The response of woody borreria (*Hedyotis verticillata* Lam.) towards curry leaves (*Murraya koenigii* (L.) Spreng.) aqueous extract at the vegetative growth

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Abstract. The massive use of synthetic chemical herbicides in crop production to meet the increasing demand had caused negative impacts on the environment and ecosystem. Allelopathy approach of potential crops is a suitable alternative that should be explored to have sustainable weed management. The present study was conducted to determine the phytotoxic effect of aqueous curry leaves (*Murraya koenigii* (L.) Spreng.) extract on the emergence and seedling growth of the bioassay species, woody borreria (*Hedyotis verticillata* Lam.) under laboratory and nursery condition. The seed and seedling of bioassay species were treated with different concentration of aqueous curry leaves extract at 0, 20, 40, 60, 80 and 100g/L (laboratory) and 0, 50, 100, 150, 200 and 250g/L (nursery). The results showed that the aqueous curry leaves extract possesses a significant inhibition on seed emergence, shoot fresh weight and radicle length of *H. verticillata* at 100g/L concentration, where it reduced by almost 100% when applied as pre-emergence under laboratory condition. Meanwhile, the curry leaves extract at highest concentration of 250g/L significantly reduced the weed shoot fresh weight by 31% as compared to control and 50g/L concentration when treated at soil surface as post-emergence under nursery condition. Conversely, there is insignificant reduction in shoot height and root length of *H. verticillata* across all the applicable rates. These results suggest that curry leaves can be a good source to develop pre-emergence natural herbicide for weed management which is also eco-friendly for the environment and human beings.

1. Introduction

Agriculture is one of the significant sectors that contribute to nation’s economy. Herbicide becomes the main target of usage and highly dependent when managing weeds on farms. Malaysia accounted for 83% of the total pesticide usage in the year 2014 [1]. Glufosinate-ammonium, paraquat, metsulfuron-methyl, and glyphosate is the most commonly used herbicides in Malaysia [2]. According to Taylor et al. [3], chemical compounds present in herbicide, unfortunately could degrade and undergo several interactions of chemical transformations, transferred to a surrounding environment where reaching other ecosystem outside area of application hence exert harmful effects on non-target species and further leading to the deterioration in quality of life. Instead of the negative impacts on human and the environment, weed resistance also develops by continued use of the same herbicide in the field thereby causing serious competition among weed species [4]. Hence, the discovery of strong allelopathic properties in some potential crops has become an interesting component of natural
herbicides development [6]. Isolation and identification of several phytotoxic substances or allelochemicals from plant tissue extract is one of the effective approaches for weed control [5]. These allelochemicals may cause suppression of germination and interfere with other plants or affect growth and yield of crops.

The curry leaves tree (Murraya koenigii (L.) Spreng.) is belongs to Rutaceae family and it is a native plant to India, Sri Lanka, Bangladesh, and the Andaman Islands [6]. The leaves of M. koenigii contain proteins, carbohydrate, fiber, minerals, carotene, nicotinic acid, Vitamin C, Vitamin A, calcium and oxalic acid. Bark contains carbazole alkaloids like murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline and xynthyletin. Carbazole alkaloids which are also abundantly present in the leaves, fruits, and roots of this plant have been reported for their antidiabetic, anticancer, antibacterial, anti-nociceptive and antioxidant activities [7]. This indigenous plant was reported to have a potential source of natural essential oil to control insects such as cockroaches, mosquitoes, and others [8]. The M. koenigii extracts also was found to be effective against Aedes aegypti [9] and Culex mosquito larvae [10]. However, there were no previous study on herbicidal activity of M. koenigii leaves extract as natural herbicide tested on any bioassay species.

