 1998; Khanh et al., 2005). The quantity of allelochemicals released or those created by plants or microbes varies by species, chemical composition, and environmental conditions (Putnam, 1988). Allelochemical-based herbicides are natural products and thus could be broken down easily by microorganisms, making them less persistent in the environment (Chon et al., 2002; Singh et al., 2003; Xuan et al., 2005; Khanh et al., 2007).

In agriculture or environment allelopathy may serve as a supplement to the use of synthetic herbicides for weed control, yield improvement without environmental cost. Increasing the efficacy of cover crops if they possess allelochemicals that can inhibit the germination and growth of some weed species, then the reliance on synthetic herbicides could be reduced, saving money and preserving the environment (Iqbal et al., 2004). Finally, as natural products to secure the world's food supply for future generation that it is one of the most important considerations for scientists. The aim of this part of the research is to get an understanding of allelopathy activity properties in *M. malabathricum*. The results from this study may suggest a comparison of allelopathic activities between leaves and root and also if this property can be a possible mechanism controlling the timing of weed germination and seedling establishment.

Materials and methods

Plants were harvested at a vegetative stage from a field of forest at Malaya University. These plants were immediately washed with tap water to remove any soil or other adhered material and leaves, stems and roots are separated.

Aqueous extract: All the materials of leaves, stems and roots were chopped into small pieces (1–2 cm size).

Fresh material: Twenty grams of fresh materials (leaves, stems and roots) was extracted with 100 ml distilled water at 25°C for 24 h in a shaker.

Dried powder: Plant Materials were dried in an oven at 40°C for 48 h, ground up to a fine powder using a Wiley mill to pass a 1 mm screen and then stored in a refrigerator at 2°C in dark until used (Chung et al. 2001; Chon et al. 2002). Twenty grams of dried leaves, stems and roots are extracted by soaking in 200 ml deionized water at 25°C for 24 h in a shaker to give a concentration of 20 g dry tissue /100 ml (200g/ liter).

The extracts are filtered through four layers of cheese-cloth to remove the fiber debris, and centrifuged (by Centrifuge Eppendorf, 5804 R) at 10.000 rpm and 4°C for 10 min. The supernatant was vacuum filtered again through Whatman no. 42 paper. Stock extracts were made fresh for each experiment.

Methanol extract: Fresh plant parts (3 kg) were dried at room temperature. Dried plants were milled and homogenized using a blender and extracted with exhaustive using the Soxhlet extraction apparatus. Initially, an amount of 500 g of dried of *M. malabathricum* (separately for leaf, root and stem) extracted by using n-hexane for 16 hours in the Soxhlet extraction apparatus. After that hexane extracts were concentrated then the slurry was filtered. The filtrate concentrated under reduced pressure using the rotary evaporator. The n-hexane solvent were be replaced by dichloromethane (CH₂CL₂) and methanol (MeOH), respectively by applying the same extraction methods as above. Hexane and dichloromethane were used in the extraction process to remove fat-soluble substances (Jefferson and Pennacchio, 2003; Dafaalla, 2004; Aziz, 2007). Root and leaf extractions were obtained by repeating all of the steps above.

Treatment concentrations were prepared using control (0) and 50,100,150 and 200 g l⁻¹ of the original dose for fresh material (root, leaf and stem); 40, 80,120, 160 and 200 g l⁻¹ for dried powder material (root, leaf and stem) and methanol extract concentrations of 10.8, 18 and 30 g l⁻¹ (leaf and root). All extracts were diluted appropriately with sterile distilled water to give the final concentrations.

Laboratory studies were conducted to assess the allelopathic potential of *M. malabathricum* on radish (*Raphanus sativus* L.) and barnyard grass (*Echinochloa crus-galli* L. Beauv.) respectively as indicator plant and weed species to see if *M. malabathricum* could exhibit any differences in the inhibition of barnyard grass growth and development. Radish was used as an indicator plant in this experiment because it is very sensitive to allelochemicals at low concentrations. Barnyard grass is a common weed of rice fields in Malaysia, was selected and used in the present study. Seeds of the weed was collected locally from the plants growing in the farmers' fields. These are stored at -5 °C until use. Seeds of the weed scarified with concentrated sulphuric acid (barnyard grass for 90 s) followed by washing with distilled water several times (Dang Khanh et al., 2005). With using above methods, the same concentrations were prepared separately from different all part of plants (root, stem and leave) and applied on the indicator

plants and weed species. Thirty (radish or barnyard grass) seeds were sown in 9 cm Petri dishes lined with filter paper, and 10 ml of each treatment solution was added. The dishes were transferred to a growth chamber (set at 25 °C, 4000 lux, lit time: 09:00–17:00 h). Germination (daily), shoot and root length, and dry weights after 8 days were calculated for all treatments. Five replicates were maintained per concentration and plant part in a completely randomized manner in a growth chamber.

