Allelopathic activity of Tulsi (*Ocimum sanctum L.)* and Neem (*Azadirachta indica A.Juss*) leaves extract on Goosegrass (*Eleusine indica)*

By

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Agrotechnology) with Honours

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DECLARATION

I hereby declare that the work embodied in this 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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I certify that the report of this final year project entitled "Allelopathic activity of Tulsi (*Ocimum sanctum L.)* and Neem (*Azadirachta indica A.Juss*) leaves extract on Goosegrass (*Eleusine indica*)" by RIVITRA A/P VINTISEN matric number F15A0199 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agrotechnology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Allelopathic activity of Tulsi (*Ocimum sanctum L.)* leaves and Neem (*Azadirachta indica A.Juss*) leaves extract on Goosegrass (*Eleusine indica*)

Agrochemical plays a significant role in agriculture fields. However, the negative impacts of agrochemical to the environment are inevitable. The effects of agrochemicals to the soil, water and air is polluting the environment yet the application of agrochemical is not reduced. Hence, the present study was conducted to investigate the effectiveness of aqueous Tulsi and Neem leaves extract as bioherbicide on goose grass. Experiment was conducted by various concentrations (2%, 4%, 6%, 8% and 10%) of aqueous Tulsi (*Ocimum sanctum L*) and Neem (*Azadirachta indica A.Juss*) leaves extracts on goose grass (*Eleusine indica*) weed. The seed germination and growth of treated weeds was determined through conducting this experiment under laboratory and nursery conditions. Tulsi and Neem leaves were dried and grind into powder. Stoke solution of 50gram of powder into 250ml distilled water was prepared separately for both extracts. It is then kept in orbital shaker for 24 hours under 250rpm for continuous agitation. Dilution process was conducted to make various concentrations of 2%, 4%, 6%, 8% and 10%. The results obtained by determining the seed germination percentage, shoot elongation, shoot fresh weight and root length of the goosegrass under both nursery and laboratory conditions. The aqueous leaf extract of Tulsi and Neem was found to have allelopathic effect on shoot and root elongation, seed emergence, and shoot fresh weight by inhibiting the growth. The results proven that the inhibitory effect was much more pronounced by combined extract with the highest concentration.

Keywords: *Allelopathy, Tulsi, Neem, weed control, seed germination, aqueous extracts*

Aktiviti allelopathic daun Tulsi (*Ocimum sanctum L.)* dan Neem (*Azadirachta indica A.Juss*) daun ekstrak pada Goosegrass (*Eleusine indica*)

Agrokimia memainkan peranan penting dalam bidang pertanian. Walau bagaimanapun, kesan negatif agrokimia terhadap alam sekitar tidak dapat dielakkan. Kesan agrokimia ke tanah, air dan udara mencemarkan persekitaran namun penggunaan agrokimia tidak berkurangan. Oleh itu, kajian ini dijalankan untuk mengkaji kesan pelbagai konsentrasi (2%, 4%, 6%, 8% dan 10%) daripada Tulsi berair (Ocimum sanctum L) dan Neem (Azadirachta indica A.Juss) rumput angsa (Eleusine indica) rumpai. Percambahan benih dan pertumbuhan rumpai terawat akan ditentukan melalui menjalankan eksperimen ini di bawah keadaan makmal dan nurseri. Tulsi dan daun Neem dikeringkan dan digiling menjadi serbuk. Penyelesaian Stoke 50gram serbuk ke dalam air suling 250ml disediakan secara berasingan untuk kedua-dua ekstrak. Ia kemudiannya disimpan dalam shaker orbit selama 24 jam di bawah 250rpm untuk gangguan yang berterusan. Proses pencairan dijalankan untuk membuat kepekatan sebanyak 2%, 4%, 6%, 8% dan 10%. Keputusan diperolehi dengan menentukan peratusan percambahan benih, memanjangkan pemanjangan, beart basah dan panjang akar goosegrass di bawah kedua-dua keadaan nurseri dan makmal. Ekstrak daun berair Tulsi dan Neem didapati mempunyai kesan allelopathic pada pucuk dan pemanjangan akar, kemunculan benih, beart basah dengan menghalang pertumbuhan. Hasilnya membuktikan bahawa kesan penghambatan itu jauh lebih ketara oleh ekstrak gabungan dengan kepekatan tertinggi.

Kata kunci: *Allelopathi, Tulsi, daun semambu, kawalan rumpai, percambahan benih, ekstrak akueus*

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LIST OF ABBREVIATION

N	North
E	East
CRD	Completely Randomized Design
ANOVA	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
SE	Standard Error

CHAPTER 1

INTRODUCTION

1.1 Research Background

Agrochemical is a vast and profitable industry in Malaysia, as most of the farmers or agricultural companies' first preferences would be most likely to be agrochemicals. Due to Malaysia's tropical environment of evergreen and warm with a humid temperature promotes the rapid growth of undergrowth and the life cycles of farm weed (Ali & Shaari, 2015). Therefore, weed control is an important activity that required greater attention. Generally, the best solution for this problem is chemical herbicide because of its effectiveness and price rate. Whereas this solution does come with adverse effects. Chemical herbicides are actually threating to the environment and human health (Jung Lee & Ngim, 2000; Martins & Christoffoleti, 2014). Furthermore, the chemical waste or runoff highly contaminate the water and increase the salinity of the soil which leads to soil degradation (Fujii, 2003). As an indirect effect, it is capable of harming the livestock and affects the vegetation. Chemical herbicides that are well-known in Malaysia are Paraquat Di-Chloride 13%; MSMA 55%; Glufosinate-Ammonium 13.5% (Basta 15); Glyphosate Isopropylamine 41% (Round-up) and many more. Herbicides that used to kill

bushes and small trees comprised of 2,4.D-Amine 48%; Diuron 80%; Metgsulfuron-Methyl 20% (Ally) (Ali & Shaari, 2015). Even though, the results of the chemical herbicides are satisfying; the negative effects are a serious concern. Hence natural herbicide will be the best solution for weed control.

The allelopathy phenomenon is a situation where a plant species chemically interferes with the germination, growth or development to other plant species, which has been known for over 2000 years (Megh & Samunder, 2014). Allelochemicals in plants are an important element in this situation. When the allelochemicals contact with susceptible plants, germination, growth, and development may be affected. Allelochemical can reduce seed germination, coleoptiles, and radical elongation and root or shoot growth inhibition and block the nutrient uptake (Schulz, Marocco, Tabaglio, Macias, & Molinillo, 2013). Recently, studies are being conducted on allelopathic interaction between plants that will be exposed to laboratory conditions and acclimatization. The idea of using allelopathic crop plants to inhibit the growth of weed in the farm is a great weed management strategy. "In agricultural systems, allelopathy can be part of the interference between crops and weeds and may, therefore, affects the economic outcome of plant production," (Megh & Samunder, 2014). This is an effective method to keep the weeds at bay. By planting the allelopathic crop plants at a plantation in a rotational sequence, its residue or mulch inhibiting weed emergence and subsequently their growth. In short, allelopathic crops is an alternative for weed control without any chemical application.

Tulsi (*Ocimum sanctum*) is an aromatic shrub in the basil family Lamiaceae. It is believed to be originated in north-central India, whereas now it grows native throughout all over the world (Bast, Rani, & Meena, 2014). This is honored as a sacred plant of India because of its great spiritual, medicinal and therapeutic value in Hindu belief (Upadhyay,

2017). In the context of Ayurveda, Tulsi is known as “The Incomparable One,” “Mother Medicine of Nature” and “The Queen of Herbs,” and it is also celebrated as an “elixir of life” that is without equal for both its medicinal and spiritual properties (Wakchaure, Ganguly, & Kumar, 2016). The essential oil found in this plant has phenolic constituents such as eugenol, thymol where the sesquiterpene alcohols as single major oil constituents and terpene compounds as minor constituents (Islam & Kato-Noguchi, 2014). Soil incorporate with basil plant tissues capable of increasing the mineralization and availability of macro- and micronutrients while decreasing the soil bacterial colonies (Chouliaras et al., 2007).