Weed management is essential for agricultural crops that have significant value that contributes majorly in the industry with their economic value, such as rubber and oil palm. According to Kuan et al. [11], the difficulties faced as the infestation of weeds at oil palm and rubber plantation is hard to quantify and likely to affect the productivity in farm due to their long economic lifespan (20 - 30 years). Hedyotis verticillata Lam., basically known as woody borreria belongs to the Rubiaceae family which from the category of broadleaf weed. It was reported to infest rubber and oil palm plantation in Malaysia and develop multi-resistance towards chemical herbicide, such as paraquat and glyphosate [3]. According to Ong and Teo [12], as this weed started to mature, it is difficult to control due to stems started turn woody and within 3 weeks of maturity, the plant would produce multi-seeded capsules [13]. Therefore, this study was conducted to determine the phytotoxic effect aqueous extract of Murraya koenigii (L.) Spreng. leaves towards seed germination and seedling growth of Hedyotis verticillata Lam. under laboratory condition and nursery environment.

2. Materials and Methods

2.1 Plant materials
M. koenigii leaves were collected at a wasteland area from Taiping, Perak, Malaysia (4.8519°N, 100.7416°E) and seeds of H. verticillata was collected at rubber plantation of Jeli, Kelantan, Malaysia (5.7007°N, 101.8432°E). The seed coat of the bioassay species was removed by using sandpaper. The seed that exhibit 80% - 90% of germination rate were used for this study.

2.2 Extracts preparation
The collected M. koenigii leaves were washed with clean tap water to remove dust particles. Then it was dried under shade for seven days and oven dried at temperature of 45°C before grinding. The dried samples were ground into fine powder (20 mesh) using a laboratory blender and stored in an air-tight plastic container, labelled and kept at 4°C until use. Then, 50g of the powder was added with 100 ml of distilled water to obtain 50% of stock solution and then agitated vigorously for 24 hours at 200rpm at 25°C on an orbital shaker [14]. The supernatants obtained was filtered using a double layer of Muslin cloth. The stock solution of M. koenigii leaves powder was diluted with distilled water to obtain different concentrations of 20, 40, 60, 80 and 100 g/L for seed germination tested under laboratory condition (pre-emergence) while 50, 100, 150, 200 and 250 g/L for seedling bioassay tested under nursery condition (post-emergence).
2.3  **Seed germination test**
A total of 20 *H. verticillata* weed seeds were placed randomly on 90 × 15mm petri dishes that lined with two layers of filter papers and moisten with 5 ml of aqueous *M. koenigii* leaves extract at different concentration (0, 20, 40, 60, 80, 100 g/L). Each treatment was applied by using 1000µL micropipette. All petri dishes were tightly sealed with parafilm to avoid contamination and evaporation of water, then the setup was kept in at room temperature in the laboratory with 12 hours’ photoperiod for 14 days [15]. Seeds were considered germinated when attained a length of. At the end of the incubation period, the emergence of germination seeds, shoot fresh weight and radicle length was measured and recorded. The data was expressed as a percentage of control.

2.4  **Seedling growth test**
A total of 60 seeds of bioassay species of *H. verticillata* were each single sowed in paper cups with a diameter of 7cm. A three-quarter of paper cups was filled with 100g of topsoil [15]. The seeds were allowed to germinate and grow for about 1 week old and transplanted when the plant has 3 to 4 leaves [2]. The weed seedlings were allowed to acclimatize for 2 days [16] before treatment application [. Then, the soil surface for each cup was treated with different concentrations of aqueous curry leaves extract at 0, 50, 100, 150, 200 and 250g/L by using a 1000µL micropipette with spraying volume of 450L/ha (0.75ml/cup) [12]. The seedlings were placed under nursery condition at the temperature range of 27°C to 30°C and relative humidity of 78-80% [16]. After one month of treatment application, the shoot fresh weight, shoot height, and root length was measured. All data were expressed as the percentage of control to determine the level of inhibition on weed seed after treatments application.

2.5  **Statistical Analysis**
The experiment was arranged in completely randomized design (CRD) with three replications. All data were subjected to one-way analysis of variance (ANOVA) using SPSS version 20. The Tukey HSD was used to compare the mean among the treatments. Differences were regarded as significant when the p-values were less than 0.05 (P < 0.05).