Data analysis

Germination counts were performed for a period of 8 days for the radish and for the weed species, although calculations were based on the longest time taken to achieve maximum germination. The following parameters, previously reported by others, such as Jefferson et al. (2003), were calculated for all four species:

Final germination (FG) %: The maximum average percentage of seeds that germinated during the experiment.

Mean period of final germination (MPFG) =

Rate of germination (RG) =

Percentage inhibition or stimulation =

where,

$$\left(\sum_{i=1}^d NiDi \right) / FG$$

N is daily increase in seedling number

$$\sum_{i=1}^d \frac{Ni}{Di}$$

D is number of days from seed placement

$$\left(100 - \frac{FG \text{ in aqueous extracts } (\%)}{FG \text{ in distilled water } (\%)} \right),$$

Homogeneity of variances was tested, those data not normally distributed were transformed; retransformed data are presented in the results. ANOVA was performed on the data. Differences between means were determined using Tukey's Compromise test.

Results and discussion

Results showed that the allelopathic activity was significantly ($p < 0.05$) similar in different plant parts on seed germination and germinates of radish and barnyard grass when treated with different concentrations of *M. malabathricum* extracts which were isolated from root, stem and leaf using aqueous or methanol solvents (data not shown).

Radish seed germination. Final germination and germination inhibition/stimulation

Analysis of variance and Tukey' test (HSD) showed that the final germination (FG) with the application of distilled water (control) was 84 %. Application of 200 g.l⁻¹ aqueous extract of dried plant parts (root or stem or leaf) had a significant ($p < 0.05$) stimulatory effect (40.4 %) on seed germination of radish seeds (Table 1). Further comparison by HSD method showed there were not significantly ($p < 0.05$) differences of FG or the percent of germination inhibition between the rest of aqueous extracts and control samples (Tables 1, 2). All the methanol extract obtained from the leaves and roots of *M. malabathricum* ($p < 0.05$) significantly inhibited the germination of radish seed. The percent of inhibition of germinations were highest percent 76 and 86 respectively at methanol extract concentration of 14.28 and 30 g.l⁻¹ With increase in the concentration of the extracts led to a significantly ($p < 0.05$) decrease in FG from 90 % under control to the lowest

Table 1 Effect of *Melastoma malabathricum* aqueous extracts of overall plant (root, stem and leaf) on germination parameters of radish

Aqueous extract concentration (g.l ⁻¹)	RG (seed day ⁻¹)		MPFG (days)	FG%	Inhibition (%)	Root length (cm)	Shoot length (cm)	
	RG†	RG						
Control Fresh extract(%)	0	6.32 A	40.23	1.96 C	83.70 A	-	8.06 A	3.78 AB
	50	6.22 A	38.91	2.11 C	88.15 A	-5.30	6.90 A	3.47 AB
	100	6.25 A	39.46	2.08 C	86.67 A	-3.55	7.94 A	3.70 AB
	150	5.88 A	34.91	2.42 BC	85.56 A	-2.22	7.26 AB	3.91 AB
	200	5.45 A	30.40	3.81 AB	87.41 A	-4.432	5.58 AB	4.54 AB
Dried extract (g.l ⁻¹)	40	6.00 A	36.53	2.22 BC	83.76 A	-0.07	5.36 AB	3.38 B
	80	6.11 A	38.08	2.29 BC	87.78 A	-4.87	6.08 AB	4.23 AB
	120	5.79 A	34.81	2.63 ABC	87.78 A	-4.87	7.28 A	4.67 A
	160	5.88 A	35.49	2.55 BC	87.78 A	-4.87	6.22 A	4.42 AB
	200	3.33 B	14.36	3.28 A	49.89 B	40.39	3.211 B	3.99 AB
Sx , at alpha = 0.05		0.148	0.146	4.33		0.724	0.268	

† displays transformed data and the means with the same letter means no significant at $p < 0.05$ by HSD test