Azadirachta indica (neem) is another very important with high medical values which are traditionally being used to treat many kinds of injuries and diseases. This neem plant belongs to the Meliaceae family. It is native to India, Bangladesh, Thailand, Nepal, and Pakistan (Hossain, Al-Toubi, Weli, Al-Riyami, & Al-Sabahi, 2013). Due to neem’s medicinal values, it has been declared worldwide as the “Tree of the 21st century” by the United Nations. While, in India, it is called “Divine Tree”, “Life-giving tree”, “Nature’s Drugstore”, “Village Pharmacy” and “Panacea for all diseases”(Chattopadhyay, 1999). Almost all the parts of the Neem plant have its own uses which are being utilized in human health and the environment. The phenolic compounds of Neem contribute into the have demonstrated antioxidant, immunomodulatory, anti-inflammatory, antiulcer, antimutagenic and anticarcinogenic properties of Neem plant. The crucial compound contained in Neem is Azadirachtin. The oil extracted from the seeds of Neem able to perform biocidal activity against nearly 200 medical and veterinary arthropods, without any adverse effects towards most non-target organisms (Mulla & Su, 1999).

Goose grass which is scientifically known as (*Eleusine indica*) mentioned as one of the worst weed in the world. It is well known for infesting annual and perennial crops,

vegetables and roadsides plants. It is the major enemy to the economical crop by invading in crop plantation and inhibit the growth and production of the weed. Reports have also claimed that in 2009, glufosinate-ammonium failed to adequately control goose grass populations in Kesang, Malacca, and Jerantut, Pahang (Jalaludin, Ngim, Bakar, & Alias, 2010). At both the places, on-site field trials were conducted to assess the efficacy of glufosinate-ammonium towards goose grass. Goose grass is highly prolific and a single plant is capable of producing 140 000 seeds (Kanzler & van Staden, 1984).

1.2 Problem Statement

The application of agrochemicals is significantly growing, especially, for pesticides and herbicides as modern practices being utilized in agricultural lands. According to a survey done by Raja Abdul (2017), the cheapest and popular herbicide that found in Malaysia is Glyphosate 48 which the price value fell under the range of RM47-RM 58. Due to the inexpensive prices and the effectiveness of chemical herbicides, it has become a reliable method for the farmers particularly for weed control management. Weed control management is important because it reduces the growth of the plant and lowers the production. In Malaysia, goose grass is one of the noxious weeds in orchards, vegetable farms, oil palm and rubber plantations (Jung Lee & Ngim, 2000). "Goosegrass (*Eleusine indica*), regarded as one of the world's worst weeds, is highly pernicious to cash crop-growers in Malaysia" (Jalaludin et al., 2010). The population of this weed usually control by using chemical herbicides. Irrespective of its role, the usage of these chemical herbicides are causing environmental damages such as reducing soil fertility, contaminating groundwater with nitrate (Freedman, 2018). Hence, researchers are keen to find a solution for this environmental problem by familiarizing with natural

herbicide. The use of herbicides has generally provided farmers with a very reliable and cheap method of weed control.

1.3 Hypothesis

H_0 = There is no effect of Tulsi and Neem leaves extracts on seed germination and plant growth of goose grass weed

H_1 = There is the effect of Tulsi and Neem leaves extracts on seed germination and plant growth of goose grass weed.

1.4 Objectives

I. To determine the allelopathic effects of Tulsi and Neem leaves extract on seed germination and plant growth of goose grass weed.

II. To evaluate the effective concentration of Tulsi and Neem leaves extract that affect the seed germination and plant growth of goose grass weed.

1.5 Scope of study

This study comprises the chemical compound of Tulsi and Neem leaves extracts on suppressing the weed growth. Different concentrations by using 2%,4%,6%,8%, and 10% were performed to identify the best plant and suitable concentration that has the potential to be used as a natural herbicide.

1.6 Significant of study

The negative impacts of agrochemical that being used for weed control need to be fixed by the natural herbicide to protect our environment and human health. Through good knowledge and understanding of the plant physiology of Tulsi and Neem leaves extracts composition as the herbicidal activity on the goose grass can be helpful to develop effective bio-herbicide.

CHAPTER 2

LITERATURE REVIEW

2.1 Tulsi Plant (*Ocimum sanctum L.)*

2.1.1 Origin

Ocimum sanctum L. is an annual herb that belongs to Laminacea family with 150 varieties in total (Javanmardi, Khalighi, Kashi, Bais. H, & Vivanco, 2002). The Tulsi is commonly known as Ajaka, Brinda, Green Holy Basil, Hot Basil, Indian Basil, Kala Tulsi, Krishna Tulasi, Manjari, *Ocimum sanctum*, Ocimum tenuiflorum and many more (Singh, 2018). "Tulsi or Basil" is ingenuous throughout India because of its ancient history is known since long ago, the Vedic age for its multi-purpose function (Cohen, 2014). Generally, Tulsi is utilized as a part of various structures; fluid concentrates from the leaves (crisp or dried as powder) are utilized as a part of home-grown teas or blended with different herbs or nectar to upgrade the therapeutic esteem (Yamani, Pang, Mantri, & Deighton, 2016). It is broadly distributed shrub throughout in the tropical and subtropical Asia (Islam & Kato-Noguchi, 2014). *Ocimum sanctum* produces a spicy scent when it is bruised; it has a pungent and bitter taste. This plant can grow up to 0.5-1.5

meters in height and has red or purple quadrangular branches (Zhou & Yu, 2006). As the flowers are tiny, purple in colour and the inflorescence is a long spike about 12-14 cm in length. This plant also has small reddish grey fruits with smooth nut lets (Krishna, Ramesh, & Kumar, 2014).

2.1.2 Constituents

The leaves of Tulsi consist of essential oil that contains eugenol, eugenal, carvarol, methylchavicol, limatrol and caryophyllene (Jain & Argal, 2013). These chemical compounds exhibit great anti-microbial properties in food production and cosmetics production (Anon, 2017). Whereas the roots contain sitosterol (Krishna et al., 2014). The medicinal effects are mostly due to rhymol, eugenol and camphor (Upadhyay, 2017). The chemical composition of Tulsi plant is highly complex, containing many nutrients and other biological active compounds (Upadhyay, 2017). Tulsi has higher capacity to take Carbon Dioxide (CO2) than the other plants. It has approximately 300 time more capacity to take CO2 from the environment (Khambholja, 2012). Many studies have shown that Ocimum group contain insecticidal and allelopathic contents. Whereas Ocimum basilicum is mostly used as spice or natural seasoning. There are very less amount of experiment has been done to investigate the potential of the O. basilicum as an insecticide or herbicide (Umerie, Anaso, & Anyasoro, 1998).

In relation of Tulsi plant with allelopathy activity, in an experiment conducted to study the allelopathic activity of Tulsi and Wild indigo leaves extracts on certain legumes and weeds, it showed that Wild indigo shown more inhibitory effect than Tulsi. The lower concentrations of Wild indigo leaves extracts inhibited the weed seed gemination whereas Tulsi leaves extract failed to inhibit the growth of the weed seed effectively in higher

concentrations (Pandya & Purohit, 2013). Durrani & Prasad (2009), shown in their study that Tulsi extract exhibited stimulating effect on root weight of turnip where it successfully inhibited up to 80% with greater concentrations. The author mentioned that the biochemical interactions formed when the allelochemicals present in the plant extracts of the basil plant make contact with the embryo of the seeds during treatment. This will affect the germination, survival, growth and development of turnip plants raised from these (Durrani & Prasad, 2009). Through this it is understandable that Tulsi is also capable of stimulating the growth of a plant. At the same time, in a journal published in 2017 related to Tulsi allelopathy, the results proven that aqueous extract of the aboveground parts of sweet basil, significantly reduced the seed germination of the poaceous crops such as sorgum, maize and wheat. Aqueous Tulsi extract has significantly decreased the height of plant, number of leaves and root length of crop seedlings, seedling fresh weight and dry weight of all the test plants.

Irrespective to these finding, there is also study enlighten that Tulsi plants do possess many of inhibitory traits, but no allelopathic evidence on weeds was found in the study (Megh & Samunder, 2014). In this study, the effect of Tulsi leachate on the emergence on 10 weed species was studied in laboratory and greenhouse conditions. As this study was also related to intra-and inter-specific competition between the plants. Eventually, the study suggested that insignificant effect on the weed seed emergence with the Tulsi leachate may not due to any allelopathic effect but the dominant role of competition when the weed and Tulsi were grown in different plant ratio.