3.  **Results and Discussion**

3.1  **Pre-emergence application of aqueous *M. koenigii* leaves extract on *H. verticillata* under laboratory condition.

3.1.1.  **Seed emergence.** The mean value of seed emergence (% of control) of *H. verticillata* after treated with aqueous *M. koenigii* leaves extract are shown in Figure 1A. *H. verticillata* was found to be very sensitive to aqueous curry leaves extract at 40 to 100 g/L concentration. The inhibitory effect of aqueous curry leaves extract at these range of concentration were markedly stronger (P<0.005) as compared to control and 20g/L concentration where it inhibits the seed germination by >95%. However, there was no significant reduction among treatments from 40g/L to 100g/L of aqueous *M. koenigii* leaves extract. It is interesting to note that the seed germination of bioassay species was inhibited by 50% at a low concentration of 20g/L when treated as pre-emergence. This result was similar to a previous study conducted by Maharjan et al. [17] where seed germination of *Brassica campestris* inhibited by 50% at a low concentration of 2% of aqueous *Parthenium hysterophorus* L. leaves extract. According to Fabrizio et al. [18], the phytotoxicity of Mediterranean plants extracts of *Calamintha nepeta*, *Euphorbia rigida* and *Hypericum hircinum* completely inhibit the seed germination of lettuce at 25% concentration. Similar inhibition effects were observed when the extract was applied at 55%-75% concentration on *Chenopodium album*, *Sinapis alba*, and *Echinochloa crus-galli*. The previous study conducted by Aurelio et al. [19] reported that aqueous leaf extracts of *Cynara cardunculus* L. at 40% and 80% significantly reduce seed germination of *Amaranthus retroflexus* (58.1%), *Diplotaxis erucoides* (43.9%) and *Portulaca oleracea* (42.5%). Asif et al. [20]
also reported that at 25% of aqueous *Jatropha curcas* extract, the percentage of seed germination and germination index of *P. hysterophorus* L. significantly decrease in response to higher concentration.

### 3.1.2 Shoot fresh weight

The effect of aqueous *M. koenigii* leaves extract on the shoot fresh weight of bioassay species are shown in Figure 1B. Shoot fresh weight of *H. verticillata* was greatly reduced when the concentration of aqueous curry leaves extracts increased started from 60-100g/L concentration. At these concentration, *H. verticillata* was significantly inhibited by almost 100%. This results was in line with the studies conducted by Maharjan et al. [17] and Han et al. [21] where the degree of phytotoxicity of ginger (*Zingiber officinale*) and *Parthenium hysterophorus* extracts increased with incremental of extract concentration. In addition, Risalini [22] reported that neem extract at highest concentration of 10% significantly suppressed the shoot fresh weight of *Borreria latifolia* and *Eleusine indica* by almost 90%.

### 3.1.3 Radicle length

Radicle length of *H. verticillata* are very susceptible to aqueous *M. koenigii* leaves extract under laboratory condition (Figure 1C). Similar to seed germination, it was found that the radicle length of bioassay species was strongly inhibited at 40-100g/L concentration with 77-97% of inhibition as compared to control and 20g/L concentration. Besides, as the concentration increased, the radicle length of *H. verticillata* seemed stunted and turned brown. According to a study conducted by Sang-Uk, et al. [23], root systems especially root tips of alfalfa (*Medicago sativa* L.) were stunted and swollen after treated with the aqueous alfalfa leaf extracts at 30 g/L and coumarin at 10−3 M. Chuah et al. [24] reported that *Chrysopogon serrulatus* extract was strongly suppressed the radicle growth of *Asystasia intrusa* by >85% at low concentration of 0.5%. The previous study conducted by Norhafizah et al. [15] found that the radicle length of *H. verticillata* was inhibited by 50% at a concentration ranging from 3 to 14g/L aqueous root extract of *Pennisetum purpureum*.