Table 2 Effect of methanol extracts of *Melastoma malabathricum* on seed germination in radish

Concentration (g.l ⁻¹)	Rate of germination (seeds day ⁻¹)	Mean period of final (days)	Final germination%	Germination inhibition / stimulation (%)	Root length (cm)	Shoot length (cm)	
Control	0	26.50 A	2.56 BC	90 A	---	10.70 A	3.80 A
Methanol Extract	10.8	11.64 B	3.64 AB	79.44 A	11.73	4.93 B	3.85 A
	14.3	2.36 C	1.50 C	21.67 B	75.93	0.53 D	1.03 C
	18	5.78 C	4.88 C	61.11 A	32.10	2.51 C	2.20 A
	30	1.42 C	2.09 C	12.78 B	85.80	1.04 CD	1.60 BC
Sx, p<0.05	1.082	0.32	8.83	---	0.4145	0.384	

† displays transformed data and the means with the same letter means no significant at p<0.05 by HSD test

value (12.78 %) with the application of highest methanol extract concentration (Table 2).

Effect on rate and mean period of germination

The rate of germination (RG) was 40.23 seeds day⁻¹ under control. Increasing concentration of root, leaf and stem extracts significantly decreased the rate of germination. Only the application of 200 g.l⁻¹ dried plant extract reduced significantly (p<0.05) the RG to 14.36 seeds day⁻¹ (Table 1). The highest concentration of aqueous extract resulted in the slowest rate of germination. A similar trend of adverse influence on RG due to application of methanol extraction of leaves and root extracts was also recorded (Table 2). Increasing concentration of root and leaf extracts significantly decreased the rate of germination from 26.50 to 1.42 seeds day⁻¹ respectively under control and the application 30g.l⁻¹ of methanol extract. Further increase in the concentration of extracts from 14.28 g.l⁻¹ did not influence significantly (p<0.05) on the rate of germination seeds day⁻¹ (Table 2). The mean period taken for final germination (MPFG) under control was 1.96 days

which increased to 3.28–3.81 days with the application of 200 g.l⁻¹ fresh and dried aqueous extracts respectively.

The highest MPFG was observed at the concentration of extract which isolated with methanol solution of 10.8 g.l⁻¹. However, MPFG differences were not significant between treatments and control in higher concentration of the methanol extract treatment than 10.8-1. Further increase in the concentration of extracts did not influence the mean period of germination (Table 3). This indicates that the chemicals responsible for stimulatory effect at lower concentrations became inhibitory at higher concentrations.

Effect on root growth, shoot growth and total dry matter

Root length of the seedlings was significantly influenced (p<0.05) by the root, stem and leaf aqueous extract specially at dried materials and at 200 g.l⁻¹ concentration (Table 1). This effect was more obviously in the application of methanol extracts of plant (leaf and root). The root lengths were differed from 10.7 cm under control to 1.04 cm in the dried material extract concen-

S_x = 0.370, p<0.05

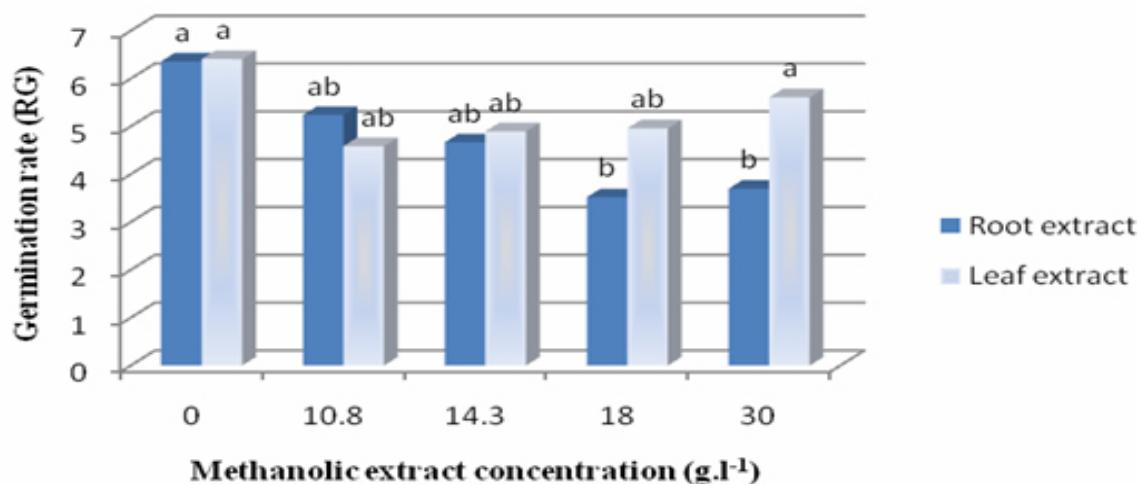


Figure 1 Rate of germination of barnyard grass seeds when treated with different concentrations of *M. malabathricum* extracts which were isolated using methanol solvents

