The Response of Seed Germination and Seedling Growth of Woody Borreria (*Hedyotis verticillata* Lam.) Towards Aqueous Curry (*Murraya koenigii*) Leaves Extract

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DECLARATION

I hereby declare that the work embodied in this thesis report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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ABSTRACT

The chemical herbicide is favorably used in a massive field of the agriculture industry in Malaysia but in contrast, it gives few negatives side impact to environment and ecosystem. It has diverse immense consequences on the human being, soil, groundwater, disturbing conservation of ecosystem especially impact to nature. Allelopathy approach of potential crop is a suitable alternative that should be explored to have sustainable weed management. The present study is to determine the phytotoxic effect of aqueous curry (*Murraya koenigii*) leaves extract on the emergence and seedling growth of the bioassay species, woody borreria (*Hedyotis verticillata*) 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 optimal concentration for shoot fresh weight, shoot height and root length is at 250g/L of aqueous curry leaves extract with 21% inhibition level under nursery condition. Conversely, there is an only slight reduction in shoot fresh weight, shoot height and root length of *H. verticillata* when treated at soil surface as post-emergence under nursery condition. The results obtained might vary between laboratory and nursery as soil media tend to have a flexible chemical reaction in response to aqueous curry leaves extract. The inhibition level increase with incremental of extract concentration as the probable reason for inhibitory activity of aqueous curry leaves extract may be due to the presence of allelochemicals. These results suggest that curry leaves can be a good source to produce natural herbicide for weed management while it is also an eco-friendly compound for the environment and human being.

Keywords: Curry leaves, allelopathy, woody borreria, phytotoxic effect, aqueous extract.

Tindak balas percambahan benih dan pertumbuhan anak pokok woody borreria (*Hedyotis verticillata*) terhadap ekstrak akueus daun kari (*Murraya koenigii*)

ABSTRAK

Herbisid kimia selalu digunakan dengan baik dalam dunia industri pertanian di Malaysia tetapi sebaliknya, ia memberi kesan negatif terhadap alam sekitar dan ekosistem. Terdapat akibat yang mendalam kepada manusia, tanah, air bawah tanah, konservasi ekosistem terutama sekali kepada alam semula jadi. Pendekatan alelopati melalui tanaman yang berpotensi adalah alternatif yang sesuai yang perlu diterokai untuk mencapai pengurusan rumpai yang mapan. Kajian ini adalah untuk menentukan kesan fitotoksik ekstrak akueus daun kari terhadap percambahan dan pertumbuhan anak pokok spesis bioasai, woody borreria (*Hedyotis verticillata*) di dalam makmal dan keadaan nurseri. Biji benih dan anak pokok spesis bioasai telah dirawat dengan ekstrak akueus daun kari pada kepekatan yang berbeza iaitu pada 0, 20, 40, 60, 80 dan 100g/L (makmal) dan 0, 50, 100, 150, 200 dan 250g/L (nurseri). Keputusan menunjukkan ekstrak akueus daun kari mempunyai perencatan yang signifikan terhadap percambahan benih, berat bersih dan panjang radikal *H. verticillata* pada kepekatan 100g / L, dimana dikurangkan hampir 100% apabila digunakan sebagai pra-cambah di bawah keadaan makmal. Sementara itu, kepekatan optimum untuk merencatkan berat bersih, tinggi pucuk dan panjang akar adalah pada 250g/L ekstrak akueus daun kari dengan 21% perencatan di bawah keadaan nurseri. Sebaliknya, terdapat sedikit pengurangan pada berat bersih, ketinggian pucuk dan panjang akar *H. verticillata* apabila dirawat di permukaan tanah sebagai pasca-cambah di bawah keadaan nurseri. Keputusan yang diperolehi adalah berbeza antara makmal dan nurseri kerana media tanah cenderung mengalami reaksi kimia yang fleksibel sebagai tindak balas terhadap ekstrak akueus daun kari. Peningkatan perencatan ini seiring dengan peningkatan kepekatan ekstrak daun kari yang mungkin disebabkan oleh kehadiran alelokimia. Hasil ini mencadangkan bahawa daun kari boleh menjadi sumber yang baik untuk menghasilkan herbisid semulajadi untuk pengurusan rumpai manakala ia juga merupakan sebatian mesra alam untuk alam sekitar dan manusia.

Kata kunci: Daun kari, alelopati, woody borreria, kesan fitotoksik, ekstrak akueus

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LIST OF ABBREVIATIONS

N	North
E	East
CRD	Completely Randomized Design
ANNOVA	Analysis of variance
SPSS	Statistical Product and Service Solution
HSD	Honest Significance Difference
df	Degree of freedom
F	F-test
Sig.	Significant
SD	Standard deviation

LIST OF SYMBOLS

=	Equals
≠	Not equal to
<	Less than
>	More than
±	Plus Minus
%	Percentage
°	Degree
°C	Degree Celsius
rpm	Revolutions per minute
mL	Milliliter
g	Gram
cm	Centimeter
mg	Milligram
/	Per
µL	Micro liter
g/L	Gram per liter

CHAPTER 1

INTRODUCTION

1.1 Research Background

H. verticillata is one of the noxious perennial weeds used to be a nuisance to farmers and likely to build herbicide resistant significantly infesting oil palm and rubber plantation. This scenario happens when the particular herbicide is applied continuously for at least three to five years contribute to herbicide resistant to occur in certain sensitive species (Manaco, Weller & Ashton, 2002). According to Ong and Teo (1990), 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 (Jacob, Raju & Radhakrishna, 2018). It is also found to developed resistance biotype which multiple-resistance towards both glyphosate and paraquat (Chuah, Noor-zalila, Cha & Ismail, 2005)

Agriculture development is one of the significant sectors that contribute to nation's economy while herbicide becomes the main target of usage and highly reliant when dealing with weed management in Malaysia which accounted for 83% of the total pesticide usage in the year 2014 (Dilipkumar, Chuah, Goh & Ismail, 2017).

Glufosinate-ammonium, paraquat, metsulfuron-methyl, and glyphosate is the most commonly used herbicides in Malaysia (Kuntom, Tan, Kamaruddin & Yeoh, 2007; Chuah et al., 2005). According to Taylor, Klaine, Carvalho, and Barcelo (2003), 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 (Taylor et al., 2003). The side effect of chemical herbicide will also deteriorate the quality of life.

Hence, the discovery of strong allelopathic properties in some potential crops has become an interesting component of natural herbicides development (Batish, Lavanya, Singh & Kohli, 2007). Isolation of several phytotoxic substances from plant tissue may cause suppression of germination and also become a growth inhibitor (Turk & Tawaha, 2003; Soyler, Canihos, Temel & Hajyzadeh, 2012). Identification of allelopathic plants for selection with biological active natural products is commonly a useful approach (Duke, Dayan, Romagni & Rimando, 2000). It is a source of interest for herbicidal compounds approach nowadays which likely to identify phytotoxic activity in potential plants (Ma, Wu, Bai, Zhou, Yuan & Hou, 2011).