2.1.3 Uses

The chemical compounds contained in Tulsi make it undergoes variety of biological or pharmacological activities such as antibacterial, antiviral, antifungal, anti-protozoal, anti-malarial, anthelmentic, anti-diarrhoeal, analgesic, antipyretic, anti-inflammatory, anti-allergic, antihypertensive, cardio protective, central nervous system (CNS) depressant, memory enhancer, antihypercholesterolaemic, hepatoprotective, anti-diabetic, anti-asthmatic, anti thyroidic, antioxidant, anticancer, chemopreventive, radio protective, immunomodulatory, anti-fertility, antiulcer, anti-arthritic, adaptogenic / anti stress, anti-cataract, anti leucodermal and anticoagulant activities (Cohen, 2014). Ocimum capable of suppressing the growth of all weed species, except yellow nutsedge, but plant height of basil was unaffected by weeds (Megh & Samunder, 2014). Tulsi contains secondary metabolites like a steroid ursolic acid and n-triacontanol. Eugenol (70.5), its methyl ether (4.8), nerol (6.4), caryophyllene (7.5), terpinen –4-(0.4), decylaldehyde (0.2), selinene (0.4), pinene (0.4), camphene (2.0) and apinene (3.5%) (Pandya & Purohit, 2013). In Africa, it has been used to expel worms (Cohen, 2014).

2.2 Neem (*Azadirachta indica A.Juss)*

2.2.1 Origin

Neem is scientifically known as *Azadirachta indica*, an evergreen tree that belongs to the Meliaceae family that includes about 50 genera and 550 species. (Saleh Al-Hashemi & Hossain, 2016). It is native to India, Bangladesh, Thailand, Nepal, and Pakistan. It grows well in tropical and sub-tropical regions (K & Krishnaiah, n.d.). This plant is

commonly known as neem and considered as highly valuable plants because of its broad spectrum of function. Neem tree can grow 25 meters in height with a semi- straight trunk. It is an angiosperm and normally starts fruiting after 3 or 5 years. The bark of this tree is grey and rough and the leaves are up to 30cm long. The leaves have margin notched like a saw with teeth pointing toward the apex that is 7cm long by 2.5cm wide (Saleh Al-Hashemi & Hossain, 2016). This tree able to grows well in minimum rainfall.

2.2.2 Constituents

The most commonly active compounds found in neem are azadirachtin, nimbin, and nimbidine as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7- benzoylgedunin, 17-hydroxyazadiradione and nimbiol (Hossain et al., 2013). These compounds are released into the environment, either as exudates from living tissues or by decomposition of plant residues in sufficient quantities to affect neighbouring or successional plants (Ashrafi, Rahnavard, Sadeghi, Alizade, & Mashhadi, 2008). The extract of neem leaves displays strong phytotoxicity and possesses the growth inhibitory ability of noxious weeds because of the number of useful chemicals that contained in the leaves has multiple uses and very adaptable to diverse habitats and climatic conditions (Ashrafi et al., 2008). However, in the aspect of the allelopathic potential of *Azadirachta indica* not much evidence or information is available. In Tran Xuan et al (2004), it is mentioned that allelochemical such as gallic acid, p-coumaric acid, p-hydroxybenzoic acid, vanillic acid, benzoic acid, and trans-cinamic acid were found in both neem bark and leaves that are suspected to be transform when exuded into soil condition gave different inhibitory activities between bioassay and soil treatments (Xuan et al., 2004).

Ashrafi et al. (2018), germination test and growth test can have conducted along with measurement of osmotic potential test. The effects of osmotic potential of test solution on the bioassay in these experiments were analysed. The conclusion made was that the osmotic potential of the solutions did not significantly affect the germination, root growth and shoot growth of the weed plant. According to the journal, the allelopathic potential of n-hexane-soluble, acetone-soluble and water-soluble fractions obtained from extracts of shoots of *A. indica* successfully inhibited the germination of seeds. Whereas the results show that the activities of the n-hexane- and acetone-soluble fractions were weak and the seed germination in water-soluble fraction was responsive outcome. As for the seedling growth assay, all three fractions suppressed the growth of the roots, with the highest inhibition rate was marked by water- soluble fraction. In this study, seedlings of each test species used in these experiments were grown in a single petri dish without intraspecies competition. By this the author proven that the germination and growth inhibition of the species caused purely by the allelopathic reaction. The result shown to be very concentration-dependable (Ashrafi et al., 2008). Therefore, it can be said that allelopathic species can be useful for weed management.

In the article titled, "Allelopathic Effect of Aqueous Extracts of Neem (Azadiracta indica) and Eucalyptus (Eucalyptus citroides) on the Growth and Germination of Wheat (Triticum aestivum var-desi) ", by different aqueous concentrations of the leachate of neem and eucalyptus were applied on wheat seed grains. Seed germination test and weed growth test in conducted in laboratory and field. After the analysis, the result shown that the aqueous leaf extract of neem and eucalyptus inhibited the germination and growth of the wheat. This may because of the phytotoxic chemicals released by the leaves of neem. The author also provided evidence to prove the result he obtained is valid by saying that in Bora et al. (1999), who found that the inhibitory effect of leaf extracts of Acacia

auriculiformis on germination of some agricultural crops was proportional to the concentration of the extract (Bora, Sing, Borthakur, & Bora, 1999). In article written by Fag C (1994), it is argued that the allelopathic effect might be due to synergistic effect rather than single one (Fagg & Stewart, 1994). The allelochemicals such as includes phenols, alkaloids, long-chain fatty acids, terpenoids and flavonoids are responsible for allelopathic effects of the plants to be toxic to germination and plant growth. Faroz Ahmad Ahangar (2013) concluded that these plants which are neem and eucalyptus should not be grown near agricultural fields in order to increase the agriculture; crops (Ahmad, Rao, & Mamta, 2013).

2.2.3 Uses

Currently, the function of neem can be seen mostly in toothpaste, soaps, and lotions. This is due to the chemicals present in the neem plant. Almost every part of *Azadirachta indica* tree have multifarious uses and widely used in health care and agriculture. Dillion, Ahlawat & Pinderr (2009) in their NEEM treatise book reported that people have long recognized that the leaves, bark, wood, and fruit of neem tree either repel or otherwise discourage insect pests, and they incorporated these plant parts into traditional soil preparation, grain storage, and animal husbandry practice. Azadirachtin has initiated a broad range of academic and industrial interest as a potential potent insect and weed control molecule.

2.3 Goosegrass (*Eleusine indica*) weed

Eleusine indica commonly known as goosegrass, is a monocot weed belonging to the Poaceae family and an important C4 grassy weed (Dilipkumar et al., 2015). This grass is also classified as one of the five most troublesome weeds in the world (G. Holm, L.Plucknett, V.Pancho, & P.Herberger, 1978). The distinguish character of these weed is that it has very flat stem and as mature the crown and the lower parts of the weed grass will be in white color (Steckel, n.d.). This grass can propagate in enormous amount in short time of period and also very competitive. Over 20 years, glyphosate is broadly used throughout the world to control goose grass that compete with the plantation crops. In recent years study found that the goose grass become resistant to glyphosate herbicide.

It is said that in Malaysia, this weed first evolved with multiple resistance towards herbicides in 1997 itself and infested countless orchards (Lee, 2013). This species is now becoming the most dominant weed in invading the aerobic rice system. The seed of this plant is reported as 140,000 seeds per plant which cause this feed to be a fast- grower weed. In plasticulture production systems, goose grass can be controlled with the use of plastic mulches and hand pulling of small plants that emerge in the planting holes during the cropping period (Boyd, Fnu, Marble, Steed, & Macrae, 2010). Seed germination is a key event in determining the success of a weed in an agro-ecosystem and it may be regulated by several factors, including temperature, light, soil salinity, moisture, and pH (Chauhan & Johnson, 2008). When spread on the soil surface, 80% of the seeds germinated. Seeds that failed to germinate will most likely to be due to the greater depth of seed in the soil. The lack of germination at depth might be because of hypoxia and low rates of gaseous diffusion in soil. Germination and emergence rate of weed seeds are influence greatly by climate, biological characteristic of the various species and also

because of the soil granulometric composition (Benvenuti, 2003). This tolerance of a wide range of conditions for germination suggests the reason of E.indica is found so widely invading the farming system especially paddy field.