### 3.2 Post-emergence of aqueous *M. koenigii* leaves extract on *H. verticillata* under nursery condition

#### 3.2.1 Shoot fresh weight

The effects of aqueous *M. koenigii* leaves extract on the shoot fresh weight of bioassay species under nursery condition were shown in Figure 1D. The mean value of shoot fresh weight (% of control) shows a reducing trend as the concentration of aqueous curry leaves extract increased. At the highest concentration of 250g/L, the shoot fresh of *H. verticillata* was significantly inhibited by 70% compared to control and 50g/L concentration. Similarly, previous study conducted by Arshad et al. [25] reported that it was 46 to 67% reduction in shoot and root biomass of *P. hysterophorus* seedlings after treated with 100% concentration of aqueous *Alstonia scholaris* extract by foliar spraying. In addition, Khaliq et al. [26] observed that aqueous *Eucalyptus globulus* extract was more effective in retarding the dry matter accumulation of horse purslane (*Triantehma portulacastrum* L.) and jungle rice (*Echinochloa colona* (L.) Link) seedlings by 49% and 59%, respectively.

#### 3.2.2 Shoot height and root length

A similar trend also was obtained in terms of shoot height and root length of *H. verticillata* where there was no significant reduction on these two parameters across all extract concentrations (Figure 1E and 1F). In this study, the shoot height was reduced by 5 - 8% across all extract concentration. Norhafizah et al. [15] also observed the similar pattern where the shoot height of *H. verticillata* was insignificant decreased when the concentration of *P. purpureum* extract increased. They found that at highest concentration of 150g/L the shoot height of the weed was only reduced by 20%. However, Khaliq et al. [26] reported that shoot length of flat sedge (*Cyperus eragrostis*) was suppressed by 53% when treated as post-emergence with sorghum (*Sorghum bicolor*) aqueous extract. In comparison with the synthetic herbicides (Buctril-Super® and Chwastox®), the sunflower (*Helianthus annuus*) aqueous extract failed to kill 100% of *Rumex dentatus*, but the highest tested extract concentration completely overcame weed crop competition by reducing the weed
biomass and increasing wheat yield significantly [27]. However, according to Dilipkumar et al. [28], the emergence and seedling growth of barnyardgrass were stimulated by 5 to 50% with increased of sunflower leaf extracts rates from 25 to 100% (w/v) in both soil series (Marang series and Seberang series). In this study, less inhibition level of aqueous M. koenigii leaves extract might be due to soil condition and reaction of phytotoxic compounds. Nonetheless, soil possesses the ability to detoxify allelochemicals, so the bioassays under controlled conditions in the absence of soil might be misleading due to an overestimation of the allelopathic potential [29].

4. Conclusion

It can be concluded that aqueous M. koenigii leaves extract has the potential to inhibit the germination and growth of H. verticillata. From the results obtained, the optimal concentration of aqueous M. koenigii leaves extract for weed inhibition was 100g/L for laboratory condition while 250g/L for nursery condition. The aqueous M. koenigii leaves extract possesses a significant inhibition of seed emergence, shoot fresh weight and radicle length of H. verticillata at 100g/L concentration where it inhibits the weed at the vegetative growth by almost 100% when applied as pre-emergence. Conversely, there is insignificant reduction on the weed growth when treated onto soil surface as post-emergence, suggesting that the aqueous M. koenigii leaves extract was more suitable to be applied as a pre-emergence natural herbicide for weed control.

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Figure 1. Effect of aqueous *M. koenigii* leaves extract on the emergence (A), shoot fresh weight (B) and radicle length (C) of *H. verticillata* under laboratory condition; and shoot fresh weight (D), shoot height (E) and root length (F) of *H. verticillata* under nursery condition. Data are the means of three independent replicates with standard deviation shown by vertical bars. Means with different letter(s) indicate significant difference by Tukey’s test at $P \leq 0.05$. 