Previous studies are much more exploring on the aqueous extract of curry leaves on medicinal, nutraceutical properties, cosmetic uses and also as insecticide purposes. India, Sri Lanka, Bangladesh, and the Andaman Islands are places which native to the curry leaf tree (Suman, Omreb & Sandhya, 2014). A comparative study was conducted under laboratory conditions to determine the phytotoxic effect on mustard (*Sinapis alba*) seed germination and seedlings growth using aqueous sunflower extract is typically tested with series of concentration (2.5, 5 and 10%) as treatments (Bogatek, Gniazdowska, Zakrzewska, Oracz, & Gawronski, 2006). Therefore, the potential of the phytotoxic effect of aqueous curry leave extract was studied on seed germination and growth of *H. verticillata* weed as a natural herbicide.

1.2 Problem Statement

Agrochemical application of agriculture field in Malaysia has diverse immense consequences on the human being, soil, groundwater, conservation of the ecosystem and especially to nature. The herbicide is commonly used to control weeds which will give direct impacts or indirect impacts to human health especially farmers thus deteriorate quality of life. Weed resistance also develops by continued use of the same herbicide in the field likely happens to *H. verticillata* which usually infest oil palm and rubber plantation. Weed species tend to experience the evolution of herbicide resistance due to continuous use of herbicides. Henceforward, to deal with bad effects caused by chemical herbicide, plant extract was used as an herbicide (Kakati & Baruah, 2013). Therefore, this study is an approach to explore the potential of phytotoxic effect of aqueous curry leaves extract on seed germination and seedling growth of *H. verticillata* as a natural herbicide.

1.3 Hypothesis

H_O : Aqueous curry leaves extract cannot inhibit the seed germination and seedling growth of *H. verticillata* .

H_1 : Aqueous curry leaves extract can inhibit the seed germination and seedling growth of *H. verticillata* .

1.4 Objective

To determine the phytotoxic effect of aqueous curry leaves extract on seed germination and seedling growth of *H. verticillata* .

1.5 Scope of study

This study was conducted to investigate the effect of aqueous curry leaves extract with the different concentration on seed germination and seedling growth of *H. verticillata*. The treatment of aqueous curry leaves extract was applied as a pre-emergence application (laboratory) and post-emergence application (nursery) on the bioassay species.

1.6 Significance of study

The findings of this study would contribute rebound of benefit to the environment and society considering that natural herbicide helps in controlling weeds resistance. The development of the agricultural industry need for more effective natural friendly alternative approaches in controlling weeds. It will be an alternative to chemical weed control by utilizing allelopathic activity in aqueous curry leaves extract on *H. verticillata*. Allelochemicals compounds are found to have low phytotoxic residue amounts in water and soil which facilitate wastewater treatment and recycling management either stimulate or inhibit plant germination and growth without affecting the development of crops (Macias, Marin, Oliveros-Batidas, Varela, Simonet, Carrera & Molinillo, 2003; Zeng, Malik & Luo, 2008). There are no residual or toxic effects of allelochemicals, so it will become a good substitute for synthetic herbicides, although the efficacy and specificity of many allelochemicals are limited (Bhadoria, 2011). Allelochemical can potentially use as growth regulators, herbicides, insecticides, and anti-microbial crop protection products. Thus, the potential crop was assayed for their allelopathic activity and as the potential source of new natural herbicide for weed control.

1.7 Limitation of study

The study only focuses on the effect of aqueous curry leaves extract towards physical growth of *H. verticillata*. However, the fundamentals of the chemical reaction of the inhibition process are not included in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Woody borreria (*Hedyotis verticillata* Lam.)

H. verticillata Lam., basically known as woody borreria belongs to the Rubiaceae family which from the category of broadleaf weed. It has found to become multi-resistance toward chemical herbicide infesting rubber and oil palm plantation. *H. verticillata* becomes progressively significant and used to build resistant trait toward paraquat and glyphosate herbicide. This weed found to build up multi-resistant towards glyphosate and paraquat in oil palm plantations of FELCRA at Bukit Kapah, FELDA Belara and also FELCRA Bukit Sudu (Chuah et al., 2005). It is a dicot perennial weed that contains the chemical compound of kaempferitrin the 3, 7-dirhamnoside of kaempferol isolated from the leaves. (Ahmad, Lajis & Sargent, 1994). In addition, traditional herbal medicine also uses Kaempferitrin that acts as a naturally occurring kaempferol glycoside which can be isolated from the leaves of various plants (Tatsimo, Tamokou, Havyarimana, Csupor, Forgo, Hohmann, Kuiate & Tane, 2012). Ong and Teo (1990), mention that *H. verticillata* becomes more difficult to control once it matures and stems turn woody (Appendix A: Figure A.1).

2.2.1 Origin and habitat

This weed is geographically distributed from India to the Himalayas and southern China, the Andaman and Nicobar Islands and throughout Southeast Asia (Djaja, 2002). *Hedyotis* is native to tropical and subtropical Asia and to islands of the northwest Pacific (David & Mabberley, 2008). *H. verticillata* usually grows in dry, less fertile with open localities such, in old clearings, teak forest, and thickets, on sandy soils from sea-level up to 1600 m altitude which experience lightly shaded to sunny weather (Grierson & Long, 1987).

2.2.2 Scientific classification

Table 2.2: A scientific classification of *H. verticillata* (Wikipedia, 2018).

Hedyotis verticillata .	
Kingdom	Plantae
(unranked)	Angiosperm
(unranked)	Eudicots
(unranked)	Asterids
Order	Gentianales
Family	Rubiaceae
Subfamily	Rubioideae
Tribe	Spermacoceae
Genus	Hedyotis
Species	*H. verticillata*
Binomial name	*H. verticillata* . (L.) Lam.

2.2.3 Morphology

It has a stout taproot, hispid or smooth that may develop to about 15cm to 100cm tall. It has a size measure of 20-55 mm x 1.5-10mm, oblong to linear-lance shaped, pointed at both ends, scabrid, sessile and firm of elliptical lamina leaves (Grierson & Long, 1987). It is rarely solitary for the flowers as it is in sessile axillary verticillasters meanwhile the cyme is axillary. It is hairless or pubescent of the morphology of the sepal tube with triangular lobes located at the apex where corolla is shortly campanulate. It almost reaching tips of corolla lobes for filaments long anthers whereas stigma slightly overlapping anthers shortly bifid. The capsule is ovoid, hispid and loculicidal on crown only with 2mm length (Grierson & Long, 1987). The stand out character owned by this most Asian weed species is, it has diplophragmous capsules with erect, robust or shrubs (Xing, Rui-Jiang, Mark, Paul, & Jing, 2013).

2.2 Weed management

Weed management is essential for agricultural crops that have significant value contributes majorly in the industry with their economic value such rubber and oil palm. According to Kuan, Ann, Ismail, Leng, Fee, and Hashim (1991), 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). During the first two years of rubber planting, about 24-70% field maintenance expenses required for weed control but cost implementation depends on the type of rubber plantation (Chee, 1989). Hence, the approach chosen by local farmers is to use chemical herbicides which already commercialize in the market but unfortunately, there are various effects due to deposition of residue in soil and also in crops.