Application of some pre-emergence herbicides such as pedimethalin, butachlor, thiobencarb, oxadiazon, oxyfluorfen and nitrofen were found to be useful weed control. Whereas Pendimethalin was highly effective in controlling E. indica according to Rahman (2012). Pretilachlor is another pre-emergence selective herbicide used to control of barnyard grass, psrandletop, nutgrass, duck tongue weed (Rahman, Shukor Juraimi, Suria, Man, & Anwar, 2012) (Ashrafi et al., 2008). In research conducted in 2015, show that mulches from oil palm (frond, leaflet and rachis) and rice (husk) residues exhibited phytotoxic effects on goosegrass (Dilipkumar et al., 2015).

2.4 Allelopathy

Allelopathy has been defined as an antagonistic influence of one plant or micro-organism on another of the same species. Allelochemicals can be found in most parts of plants viz. roots, rhizomes, leaves, stems, and flowers. It is released into the environment by root exudation, leaching from above ground parts, volatilization and plant decomposition material (Megh & Samunder, 2014). The article also has mentioned that these allelopathic plants are the great concern when they are present along with the crop plants. Samunder and Megh (2014) also reported that allelochemicals can also prevent cell division, pollen germination, nutrient uptake, photosynthesis, and specific enzyme functions in sensitive plants. Sharma and Singh (2003) reported the allelopathic effect of basil on some weed species, however, there is less amount of literature studies on allelopathic effects of Tulsi on weeds.

The growth inhibition of weed or grass in presence of allelochemicals could be for the reason of reduced cell division, elongation and extension rate which are growth pre-requirements (Rice & Leon, 1984) (Cruz Ortega, Anaya, & Ramos, 1988). Allelochemicals may create more than one effect of the above on the cellular processes that could be in precursor for the retarded seedling growth of barnyard grass. Be that as it may, the details of the biochemical mechanism through which allelochemicals exert a toxic effect on the development of any plant species are still not well discovered (Zhou & Yu, 2006).

CHAPTER 3

MATERIAL AND METHODS

3.1 Materials

3.1.1 Selection of plants

Selected leaves of Tulsi Plant (*Ocimum sanctum*) and Neem (*Azadirachta indica* A. Juss) were collected from Ampang, Selangor at 3°08'32.0"N 101°45'11.7"E.

3.1.2 Weed Seed Collection

The seeds of the goose grass were collected at the AgroTechno Park (ATP) UMK Jeli campus at 5°44'46.4"N 101°52'01.3"E where they grow wildly. The goose grass seed heads were plucked from the weed plant using a plant scissor. The seeds heads were kept immediately into an envelope. The seeds that fell off from the head into the envelope was then taken and placed into a new envelope.

3.1.3 Extraction Preparation

Tulsi leaves were washed and kept for complete dry in the oven. The dried-out leaves ground into powder form using a blender. It was ground into fine small particles. The powder was then placed into a fully dried container and sealed securely. The Neem leaves undergone the same process to make dried powder from the leaves. Then 50g was taken from each powder and dissolved into 250ml of distilled water and kept in an orbital shaker under 30 ºC for 24 hours (Ahmad et al., 2013) to make 20% of total concentration. The resulting extract was then filtered using a Muslin cloth to remove the debris and after that with a Whatman filter paper No. 1. The prepared 20% was then diluted with distilled water to make required concentrations (2%, 4%, 6% 8% and 10%) (Dheeba, Niranjana, Sampathkumar, Kannan, & Kannan, 2015). The dilution process for each concentration was calculated using the formula, M1V1 = M2V2, where M1= molarity of stock solution, V1= Volume of stock solution to be used, M2 = molarity of solution to be prepared, V2 = Volume of solution to be prepared.

This serial dilution was done for both Tulsi and Neem leaves extracts. As for the combined extracts, the aqueous Tulsi and Neem extracts were prepared and mixed together. For example, in order to prepare 2% of combined extract, 1% of Tulsi leaves extract and 1% of Neem leaves extracts were combined together. The combined extracts prepared according to 2%, 4%, 6%, 8% and 10% concentrations. All the extracts or treatments were then designated as shown in Table 3.1. The extracts were then stored in a refrigerator under dark place until it is required. This is to minimize the possibility of contamination and maintain its bioactivity for long period.

Table 3.1. Treatment layout of various concentration of Tulsi and Neem extract

Designation	Treatment
T1	Control (distilled water only without contained Tulsi or Neem extract)
T2	2% of Tulsi Extracts
T3	4% of Tulsi Extracts
T4	6% of Tulsi Extracts
T5	8% of Tulsi Extracts
T6	10% of Tulsi Extracts
T7	2% of Neem Extracts
T8	4% of Neem Extracts
T9	6% of Neem Extracts
T10	8% of Neem Extracts
T11	10% of Neem Extracts
T12	2% of Combined (Tulsi and Neem) Extracts
T13	4% of Combined (Tulsi and Neem) Extracts
T14	6% of Combined (Tulsi and Neem) Extracts
T15	8% of Combined (Tulsi and Neem) Extracts
T16	10% of Combined (Tulsi and Neem) Extracts

3.2 Methods

3.2.1 Seed Sterilization

The *Eleusine indica* or goosegrass seeds were surface sterilized using 2% of sodium hypochlorite (NaOCl) before conducting any test on the seeds (Chuah, Tiun, Ismail, & Science, 2011). This sterilization process will be done for 15 minutes, then the seeds were rinsed with distilled water three times to remove excess of chemical (Khan, Hussain, & Khan, 2008).

3.2.2 Seed germination test

This test is crucial to conduct for the aqueous extracts of Tulsi and Neem to show their effects on the goose grass seed germination. In order to perform this test, healthy and uniform sized seeds were chosen and presoaked in prepared Tulsi and Neem extract with different concentration for about 4 hours (Siddiqui, Bhardwaj, Khan, Meghvanshi, & Sciences, 2009). The seeds were kept in sterilized petri dishes. In total 64 petri dishes were used. First five petri dishes were treated with different concentrations of Tulsi and the next five with Neem extracts with different concentration. The following five petri dish was treated with the combined extracts with different concentration. The last or 16th petri dish was kept as a control containing the seeds treated with distilled water. Around 10 seeds will be placed on each of the petri dishes. The petri dish was kept in place at where a relative humidity of 78 – 80% and a temperature of 28 – 30 ºC with 12 hours of photoperiod.

The emergence of the radical of the seeds is considered as germination of the seed. The length of the radicle was measured with a measuring scale or ruler at 24-hour intervals over a week period. This is to show the effect of the different extract concentration. The experiment was conducted in three replications.

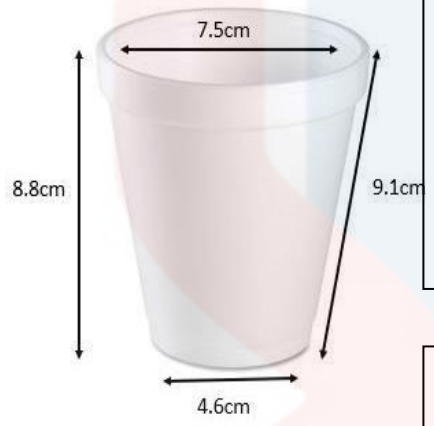
3.2.3 Weed Growth Test

Growth test is to investigate the effect of aqueous extracts of Tulsi and Neem with concentration of 2%, 4%, 6%, 8% and 10% on the growth of the goosegrass weed.18 clean plastic cups were taken and filled with soil that will also be collected at the same field of the weed. The soil filled into the cup according to the calculation shown in Figure

3.1. Then the similar size of seeds was selected and sown in the soil where each cup contains 25 seeds of goose grass. First five cups were treated with different concentrations of Tulsi extract and the next five with Neem extract. The following 5 cups were treated with combined extract with different concentrations. The last three cups will be treated with distilled water. The seeds were sown in each cup will be at equal depths. The cups were then kept in a greenhouse under suitable conditions such as the temperature about 30ºC -35ºC and humidity (Ahmad et al., 2013). The aqueous extracts of various concentrations were applied into the cups according to Table 3.1. The amount of extract applied into each cup was calculated as shown in Figure 3.2 then applied on the first three days after sowing, while the control cup was treated with distilled water to ensure it acts as a control. In each cup, the extract applied was 0.86ml by using a 1000µl pipette.