Table 3 Effect of methanol extracts of *Melastoma malabathricum* on seed germination in barnyard grass

Concentration (g.l ⁻¹)	RG (seeds day ⁻¹)	MPFG (days)	FG %	Inhibition (%)	Root length (cm)	Shoot length (cm)	
Control	0	6.40 A	4.68	88.33	0	5.48 A	4.73 A
Methanol Extract	10.8	4.91 B	5.11	75.56	12.824	2.52 B	4.44 A
	14.3	4.78 B	5.48	77.22	10.91	0.5 C	3.19 BC
	18	4.24 B	5.33	68.33	21.16	0.5 C	2.85 C
Sx	30	4.65 B	5.34	74.44	14.11	0.5 C	3.66 B
p<0.05		0.262	0.245	5.11		0.055	0.112†

† displays transformed data and the means with the same letter means no significant at p<0.05 by HSD test

tration of 200 g.l⁻¹ (Table 3). The shoot length was also changed significantly (p<0.50) from 3.80 cm under control to 1.03 cm at the methanol extract concentration of 14.28 g.l⁻¹ (Table 3). The methanol extract with concentration of 14.28 g.l⁻¹ caused to inhibit the elongation of root (95%) and shoot (73%) compared with the control whereas aqueous extract inhibited the elongation of root (60%) and shoot (11%). Chemical extracts impeded the germination and caused abnormal root elongation and seedling abnormality. Analysis of variance (data not shown) indicated that the aqueous and methanol extracts were not influenced significantly p<0.05 on the total plant dry matter.

Barnyard grass seed germination. Final germination and percent of germination inhibition /stimulation

The germination of barnyard grass (*Echinochloa crus-galli*, Beauv. var. *oryzicola* Ohwi) seed was not inhibited significantly (p<0.05) when treated with an methanol extract of the leaves or root of *M. malabathricum* (Table 3). The FG percentage of barnyard grass decreased significantly (p<0.05) from 84.33 % with the application of distilled water (control) to 68.33 % with application of 18 g.l⁻¹ methanolic extract of root or leaf with a percent inhibition of 21.16.

Effect on rate and mean period of germination

The rate of germination (RG) was 6.40 seeds day⁻¹ under control. Increasing concentration of root and shoot methanol extracts significantly decreased the rate of germination. The application of 10.8 g.l⁻¹ methanol extract reduced the RG to 4.91 seeds day⁻¹ which further methanol concentrations than 10.8 g.l⁻¹ had not any significant effect (p<0.05) on the RG (Figure 1). Table 3 showed that the MPFG increased in response to application of methanol concentration of the extracts. However, the MPFG differences were not significant at p<0.05. The same results were reported on allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination as although RG significantly reduced but no effect on MPFG was observed by application plant methanol extracts on alfalfa seeds (Jefferson and Pennacchio 2003). The mean period taken for final germination (MPFG)

under control was 4.68 days which increased to 5.48 days with the application of 14.28 g.l⁻¹ root and shoot extracts. Further increase in the concentration of extracts did not influence the mean period of germination (Table 3).

Effect on root growth, shoot growth and total dry matter

With increasing concentration, the *Melastoma malabathricum* leaf and root methanol extracts significantly reduced root and shoot lengths of barnyard grass (*Echinochloa crus-galli*, Beauv. var. *oryzicola* Ohwi) (Table 3). Although there was not observed a significant difference for percent of final germination but the application of methanol extracts were caused to increase the rate of the