2.2.1 High dependency on herbicide

High dependency on chemical herbicides is catalyzed by the availability of various product formulations in the market, encouragement of policy outlines, easy to handle, affordable price and high efficiency in giving fast response controlling weeds of the target area which favored by most of the farmers as a preference. It is crucial that farmers or applicators need to have basic knowledge about herbicide application, the product usage and the proper equipment methods. According to Christoffoleti and López-Ovejero (2004), physicochemical characteristics and dose of the product, the weed species to be controlled and the phenological stage of weed and crop are dependable factors to have herbicide efficacy. Basic important knowledge when choosing herbicide rates is to understand the herbicide efficacy on weed species over time in order to obtain the desired residual effect, in case of chemical management in pre-emergence (Carvalho, Lombardi, Marcelo, López-Ovejero, Pedro & Medeiros, 2005). According to FAOSTAT (2017), about 39,407 and 49,199 tons of active pesticide ingredients were used in Malaysia during 2006 and 2014, respectively. Among the agricultural chemicals, a large percentage (83%) of herbicide usage was reported during 2014 (FAOSTAT, 2017). Synthetic chemical herbicide likely to devoted significant weed control in the plantation where the cost of management could be minimized in comparison to hand weeding which requires greater cost investment for numerous labors, energy and time. For example, Class I herbicides, such as paraquat, are recommended to be reduced or eliminated in oil palm plantations under the stewardship program of the Roundtable on Sustainable Oil palm (RSPO) (Rutherford, Flood & Sastroutomo, 2011), although a study initiated by the Malaysia Oil Palm Board suggested that the agricultural sector is still required to use paraquat for effective economic purposes (Wahid, Kushairi, Shariff, Kuntom, Kamarudin, & Mohamed, 2011).

2.2.2 Effect of chemical herbicide

It is an unavoidable phenomenon over the agricultural world includes in Malaysia where one of the current problems is the herbicide resistance within weed species. According to Heap (1997), herbicide resistance is defined as the naturally occurring inheritable ability of certain weed biotypes within a population to survive herbicide treatment result in ineffective control of the weed population. Chuah and Ismail (2010) mention that the resistant biotypes may evolve eventually because of selection pressure of the herbicide. Effect of chemical herbicide also become a concerning issue to human health such as obesity, cancer, endocrine disruption and other diseases closely related to exposure of synthetic chemicals and pesticides. (Gorell, Johnson, Rybicki, Peterson & Richardson, 1998; Bassil, Vakil, Sanborn, Cole, Kaur, & Kerr, 2007; George & Shukla, 2011; Mrema, Rubino, Mandic-Rajcevic, Stturchio & Turci, Osculati, Brambilla, Minoia & Colosio, 2013; Araújo, Delgado, Paumgartten, 2016; WHO, 2017). In fact, it present direct impacts of herbicide residues toward soil microorganisms where studies shown that there are ultimate affects with the recalcitrant like lignin, cellulose, the rates of decomposing labile, respectively in diversity of ecosystem (Tripathi & Singh, 1992; Pandey, Sharma, Tripathi & Singh, 2007; Osono, Iwamoto & Trofymow, 2008).

2.3 Allelopathy and plant extract

It is one of the possible alternatives using allelopathy which exhibit through the use of plant extracted natural substances for attaining sustainable weed management. Natural allelopathic interactions may be directly utilized from allelochemicals usage as natural herbicides for controlling weeds (Singh, Daizy & Kohli, 2003). The element of phytotoxic substances which have the potential to inhibit germination or growth for certain target weeds need to be isolated from plant tissues (Turk & Tawaha, 2003; Soyler et al., 2012). Significant approach to identify plants with phytotoxicity assay may become herbicidal compounds of field interest (Ma et al., 2011). According to Fang and Zhihui (2015), biochemicals which known as allelochemicals produce by organisms could influence survival, growth, reproduction, and development of other organisms called as a usual biological phenomenon is a definition of allelopathy. Allelochemicals is the release of photo chemicals either give beneficial or harmful effects of one plant to another plant (Gupta, Thakur & Das, 2007). The previous study has discovered the potential of Napier grass as an insecticide against stem borer, *Chilo partellus* and sorghum in the "push-pull" system (Khan, Midega, Hutter, Wilkins & Wadham, 2006). It is a similar discovery of potential inhibition on Striga germination tested with plant water extract which may have allelopathic effects suppress on Striga germination (Ma, Cheng, Inanaga & Shui, 2004). Nazir, Uniyal, and Todaria (2006) assessed that the allelopathic ability against few traditional crops inside 3 different herbal species (*Rheum emodi, Saussaurea lappa,* and *Potentilla fulgens*) where result showed that germination of all crops was decreased expressively by aqueous extract of *S. lappa* and *P. fulgens*. Several indirect impacts on the rates of photosynthesis and transpiration (Maroua, Neziha & Rabiaa, 2013) while decrease the respiration and uncoupling oxidative phosphorylation were due to allelochemical affect the stomatal conductance by inducing ABA production. In addition, physiological alteration of plant growth pattern is interfered by the action of groups of allelochemicals causing typical allelopathic inhibitory effects (Einhellig, 1996; Parvez, Parvez, Fujii & Gemma, 2004; Kil & Shim, 2006). All functions in the plant such as photosynthesis, respiration, transpiration, resistance, and growth are affected by the presence of allelochemicals (Rice, 1984; Saxena, Sharma, Kumar, Sand & Rao, 2004). Iqbal, Cheema, and Mushtaq (2009) reported that water extracts of sorghum, sunflower, and rapeseed (*Brassica napus* L.) were used with reduced of glyphosate dosage conducted for two field studies to control purple nutsedge (*Cyperus rotundus* L) in cotton. Water extracts have been used as potential herbicides in mixture with the reduced dose of chemical herbicides (Cheema, Farooq & Khaliq, 2012). The previous study showed that allelopathic water extracts such Sorghum, sunflower and eucalyptus have reduced herbicide doses by the half of standard giving effective control over noxious weeds of major crops (Cheema, Khaliq & Mubeen 2003). The production of commercial botanical herbicide could be exploited from harmful impacts of allelopathy (Sodaeizadeh, Jaroslav, & Patrick, 2009; Sher, Aldosari, Ali & Boer, 2014). Phenolic compounds have a significant allelopathic potential for application in agriculture as herbicides, fungicides, and insecticides (Santana, Ferrera, Padrón & Rodríguez, 2009; Hussain, Barkatullah & Ahmad, 2014). Inhibition of shoot or root growth and nutrient uptake, or disturbance in symbiotic relationship may occur thereby destroying the plants' usable source of nutrients due to release of allelochemicals from plants (Conn, 1980; Khan, Marwat, Gul, & Zahid, 2005; Hassan, Muhammad, Khan, Anwar & Saima, 2008; Safdar, Tanveer, Khaliq & Naeem, 2014). Utilization of allelopathic properties of allelopathic plant species offers promising opportunities for sustainable weed management (Lorenzo, Efthymios, & María, 2013).

2.4 Curry leaves (*Murraya koeniigi*)

Murraya koenigii is commonly known as curry leaves belong to Rutaceae family and also known as "Meetha neem". *Murraya koenigii* Spreng is one of the only species which is available in India together with *Murraya paniculata* (Linn) among the other fourteen global species (Nayak, Mandal, Banerji & Banerji, 2010). According to Prasan (2012), since many centuries, curry leaf is used in Ayurvedic medicine known as a small tree of aromatic shrub with 6-meter height.