As the seed germinated, the data for seed emergence was collected. After the germination of the seed, only around 2-6 plants were allowed where the rest were eliminated to avoid intraspecific competition between the plants for nutrients uptake. The root length and the radicle length of the plants were measured. The measurement of the plant was done using ruler over 14-day of the period at an interval of 3-days (Ahmad et al., 2013; Siddiqui et al., 2009). This is to evaluate the effect of the various aqueous extracts of different concentration on the growth of the goose grass grown in the pots. The shoot fresh weight of the plants was also taken using weight balance in milligram(mg). The percentage of shoot fresh weight was calculated the following formula (Sahid, Chuah, Asmah, Cha, & Hasan, 2008).

$$\text{Fresh Weight} = \frac{\text{treated plant fresh weight}}{\text{untreated plant fresh weight}} \times 100$$



Lateral Surface Area (LSA) =

πrs, r=radius, s= slant

LSA

$= \pi(r_1+r_2)s$

$= \pi(2.3+3.75)(9.1)$

$= \pi(55.055)$

$=172.98cm^2$

r_1 = radius of upper end (d/2)

r_2 = radius of lower end (d/2)

Total Surface Area (TSA) =

LSA + πr^2

TSA = $172.98 + \pi(2.3)^2$

$= 172.98 + \pi(5.29)$

$= 172.98 + 16.62$

$= 189.60cm^2 = 0.019m^2$

- **¾ (0.019) = 142.2g of soil in each cup**

Figure 3.1: Calculation of Amount of soil in each cup.

Amount of extract required in each cup for treatments

X = amount need to applied in a cup

450L = 10, 000m^2 (1hectar)

The total surface area = 0.019m^2

$$X = \frac{0.019 \times 450,000}{10,000}$$

450 L = 450, 000 ml

= 0.855 ml

- **0.86ml per hec (required to applied in each cup)**

Figure 3.2: Amount of extract used for each treatment.

3.2.4 Statistical Analysis

Bioassay of each treatment was carried out with 3 replicates which were arranged in a completely randomized design (CRD). Statistical analysis was performed employing Analysis of Variance (ANOVA) test and multiplicative survival model using IBM SPSS 2.0 software. In order to detect the significance of differences of concentration to inhibit seed emergence or plant growth, all the values were expressed as mean ± standard deviation (Ahmad et al., 2013) (Sharma & Singh, 2003). Using t-test the significant between the extracts was also obtained. Each treatment was compared and the treatment means were then separated using the Tukey's Honesty Significance Difference (HSD) test at the 5% level of significance (Sahid et al., 2008).

Chapter 4

RESULTS AND DISCUSSION

4.1 Effects of aqueous Tulsi and Neem leaves extract on *Eleusine indica* under laboratory condition

4.1.1 Seed emergence

All the concentration of dry leaves extract of *Azadirachta indica* and *Ocimum sanctum* showed the significant effect on goose grass seed germination. The combination of both the extracts with various concentrations (2%, 4%, 6%, 8%, and 10%) also shown a significant difference for the seed emergence. Seed emergence of *E.indica* was inhibited by all the treatments (T2 to T16) and the inhibitory effects increased as the concentrations elevated. Figure 4.1 shows that the seed emergence percentage decreased as the concentrations of the extracts increased. As the concentrations progressed from 2% to 10%, it affected the seeds emergence. The figure depicted in the low concentrations (0%, 2%, 4%), Neem found to be effective than Tulsi. At the same time, the table also proven in the high concentrations (6%, 8%, 10%), Tulsi showed better performance in inhibiting the seed germination than Tulsi. Higher amount of allelochemical possess a greater

amount of inhibition towards the germination of the seeds. The chemicals secreted by the allelopathic plants which were Neem and Tulsi are important in activating the allelopathic mode of action. These plants release a diversity of allelochemicals into the environment. These allelochemicals are long-chain fatty alkaloids, which includes phenols, acids, terpenoids, and flavonoids. The aqueous leaves combined extract recorded the lowest seed emergence percentage than other two extracts (Tulsi and Neem). The 10% of combined extracts recorded the lowest seed germination percentage of 23.33% which implementing that it controlled the seed emergence by 87% against the of *E. indica* (Figure 4.3). Whereas, highest percentage is recorded by 2% of Tulsi extract with 83.33% that controlled the seed emergence only by 20% (Figure 4.3). Tukey HSD reflected that this difference between the 0% and the 2% concentrations of Tulsi is not significantly different. Therefore, it can be said that Tulsi 2% is not effective towards seed emergence of *E.indica* in laboratory condition. This result was similar to the result obtained by the other allelopathic studies (Ashrafi, Sadeghi, Alizade, Mashhadi, & Mohamadi, 2009; Ashrafi et al., 2008; Sharma & Singh, 2003). In the study conducted by Sharma & Singh (2003), the fresh Tulsi leaf extract was treated on radish seed where the result proven that there was reduction in the germination of the seed with different concentrations of Tulsi test solution, but it shown no significant different between the control treatment and the test solutions. Nevertheless, in this study, Table 4.1 proven that all three extracts (Tulsi, Neem and combined) with various concentrations successfully inhibited the emergence of the *E. indica* seeds. This implied that the allelochemicals in each of the extracts inhibited the germination of the seed. The allelochemicals that found in the plants contributed in inhibiting the germination of the seed. When comparing the three extracts (Neem leaves, Tulsi leaves and combined of Neem and Tulsi extracts), it was evident that the combined extract has controlled the seed emergence of *E. indica* in higher percentage than both the

other extracts.

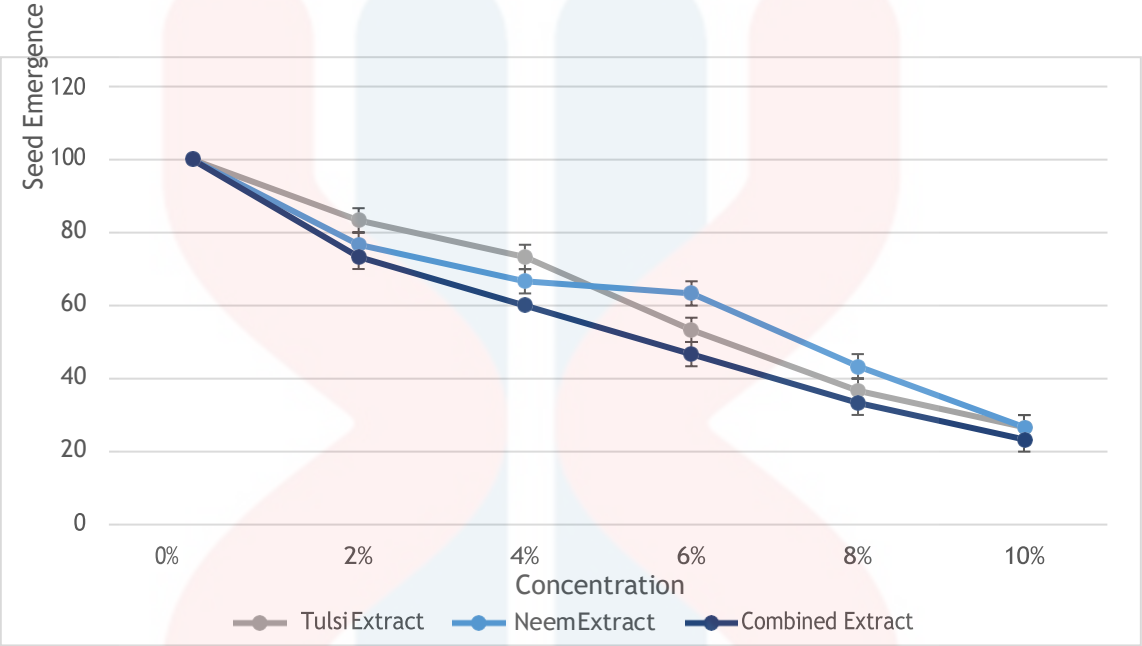


Figure 4.1. The effects of different extracts (Tulsi extract, Neem extract, Combined extract) on the seed emergence (%) of *Eleusine indica* with different concentrations in laboratory conditions. Data are the means of three independent replicated with standard errors shown by vertical bars.