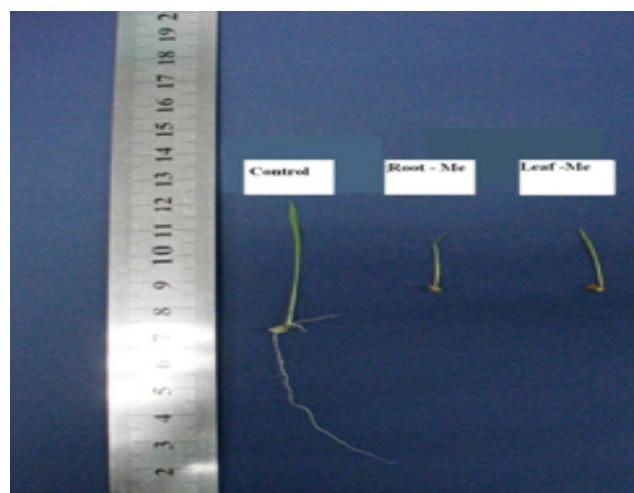


Figure 2 The consumptive root and shoot lengths of barnyard grass when treated with different concentrations of *M. malabathricum* extracts which were isolated using methanol solvents (Me). The seedling on the left side was treated with distilled water (control)

deformed or consumptive roots of seedling Figure 2.

Phenolic compounds reduced root and shoot lengths of barnyard grass. The root systems, especially root tips of barnyard grass, were stunted, swollen and shorten (91%) by the methanol *M. malabathricum* extracts at 10.8 g.l⁻¹ or more concentrations (Figure 3).

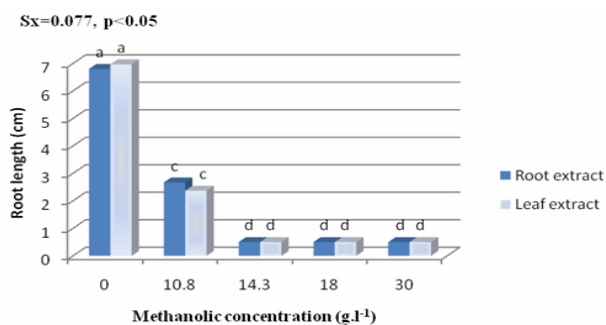


Figure 3 The seedling length of barnyard grass seeds when treated with different concentrations of *M. malabathricum* leaf extracts which were isolated using methanol solvents

Both root and shoot elongation of germinates was inhibited by aqueous and methanol extracts. The methanol extract of *M. malabathricum* was even more potent and inhibited radish seeds (85.80%), aqueous extract with 40.39 % against the percent inhibition as high as 21.91 % in methanol solvent.

Allelopathy can affect many aspects of plant ecology including occurrence, growth, plant succession, the structure of plant communities, dominance, diversity, and plant productivity. Plants that germinate at slower rates are often smaller. This may seriously influence their chances of competing with neighboring plants for resources such as water especially in arid or semi arid regions.

The allelo-chemicals present in *M. malabathricum* extracts might have appeared to inhibit or to stunt the growth of roots and shoots of germinates by at least two mechanisms. The first reason is the exist of phenolic compounds. They have been already identified and separated from *M. malabathricum* extracts (Dafaalla, 2004) and possibility they inhibited root elongation and cell division completely, indicating that the thickness of seminal roots was enlarged abnormally. It is thought that only transverse growth of root was sequentially maintained while longitudinal growth was greatly inhibited by the extracts and the phenolic (Bhowmik and Doll, 1982; Chon et al., 2002). These phenomena may account for the results obtained in the study with the extracts of *Melastoma malabathricum*. Our findings are consistent with those reported elsewhere for other species in a variety of plant families (Jefferson and Pennacchio, 2003; Iqbal et al., 2005; Erhard and Gross, 2006; Belz et al., 2007; Khanh et al., 2007). A number of important factor such as the density at which the shoot fall, root residual, decomposing rate of materials, temperature, the presence of certain micro-organisms rainfall, PH and the distance from other plants present the potential of allelopathic substances in the soil (Saxena et al., 1996; Kato-Noguch et al., 2002; Belz et al., 2007).

This research suggests that *Melastoma malabathricum* plant extracts significantly affected root growth and morphological differentiation of susceptible plants, that final result with growing the plant will be a reduction of plant biomass in the presence of either autotoxic or allelopathic

compounds. The results may have value in enabling weed control based on natural plant extracts. We have demonstrated that allelochemicals are produced in the shoot, root and of *M. malabathricum* Such chemicals are both species-specific and concentration-dependent. These characteristics may influence the density and the composition of individual plant communities. Allelochemicals may, directly, prevent or promote germination when environmental conditions are conducive to growth and establishment, therefore, influencing the number of plants of each species in a community (Jefferson and Pennacchio, 2003).

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