2.4.1 Origin and habitat

Curry leaf trees are geographically native distributed from India, Myanmar, Southern China, Indo-China, Hainan and Sri Lanka (Perera & Dahayake, 2015). Curry leaf trees are naturalized in forests and wasteland throughout the Indian subcontinent except in the higher parts of the Himalayas and from the Ravi River in Pakistan, its distribution extends eastwards towards Assam in India and Chittagong in Bangladesh, and southwards to Tamil Nadu in India (Sunita, 2018). According to Muthulinggam and Partiban (2015), the plant prefers to germinate seeds without restraint under partial shade or shade and most be found in the moist forest of 500-1600 meters' height. South Asian immigrants are responsible for the plants spread to Malaysia, South Africa and Réunion Island. (Singh, Omreb & Mohan, 2014)

2.4.2 Scientific classification

Table 2.4: A scientific classification of *M. koenigii* (Suman et al.,2014)

Murrayya koenigii	
Kingdom Plantae	Plants
Subkingdom Tracheobionta	Vascular plants
Superdivision Spermatophyta	Seed plants
Division Magnoliophyta	Flowering plants
Class Magnoliopsida	Dicotyledons
Subclass Rosidae	Order Sapindales
Family Rutaceae	Rue family
Genus Murraya J. Koenig ex L.	murraya
Species *M. koeniigi* (L.) Spreng.	curry leaf tree

2.4.3 Morphology

Curry leaf tree is a small spreading shrub which in average 2.5m high with dark green to brownish main stem color of 16cm for girth (Suman et al., 2014). The leaves have reticulate venation with 30cm long where each bearing 24 leaflets with slightly pungent smell, bitter and feebly acidic taste while the flower is bisexual having sweet aromatic of the white funnel-shaped criteria (Muthulinggam and Partiban, 2015). Perera and Dahanayake (2015) reported that fruit of curry plant is ovoid to subglobose, wrinkled or rough with glands and it is biseeded and gets purple purplish black color when ripen.

2.4.4 Uses

This indigenous plant belongs to Rutaceae family and also have a potential source of natural essential ingredient oil to ward off insects (Deepti & Nupur, 2013) such as cockroaches, mosquitoes, and others. The extracts of *M. koenigii*, *Coriandrum sativum*, *Ferula asafetida*, and *Trigonella foenumgraceum* were found to be effective and showed encouraging results against *Aedes aegypti* (Gauri, & Vijayalaxmi, 2004) and *Culex* mosquito larvae (Desai, 2002). It is usually used in cooking to boost the flavor and aroma of the food. *M. koenigii* also has many properties such as antioxidant, anti-nociceptive, lipid-lowering, helps in Alzheimer's disease, miscellaneous, gastrointestinal disorders, anti-diabetic, anti-cancer, anti-bacterial and hepatoprotective (Prasan, 2012). *M. koenigii* leaves contain murrayastine, pyrayafoline carbazole alkaloids and murrayaline. (Bhandari, 2012). Cinnamaldehyde, (Gupta & Prakash, 2009) and numerous carbazole alkaloids, including mahanimbine (Elina, Arnab, Debosree, Debasri, Aindrila, Santanu, Sanjib and Debasish, 2012) and girinimbine (Patil, Langade, Dighade & Hiray, 2012). There were no previous study on herbicidal activity of aqueous curry leaves to extract as natural herbicide tested on any bioassay species.

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Plant materials

Curry leaves were collected at a wasteland area from Taiping, Perak at 4.8519^{o}N, 100.7416^{o}E, and seeds of *H. verticillata* was collected at one of rubber plantation at Jeli, Kelantan at 5.7007^{o}N, 101.8432^{o} E. The seed coat of the bioassay species was removed by using sandpaper (Appendix A: Figure A.6). The seed that exhibit 80% - 90% of germination rate were used for this study.

3.1.2 Extracts preparation

The phytotoxic activity of curry leaves on *H. verticillata* was investigated using aqueous curry leaves extract. The collected curry leaves were washed with clean tap water to remove dust particles. The complete dried leaves were grinded into powder form by using a blender (Appendix A: Figure A.2). Then, 50g of the powder (Appendix A: Figure A.3) was then extracted with 100 mL of distilled water poured in conical flasks and then agitated vigorously for 24 hours at 200rpm at 25^{o}C on an orbital shaker (Yehia, 2016). The supernatant obtained was filtered using a double layer of Muslin cloth to obtain 50% stock solution. Stock solution extract of leaf powder was diluted with distilled water to obtain extract concentrations of 20, 40, 60, 80 and 100 g/L (Appendix A: Figure A.4) for seed germination tested under laboratory condition (pre-emergence) while 50, 100, 150, 200 and 250 g/L (Appendix A: Figure A.5) for seedling bioassay tested under nursery condition (post-emergence) (Aslani, Juraimi, Ahmad-Hamdani, Alam, Golestanhashemi, Omar & Hakim, 2015).

3.2 Methods

3.2.1 Seed germination test

A total of 20 weed seeds placed randomly on 90 × 15mm petri dishes that lined with two layers of filter papers and moisten with 5 mL of aqueous curry leaves extract at different concentration (0, 20, 40, 60, 80, 100 g/L) as 0 g/L or distilled water applied as the control treatment (Appendix A: Figure A.7) (Aslani et al., 2015). Each treatment was applied by using 1000µL micropipette. All petri dish were tightly parafilm to avoid contamination and kept in at room temperature in the laboratory with 12 hours photoperiod for 14 days (Norhafizah, Oh, Ismail & Chuah, 2013). Parafilm with colorless impermeable criteria was used to wrapped tightly petri dishes to avoid water losses (Digdem & Mehmet, 2008). There were 5 replicates of each treatment. Seeds were considered germinated when attained a length of 1mm (Mishra, 2011). 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.

3.2.2 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 (Appendix A: Figure A.9) (Norhafizah et al., 2013). The seeds were allowed to germinate and grow for about 1 week old (Appendix A: Figure A.10) and transplanted when the plant has 3 to 4 leaves (Chuah et al., 2005). The weed seedlings were allowed to acclimatize for 2 days (Chuah, Norhafizah & Ismail, 2015) before treatment application (Appendix A: Figure A.11) (Chahal, Aulakh, Rosenbaum & Jhala, 2015). Then, the soil surface for each cups 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) (Ong & Teo, 1990). There were 10 replicates of each treatment. The seedlings were placed under nursery condition at the temperature range of 27^{o}C day to 30^{o}C and relative humidity of 78-80% (Chuah et al., 2015). After one month of treatment application, the shoot fresh weight (Appendix A: Figure A.13), shoot height, and root length (Appendix A: Figure A.12) was measured. All data were expressed as the percentage of control to determine the level of inhibition on weed seed after treatments.