4.1.2 Shoot Elongation

The elongation of the shoot of *Eleusine indica* reduced by aqueous Tulsi, Neem and the combined extracts which was revealed in Figure 4.2. The length of the radicle of the seed that undergone different treatment (T2 to T16) was comparatively lesser than the control (T1). The figure revealed that the difference between the 0% and the 2% of all the three extracts (T2, T7 and T12) statistically significant. The shortest value of shoot elongation which is 2.83±0.56mm (±SE), obtained from the seed treated with T6, the aqueous Tulsi extracts with 10% concentration (Table 4.2). The 10% concentration of combined extract (T16) recorded 2.9±0.3mm which is the second lowest shoot

elongation, found to be slightly higher by 0.1mm than T6 (Table 4.2). Never the less, statistic calculation proven that the difference between the two extracts (Tulsi and combined) were significant in this parameter. By using Turkey HSD, the combined extract showed the most effective result in controlling the *E. indica* growth. This revealed that the allelochemical in the aqueous extract has caused reduction in shoot elongation. This study is also corresponding to the study of Siddiqui et al. (2009) done previously on seed germination of wheat. In the study, it is found that the length of the adicle of Triticum aestivum var- Lok-1, declined as the concentration increases aqueous leaves extract of *Prosopsis juliflora.*

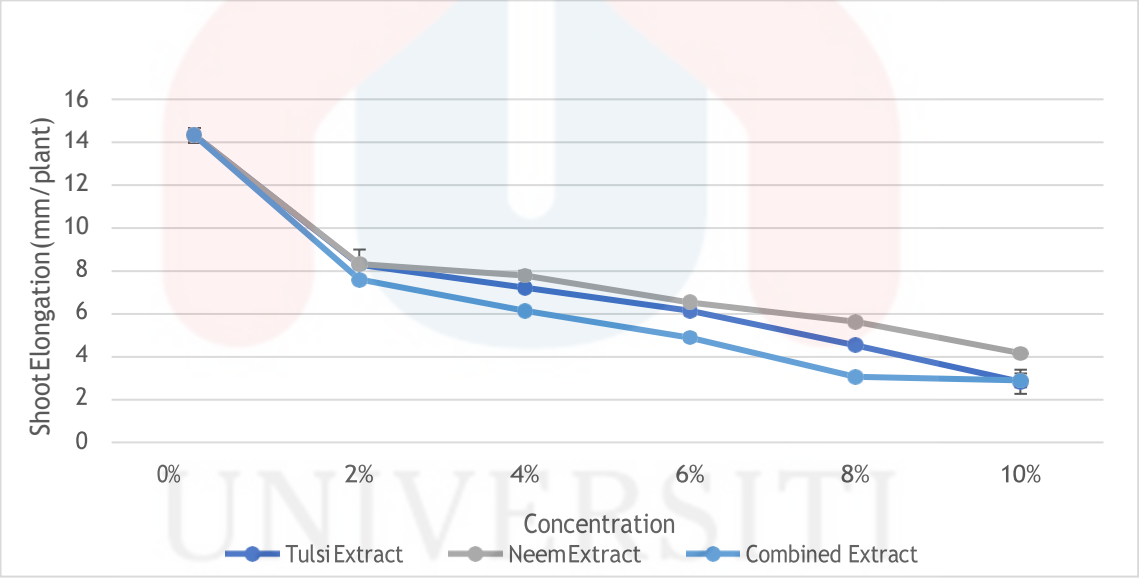


Figure 4.2. The effects of different extracts (Tulsi extract, Neem extract, Combined extract) on the shoot elongation (mm/plant) of *Eleusine indica* with various concentrations in laboratory conditions. Data are the means of three independent replicated with standard errors shown by vertical bars.

4.1.3 Shoot Fresh Weight

The shoot fresh weight of *Eleusine indica* after treated the seeds as T1 to T16, the results were depicted in Figure 4.3. The shoot fresh weight percentage declined as the

concentrations increased from 2% to 10% in all three extracts (Tulsi, Neem and combined). The figure 4.3 revealed that the most effective extract is combined extract with the presence of allelochemicals from two different extract. The figure also depicted that Tulsi extract was the least effective in controlling shoot fresh weight. As in table 4.3, the lowest data collected on the shoot fresh weight of *E.indica* was recorded by T16, the 10% combined extract with 13%. This help in deducing that the T16 in habited the plant growth by 87%. Concurrently, 10% of Tulsi extracts and 10% Neem recorded the shoot fresh weight of 22.67% and 13.67% respectively. This directly implied that the extracts restricted only 77% and 86% respectively the growth of *E. indica* (Table 4.3). Statistically, the different between Tulsi extract with the combined extract was significant while the different between Neem extract with combined extract was not significant. While, 2% concentrations of all the extracts (Tulsi, Neem and combined) recorded that the shoot fresh weight was reduce nearly to 40%. (Figure 4.3). Overall, it can be summarized that combined extract held the highest records in inhibiting the plant growth. Therefore, it can be said that combined is more effective than Tulsi extract and Neem extract.

The shoot fresh weight in the weed seed was due to the allelochemicals that affect the basic plant processes, hormonal balance, protein synthesis, respiration, chlorophyll production, and even plant water relations as well as permeability (Kasarkar & Barge, 2016). Corresponding studies were also detected that there are allelochemicals govern the weed growth and the steps in their development that involve many metabolic processes (Kim & Shin, 1998) (Jagtap, Tayade, & Athawale, 2016). It is evident that the seeds treated with combined extract have comparatively lowest fresh weight than the other two extracts. This may due to the presence of allelochemical from two different extracts that incorporated to retard the growth of *Eleusine indica.*

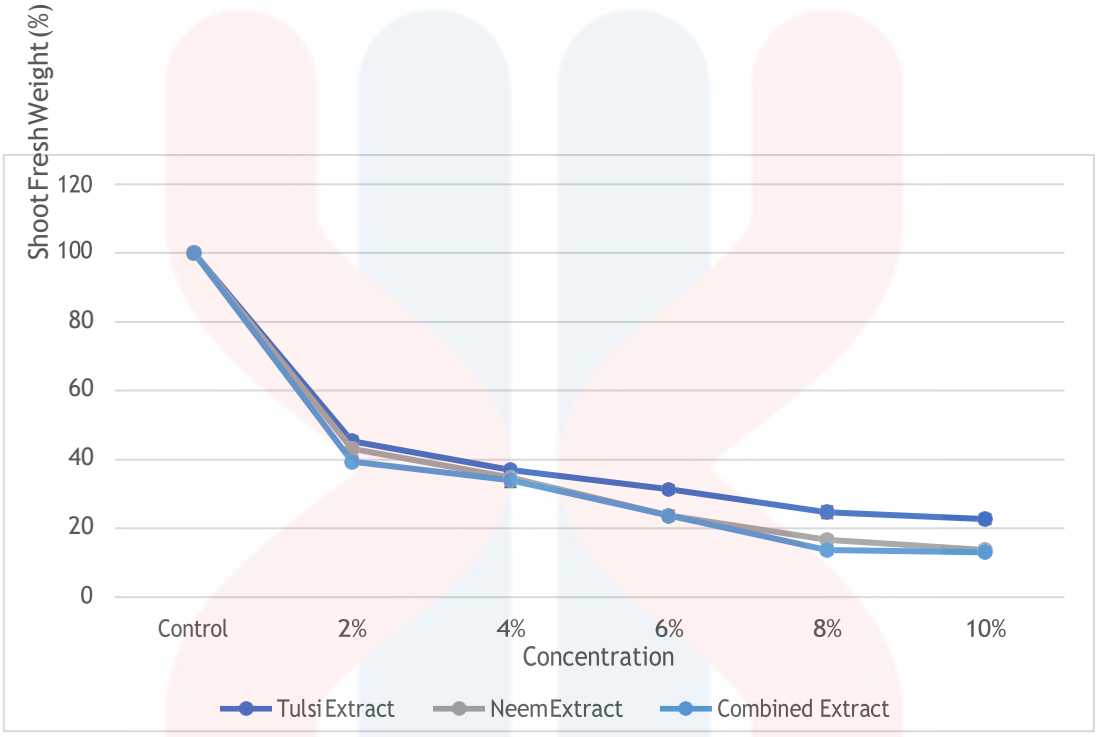


Figure 4.3. The effects of various extracts (Tulsi extract, Neem extract, Combined extract) on the shoot fresh weight (%) of *Eleusine indica* with different concentrations in laboratory conditions. Data are the means of three independent replicated with standard errors shown by vertical bars.

Table 4.1. Mean value of different concentration of aqueous Tulsi leaf extract treatment on seed emergence (%), Shoot Elongation (mm) and Shoot Fresh Weight (% of control) of *Eleusine indica* under laboratory condition. (mean± standard error)

Concentration	Seed emergence (%/plant)	Shoot Elongation (%/plant)	Shoot Fresh Weight (%/plant)
0%	100±0d	14.33±0.33e	100±0d
2%	83.33±3.33c	8.3±0.17d	45.33±1.45c
4%	73.33±3.33c	7.23±0.17cd	37±1.16b
6%	53.33±3.33b	6.13±0.21c	31.33±1.20b
8%	36.67±3.33a	4.53±0.15b	24.67±1.76a
10%	26.7±3.33a	2.83±0.5a	22.67±1.453a

Table 4.2. Mean value of different concentration of aqueous Neem leaf extract treatment on seed emergence (%), shoot elongation (mm) and shoot fresh weight (% of control) of *Eleusine indica* under laboratory condition. (mean± standard error)

Concentration	Seed emergence (%/plant)	Shoot Elongation (%/plant)	Shoot Fresh Weight (%/plant)
0%	100 ± 0^{d}	14.33 ± 0.33^{d}	100 ± 0^{e}
2%	76.67 ± 3.33^{c}	8.33 ± 0.67^{c}	43 ± 2.08^{d}
4%	66.67 ± 3.33^{c}	7.8 ± 0.21^{c}	34.67 ± 2.08^{c}
6%	6333 ± 3.33^{c}	6.53 ± 0.15^{b}	23.67 ± 1.20^{b}
8%	43.33 ± 3.33^{b}	5.63 ± 0.15^{b}	16.67 ± 0.88^{a}
10%	26.67 ± 3.33^{a}	4.17 ± 0.15^{a}	13.67 ± 0.667^{a}

Table 4.3. Mean value of different concentration of aqueous combine leaf extract treatment on seed emergence (%), shoot elongation (mm) and shoot fresh weight (% of control) of *Eleusine indica* under laboratory condition. (mean± standard error)

Concentration	Seed emergence (%/plant)	Shoot Elongation (%/plant)	Shoot Fresh Weight (%/plant)
0%	100 ± 0^{e}	14.3 ± 0.3^{e}	100 ± 0^{d}
2%	73.33 ± 3.33^{d}	7.6 ± 0.2^{d}	39.33 ± 0.88^{c}
4%	60 ± 0^{c}	6.1 ± 0.1^{c}	34 ± 2.08^{c}
6%	46.67 ± 3.33^{b}	4.9 ± 0.2^{b}	23.67 ± 1.45^{b}
8%	33.33 ± 3.33^{a}	3.1 ± 0.1^{a}	13.67 ± 1.20^{a}
10%	23.33 ± 3.33^{a}	2.9 ± 0.3^{a}	13 ± 0.58^{a}

4.2 Effects of aqueous Neem and Tulsi leaves extract on *Eleusine indica* under nursery condition.

All the conditions such as sunlight, water, type of soil was kept constant in this nursery study. The type of soil used was for all the treatment was silty soil and the pH was 6.5.

4.2.1 Seed Emergence

All the extracts (Tulsi, Neem and combined) inhibited the seed germination effectively. Figure 4.4 depicted the percentage of seed emergence that was inhibited by all the extracts. The differences were statistically significant as compared all the treatment with the 0% concentration (T1). This significant inhibitory effect was also found in different literature studies done on *Eleusine indica* (Dilipkumar et al., 2015). Based on Figure 4.4, the combined extract proven to be the most effecting in inhibiting the seed emergence in nursery conditions while Tulsi extract was the least effective. Table 4.4, Table 4.5, and Table 4.6 shown the mean values of the seed emergence of the three extracts with concentrations of 0%, 2%, 4%, 6%, 8% and 10%. Table 4.6 proven that with in the various concentration of combined extract, 8% combined extract found to be the most effective that recorded 12% of seed emergence followed by 10% of aqueous combined extract recorded mean value of 13.33%. This implied that the extracts were effective in suppressing the seed emergence of *E. indica.* As compare the seed emergence of all the extracts, it was obvious that all the 2% aqueous extracts successfully did inhibit the seed emergence up to 30%. The study showing similar results found by Ashrafi, Rahnavard, Sadeghi, Alizade, & Mashhadi (2008), where the study of the allelopathic

potential of *Azadirachta indica*, the activities of the n-hexane- and acetone-soluble fractions were weak and the expected results were only found in water-soluble fraction which proven aqueous extract is the best form to be used which made the greatest influence on the seed. As in the present experiment, the inhibition of aqueous combined extracts with 8% concentration possessed the greatest inhibition value, followed by aqueous combined extract with 10% concentration (Table 4.4). When these two concentrations were compared, the difference is not significant. Hence, the greater concentrations such as 8% and 10% of the aqueous Tulsi, Neem and the combined extract have a strong influence on the seed emergence of *E. indica*. Nevertheless, all three extracts successfully suppress the seed emergence of this Poaceae species when compared to the 0% of the concentration.

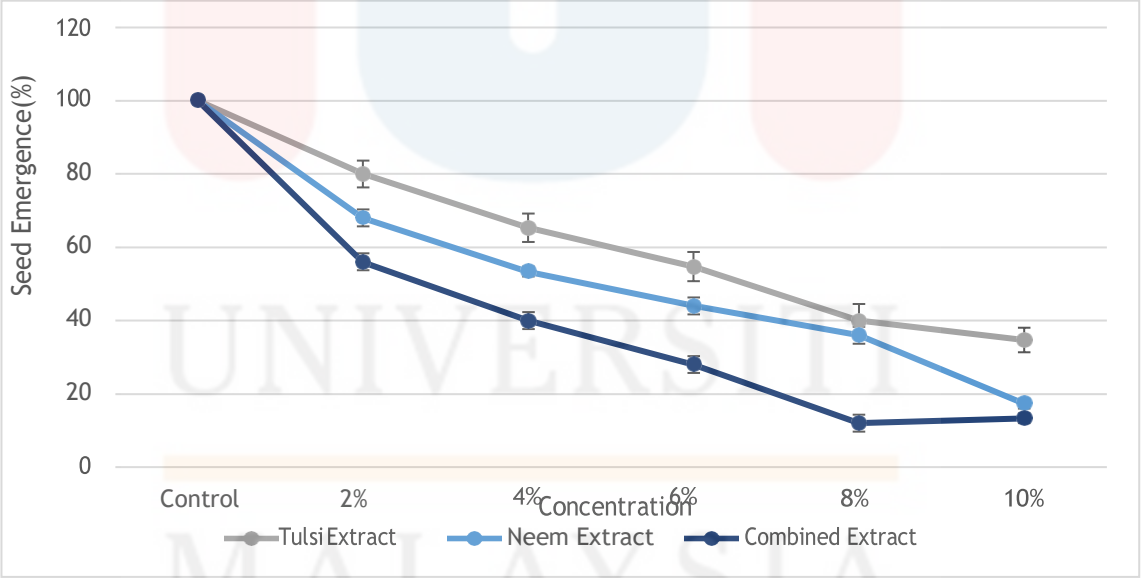


Figure 4.4 The effects of various extracts on goose grass seed in nursery conditions. Tulsi extract, Neem extract, Combined extract effects on the seed emergence of *Eleusine indica* with different concentrations. Data are the means of three independent replicated with standard errors shown by vertical bar.