3.2.3 Statistical Analysis

Bioassay of each treatment was carried out with 5 replicates for under laboratory condition while 10 replicates under nursery condition which is arranged completely randomized design (CRD). All the data on the parameters studied were subjected to one-way analysis of variance (ANOVA) using SPSS v23 for windows and differences between means for significance under significance level of $p<0.05$ were calculated. The mean among treatments was compared by using Tukey HSD. Differences are regarded as significant when the p-values are less than 0.05 ($P < 0.05$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Pre-emergence application of aqueous curry leaves extract on *H. verticillata* under laboratory condition

4.1.1 Seed emergence

The mean value of seed emergence (% of control) of *H. verticillata* shows the significantly reduced trend as the concentration of aqueous curry leaves extract increased. *H. verticillata* was found to be very sensitive to aqueous curry leaves extract at 40-100 g/L concentration (Figure 4.1A). The inhibitory effect of aqueous curry leaves extract at 100g/L was 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 curry leaves extract (Appendix B: Table B.1). 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, Shrestha, and Pramod (2007) where seed germination of *Brassica campestris* inhibited by 50% at a low concentration of 20g/L of *Parthenium hysterophorus* L. aqueous leaves extract. According to Fabrizio, Agostino, Antonio, and Maria (2012), 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*. It was found that extracts from *Azadirachta indica* (roots), *Balanite aegyptiaca* (roots), *Parkia biglobosa* (peels), *Sclerocarya birrea, Jatropha gossypifolia* (leaves) and *Theretia neriifolia* (leaves) had caused 90%-100% reduction in *Striga* seed germination (Yonli, Traore, Sereme and Sankara, 2010). The previous study conducted by Aurelio, Alessia, Gaetano, Onofri and Giovanni (2018) 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, Faizan, Sultan, Muhammad, and Farhat (2017) also reported that at 250g/L of aqueous *Jatropha curcas* extract, the seed germination (%) and germination index (%) of *Parthenium hysterophorus* significantly decrease in response to higher concentration.

4.1.2 Shoot fresh weight

The effect of aqueous curry leaves extract on the shoot fresh weight of bioassay species are shown in Figure 4.1B. Shoot fresh weight of *H. verticillata* was greatly reduced when the concentration of aqueous curry leaves extract increased. Similarly, the degree of phytotoxicity of ginger (*Zingiber officinale*) and *P.*

hysterophorus extracts increased with incremental of extract concentration (Maharjan, Shrestha & Pramod, 2007; Han, Pan, Wu, Wang & Li, 2008). Shoot fresh weight of *H. verticillata* was significantly decreased by almost 100% at 60-100g/L concentration. The previous study conducted by Risalini (2017) reported that neem extract at 100g/L significantly suppressed the shoot fresh weight of *Borreria latifolia* and *Eleusine indica* by almost 90%. Jagtap, Tayade, and Athawale (2016) also reported that significant reduction in shoot fresh weight of cowpea, black gram, moth bean and chickpea at 10%-100% of neem extract. This result also supported by Salam and Kato-Noguchi (2010) where an increased in aqueous neem extract concentration will cause stronger inhibitory activity to bioassay species.

4.1.3 Radicle length

Radicle length of *H. verticillata* are very susceptible to aqueous curry leaves extract under laboratory condition (Figure 4.1C). 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 (Appendix A: Figure A.8). According to a study conducted by Sang-Uk, Seong-Kyu, Sunyo, Hong-Gi, Byoung-Sik, and Sun-Min (2002), root systems especially root tips of alfalfa were also stunted and swollen after treated with the aqueous alfalfa leaf extracts at 30g/L. However, Chuah, Oh, Habsah, Norhafizah, and Ismail (2014) reported that *Chrysopogon serrrulatus* 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. (2013) 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*. Alagesaboopathi and Thamilazhagan (2010) also reported that a significant decreased on germination and radicle growth of black gram (*Vigna mungo*) and green gram (*Vigna radiata*) when the bioassay species were treated with aqueous leaves and root extract of Acacia. Jadhar and Gayanar, (1992) reported that increasing concentration of *Acacia auriculiformis* leaf leachates reduces the percentage of germination of plumule, radicle length of rice and cowpea.

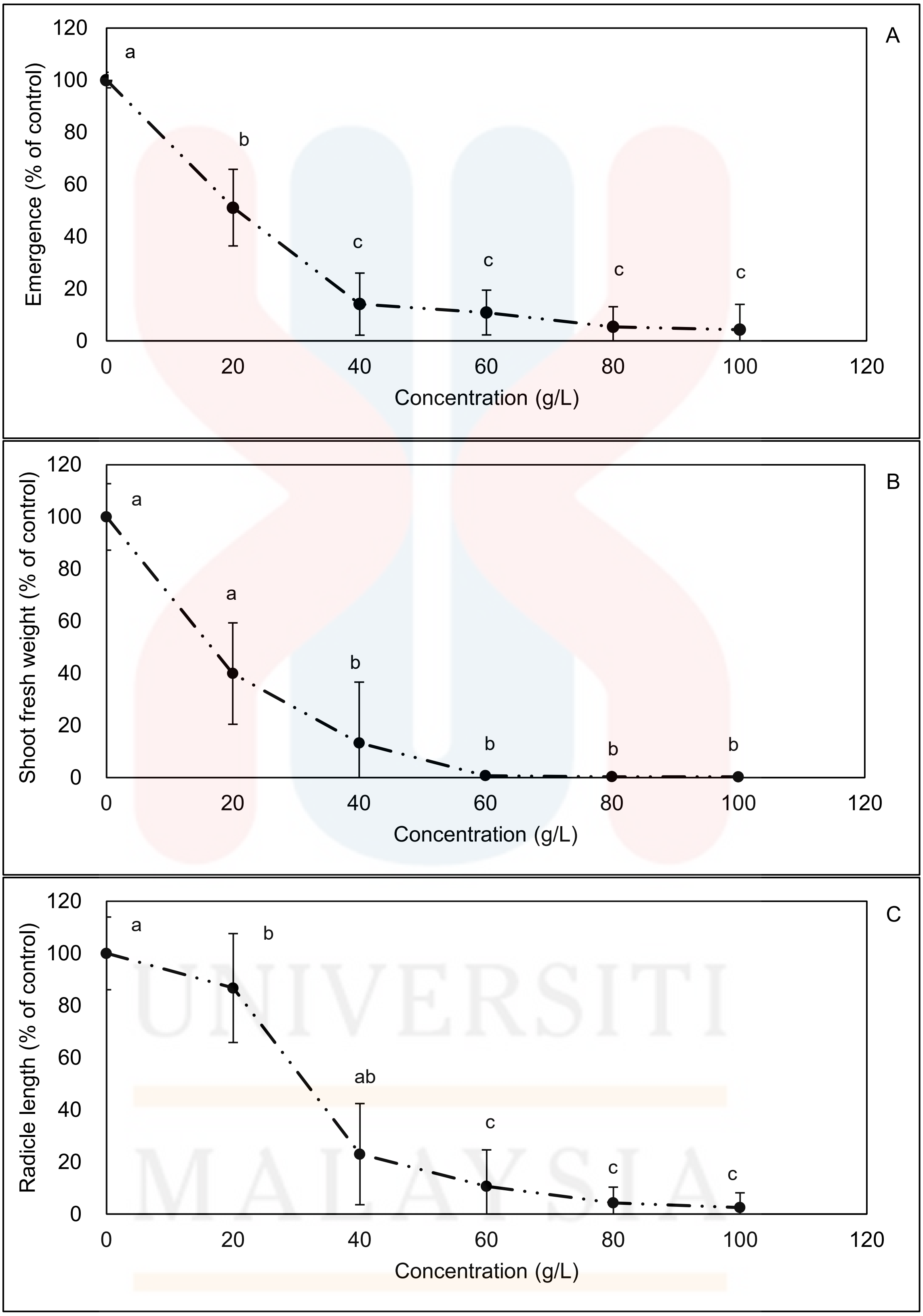

Figure 4.1. Effect of aqueous curry leaves extract on the emergence (A), shoot fresh weight (B) and radicle length (C) of *H. verticillata* under laboratory condition. Data are the means of three independent replicates with standard deviation shown by vertical bars.

4.2 Post-emergence of aqueous curry leaves extract on *H. verticillata* under nursery condition

4.2.1 Shoot fresh weight

The effects of aqueous curry leave extracts on the shoot fresh weight of bioassay species under condition were shown in Figure 4.2. 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 inhibited by 70% compared to control and 50g/L concentration (Figure 4.2A). However, the previous study conducted by Arshad, Sobiya, Rukhsana and Shazia (2010) reported that an adverse effect on *Parthenium* seedlings showed about 46 to 67% reduction in shoot and root biomass after applying 100% concentration of *Alstonia scholaris* aqueous extract by foliar spraying. The recent study showed that *Eucalyptus* aqueous extract was more effective in retarding the dry matter accumulation of horse purslane (49%) and jungle rice (59%) seedlings while winter cherry scored maximum (>60%) fresh and dry weight suppression of barnyard grass (Khaliq, Matloob & Tanveer, 2013). The result obtained was influenced by chemical interaction that occur in the soil. According to Norhafizah et al. (2013), there was phytotoxic interaction of aqueous root and clum plus leaves extract from *Pennisetum purpureum* with organic compounds or microbes available in the soil, thereby resulting in stimulatory or inhibitory effects which this response varies with bioassay species (Norhafizah et al., 2013).

4.2.2 Shoot height

A similar trend also was obtained in term of shoot height where there was no significant reduction on the shoot height of *H. verticillata* across all extract concentration. It was found that shoot height was reduced by 5 - 8% across all extract concentration. The previous study reported that shoot height of *H. verticillata* was slightly decreased when the concentration of *Pennisetum purpureum* extract increased at 150g/L, the shoot height was reduced by 20% (Norhafizah et al., 2013). However, Khaliq, Matloob, Khan, and Tanveer (2013) reported that shoot length of flat sedge was suppressed about 53% and susceptible to *Sorghum* aqueous extract at 18 L/ha spraying volume (60 mL/L of water) when applied as post-emergence. In comparison with the synthetic herbicides (Buctril-Super® and Chwastox®), the sunflower 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 (Tehmina & Rukhsana, 2007).

4.2.3 Root length

There was a declining trend represented by mean values of root length (% of control) against various concentration treatment (Figure 4.2C). However, there was no significant reduction shown among treatments seem that the root length of *H. verticillata* did not affect by different concentration of aqueous curry leaves extract (Appendix B: Table B.2). All concentrations showed the less inhibitory effect of 4% at the lowest concentration (50g/L) while 18% inhibition at the highest concentration (250g/L). However, the previous study conducted by Dilipkumar and Chuah (2013) reported that the root length of barnyard grass was decline by 74 – 84% at a concentration of 5 – 15% of the sunflower leaf extract. Less inhibition level of aqueous curry 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 (Foy, 1999; Inderjit, 2001).

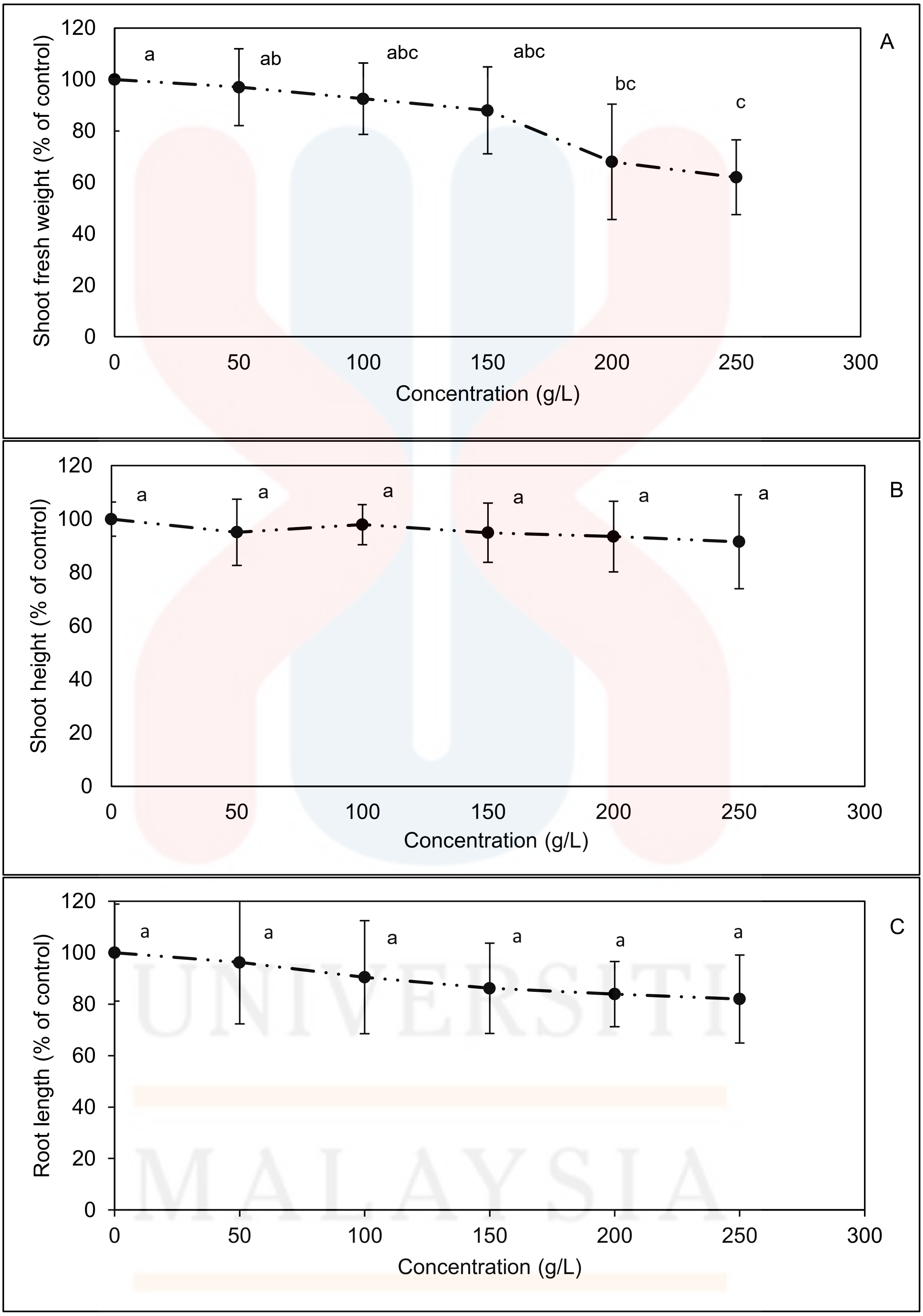

Figure 4.2. Effect of aqueous curry leaves extract on the shoot fresh weight (A), shoot height (B) and root length (C) of *H. verticillata* under nursery condition. Data are the means of three independent replicates with standard deviation shown by vertical bars.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

It can be concluded that aqueous curry leaves extract to have the potential to inhibit the germination and growth of *H. verticillata*. The objective of the study has successfully achieved. From the results obtained, the optimal concentration of aqueous curry leaves extract treatment is 100g/L for laboratory condition while 250g/L for nursery condition. The aqueous curry leaves extract possesses a significant inhibition of seed emergence, shoot fresh weight and radicle length of *H. verticillata* at 100g/L concentration, reduce by almost 100% when applied as pre-emergence under laboratory condition. Meanwhile, the effective concentration for shoot fresh weight, shoot height and root length is at 250g/L of aqueous curry leaves extract with 21% inhibition level under nursery condition. Conversely, there is only a slight reduction on shoot fresh weight, shoot height and root length of *H. verticillata* when treated at soil surface as post-emergence under nursery condition. The results obtained might vary between laboratory and nursery as soil media tend to have a flexible chemical reaction in response to aqueous curry leaves extract. The inhibition level increase with incremental of extract concentration as the probable reason for inhibitory activity of aqueous curry leaves extract may be due to the presence of allelochemicals. These results suggest that curry leaves can be a good source to produce natural herbicide for weed management while it is also an eco-friendly compound for the environment and human being.

5.2 Recommendation

This current study only focuses on the effect of aqueous curry leaves extract towards physical growth of *H. verticillata* under laboratory and nursery condition. Therefore, the fundamentals of the chemical reaction of the inhibition process need to be explored. Isolation and identification of phytochemical that present in aqueous leaves extract could be done in order to elucidate the mode of action of the identified allelochemical on selected bioassay species. This, in turn, could help in developing natural herbicide with a new mode of action that can be used in weed control.

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APPENDIX A

Figure A.1. *H. verticillata* plant

Figure A.2. Curry leaves powder

Figure A.3. Weighing curry leaves powder

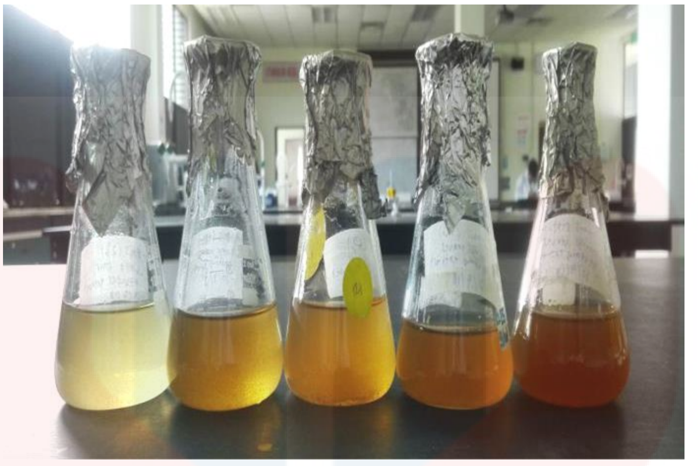

Figure A.4. Aqueous curry (*M. koenigii*) leaves extract for laboratory condition

Figure A.5. Aqueous curry (*M. koenigii*) leaves for nursery condition

Figure A.6. Scarifying weed seeds

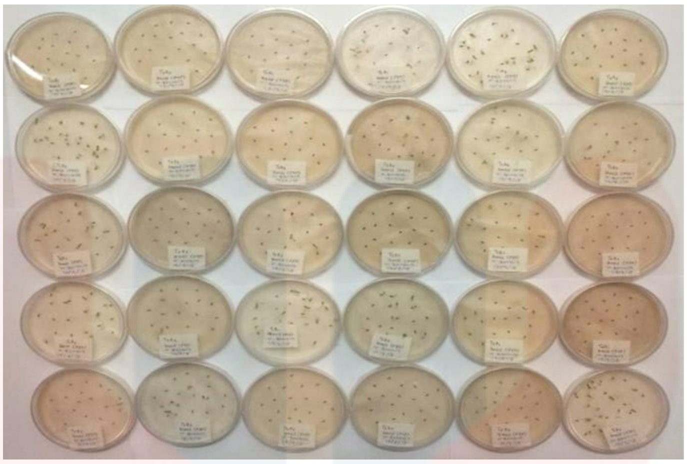

Figure A.7. Samples of weeds grown in petri dish

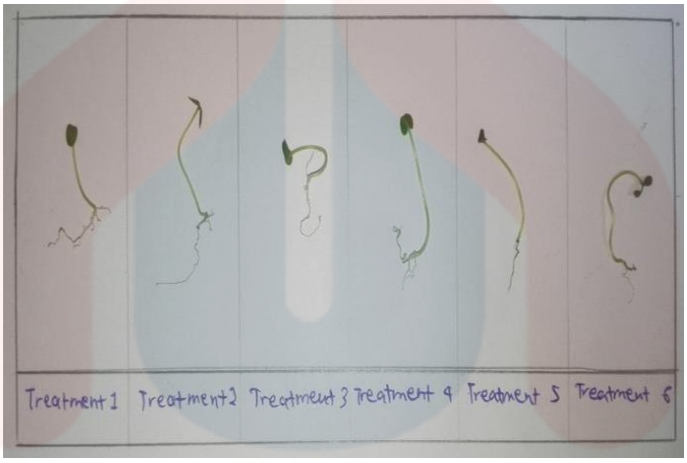

Figure A.8. Germination of *H. verticillata* under laboratory condition.

Figure A.9. Weighing top soil in paper cup

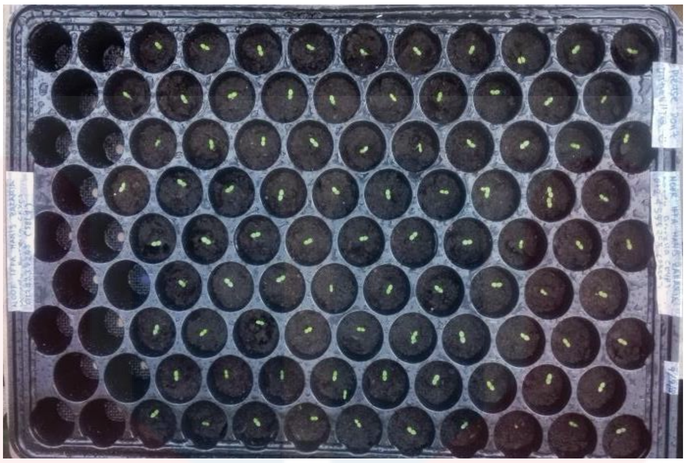

Figure A.10. Samples of weeds grown in seedling trays in nursery

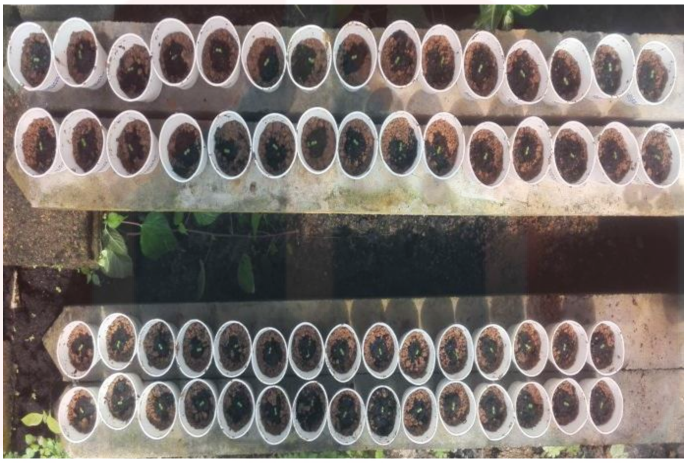

Figure A.11. Samples of weeds grown in nursery, after transplanting and application of treatment

Figure A.12. Measuring root length of *H. verticillata*

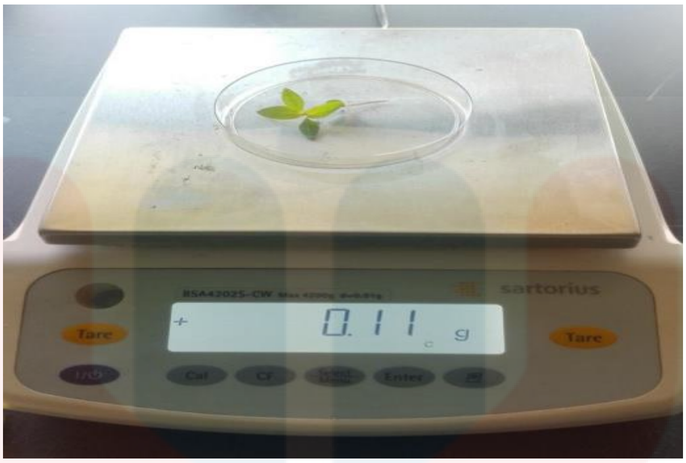

Figure A.13. Weighing shoot fresh weight of *H. verticillata*

Figure A.14. *H. verticillata* seedlings after wash with clean tap water

APPENDIX B

Table B.1. Mean value of different concentrations of aqueous curry leaves extract and control treatment on number of viable seeds in percentage (% of control), shoot fresh weight (% of control) and radicle length (% of control) of *H. verticillata* . under laboratory condition.

(mean ± standard error)

Treatments (Aqueous curry leaves extract, g/L)	Number of viable seeds (%)	Shoot fresh weight (mg/plant)	Radicle length (cm/plant)
0	100 ± 3 [a]	100 ± 13 [a]	100 ± 14 [a]
20	51 ± 15 [b]	40 ± 20 [b]	87 ± 21 [a]
40	14 ± 12 [c]	13 ± 23 [ab]	23 ± 19 [b]
60	11 ± 9 [c]	1 ± 1 [c]	11 ± 14 [b]
80	5 ± 8 [c]	0 ± 1 [c]	4 ± 6 [b]
100	4 ± 10 [c]	0 ± 1 [c]	3 ± 6 [b]

Table B.2. Mean value of different concentrations of aqueous curry leaves extract and control treatment on shoot fresh weight (% of control), shoot height (% of control) and root length (% of control) of *H. verticillata* under nursery condition.

(mean ± standard error)

Treatments (Aqueous curry leaves extract, g/L)	Shoot fresh weight (g/plant)	Shoot height (cm/plant)	Root length (cm/plant)
0	100 ± 20 [a]	100 ± 6 [a]	100 ± 19 [a]
50	97 ± 15 [ab]	95 ± 12 [a]	96 ± 24 [a]
100	93 ± 14 [abc]	98 ± 8 [a]	90 ± 22 [a]
150	88 ± 17 [abc]	95 ± 11 [a]	86 ± 17 [a]
200	76 ± 22 [bc]	94 ± 13 [a]	84 ± 13 [a]
250	69 ± 15 [c]	92 ± 18 [a]	82 ± 17 [a]

Table B.3. ANNOVA test results for seed emergence, shoot fresh weight and radicle length for *H. verticillata* under laboratory condition.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SE	Between Groups	36070.967	5	7214.193	72.262	.000
	Within Groups	2396.000	24	99.833		
	Total	38466.967	29			
SFW	Between Groups	38903.767		7780.753	42.483	.000
	Within Groups	4395.600	24	183.150		
	Total	43299.367	29			
RL	Between Groups	45422.409	5	9084.482	45.379	.000
	Within Groups	4604.350	23	200.189		
	Total	50026.759	28			

*SE = seed emergence, SFW = Shoot fresh weight, RL =Radicle length

Table B.4. Tukey HSD test for seed emergence of *H. verticillata* under laboratory condition.

Seed Emergence

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
100.00	5	4.4000		
80.00	5	5.4000		
60.00	5	10.8000		
40.00	5	14.2000		
20.00	5		51.0000	
.00	5			100.0000
Sig.		.637	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Table B.5. Tukey HSD test for shoot fresh weight of *H. verticillata* under laboratory condition.

Shoot Fresh Weight

Tukey HSD[a,b]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
100.00	5	.4000		
80.00	5	.4000		
60.00	5	.6000		
40.00	4	13.4000	13.4000	
20.00	4		39.8000	
.00	3			100.0000
Sig.		.656	.051	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Table B.6. Tukey HSD test for radicle length of *H. verticillata* under laboratory condition.

Radicle Length

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
100.00	5	2.6000	
80.00	5	4.4000	
60.00	5	10.6000	
40.00	5	23.0000	
20.00	5		86.7500
.00	5		100.0000
Sig.		.261	.998

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.800.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table B.7. ANNOVA test results for shoot fresh weight, shoot height and root length for *H. verticillata* under nursery condition.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SFW	Between Groups	7067.103	5	1413.421	4.640	.001
	Within Groups	15231.111	50	304.622		
	Total	22298.214	55			
SH	Between Groups	451.911	5	90.382	.629	.678
	Within Groups	7468.589	52	143.627		
	Total	7920.500	57			
RL	Between Groups	2109.451	5	421.890	1.169	.340
	Within Groups	15520.957	43	360.952		
	Total	17630.408	48			

* SFW = Shoot fresh weight, SH = Shoot height, RL = Root length

Table B.8. Tukey HSD test for shoot fresh weight of *H. verticillata* under nursery condition.

Shoot Fresh Weight

Tukey HSD[a,b]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
250.00	9	68.8889		
200.00	9	75.5556	75.5556	
150.00	10	88.0000	88.0000	88.0000
100.00	8	92.5000	92.5000	92.5000
50.00	10		97.0000	97.0000
.00	10			100.0000
Sig.		.057	.105	.678

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.270.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table B.9. Tukey HSD test for shoot height of *H. verticillata* under nursery condition.

Shoot Height

Tukey HSD[a,b]

Treatment	N	Subset for alpha = 0.05
		1
250.00	10	91.6000
200.00	10	93.6000
150.00	10	94.9000
100.00	9	95.1111
50.00	9	98.0000
.00	10	100.0000
Sig.		.641

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.643.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table B.10. Tukey HSD test for root length of *H. verticillata* under nursery condition.

Root Length

Tukey HSD[a,b]

Treatment	N	Subset for alpha = 0.05
		1
250.00	7	82.1429
200.00	8	83.8750
150.00	8	86.1250
100.00	8	90.3750
50.00	8	96.1250
.00	10	99.8000
Sig.		.435

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.077.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.