4.2.2 Shoot Elongation

The effects of various concentrations on *E.indica* are shown according to different aqueous extracts in Table 4.5. The extracts inhibited the radicle or shoot elongation of the seeds. It also revealed that as there was a progressive increase in concentrations (2%, 4%, 6%, 8%, and 10%), there was also an increasing trend in shoot elongation. This trend was applicable to all three aqueous leaves extracts (Tulsi, Neem and the combined). The length of the radicle of all the test plants was measured 14- day of period at interval of 3-days to observe the development of the plant. The final reading which taken on the 14th day which was used in the data analysis. The shoot elongation of the *E.indica* was restricted significantly by 10% of all the extracts. All three extracts significantly inhibited the radicle growth of the test plants. The 10% of aqueous Neem extracts held the highest percentage in inhibiting the elongation of the shoot. This may due to the allelochemicals found in the extracts. The lowest radicle or shoot elongation recorded was by the Neem extract with the concentration of 8%. The mean length recorded by this treatment was 3.7±0.37mm per plant. Nonetheless, as compare the shoot elongation was not significantly different within the various concentrations of Neem extract where the greater concentration such as 10%, 8% and 6% fall under the same subset which is depicted in Figure 4.13. However, the shoot elongation decreased significantly with the increasing concentration of all the three extracts. This exact phenomenon was studied by Mishra (2011) on the inhibition effect of goose grass extract on the seed germination and growth test of some crops. With the aid of supporting documents, it can be said that the present study suggested that the three extracts (Tulsi, Neem and the combined) have a solid allelopathic effect on the development of the weed. This will be a trigger to be used as a strategy for weed control.

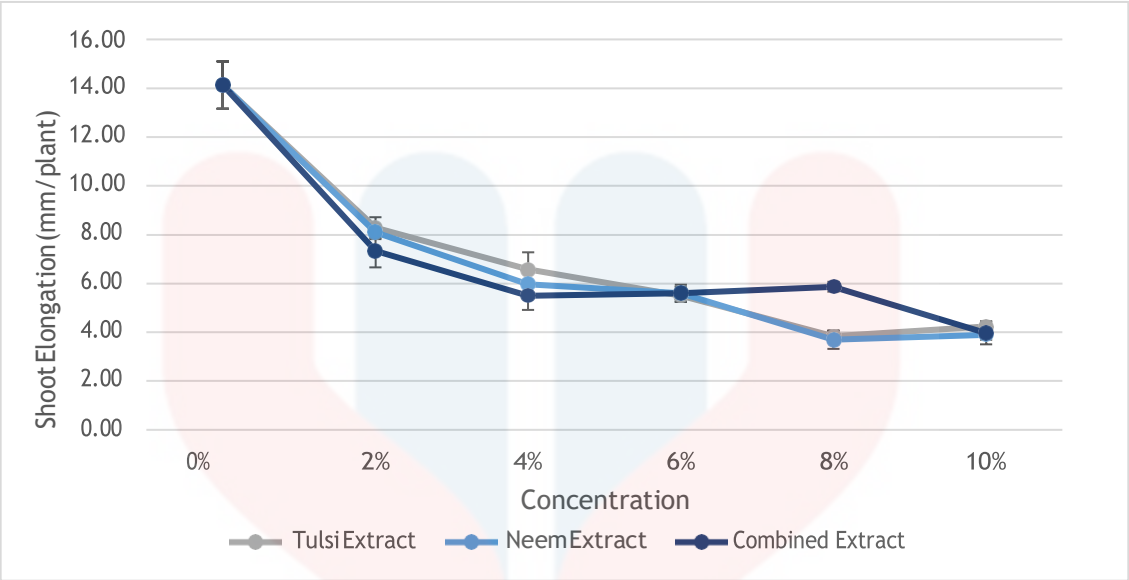


Figure 4.5. The effects of various extracts on goose grass seed in nursery conditions. Tulsi extract, Neem extract, Combined extract effects on the shoot elongation (mm/plant) of *Eleusine indica* with different concentrations. Data are the means of three independent replicated with standard errors shown by vertical bars.

4.2.3 Root length

The significant reduction in root length of *E.indica* by various (2%, 4%, 6%, 8% and 10%) three extracts. The results clearly stated in Figure 4.6. that all three aqueous leaves extracts (Tulsi, Neem and combined) with different concentrations have inhibitory properties and thus contain allelopathic substances for the plant growth. The results from Figure 4.6, showed the statistically significant inhibition by all the treatments where the 10% concentrations of all three extracts reduce the length nearly to 4mm. it also depicted that in low concentrations (0%, 2%, 4%) the Tulsi showed least effectiveness where in the higher concentrations (6%, 8%, 10%) Tulsi was the most effective. This surprising result has been discussed by Samunder Singh and Megh Singh (2014) related to how different concentrations of Tulsi influenced the inhibitory activities of seed germination and seedling growth of E.indica. Table 4.4 revealed that 0% concentration of root length recorded was 14.63±0.95mm and the 10% of Tulsi recorded the least reduction mean

value of 3.97±0.09mm. Thus, it can be said that the differences between this two group was significant. When comparing the concentrations of combined extracts, the Tukey HSD (Table 4.6) shown that except for control treatment and the 2% concentrations, the other concentrations are not significantly alike. Since there were inhibitory effects yet not significant enough, it can be said that the concentrations greater than 10% can be used to treat the plant for greater inhibitory effects. Generally, the roots are the first target tissue to confront with the phytotoxic substances, therefore inhibitory effects are more evident on roots rather than on shoots (Jagtap et al., 2016). The roots of weeds grown in control treatment were discovered to be healthy and long root growth whereas the root growth of seed treated with extracts was restricted. This significant difference in inhibition of the plant growth may due to competition among the plants in the area and allelochemicals that found in the extracts.

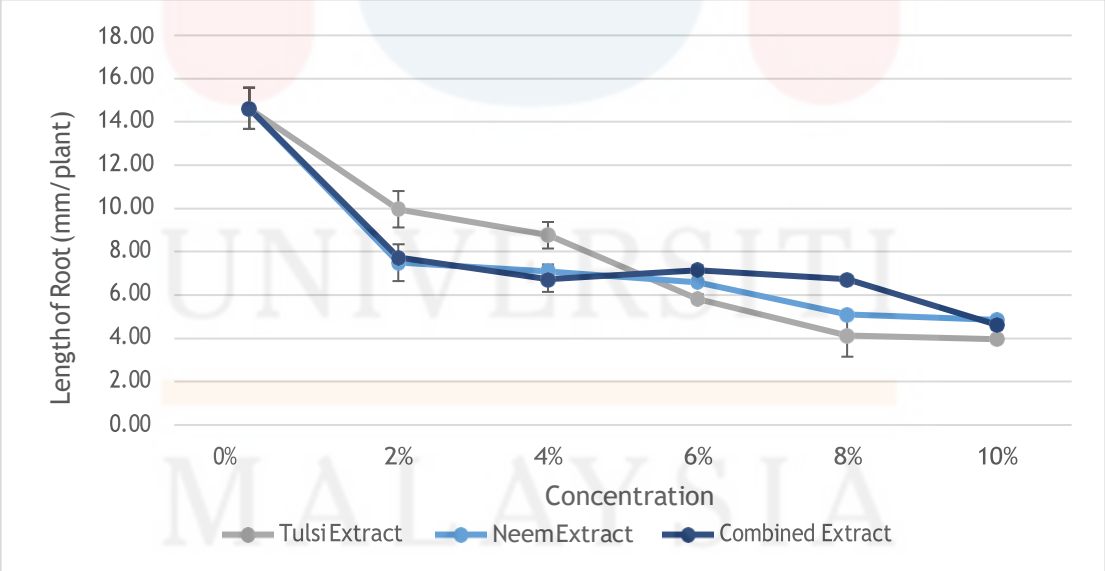


Figure 4.6. The effects of various extracts on goose grass seed in nursery conditions. (a) Tulsi extract, (b) Neem extract, (c) Combined extract on the length of the root of *Eleusine indica* with different concentrations. Data are the means of three independent replicated with standard errors shown by vertical bars.

4.2.4 Shoot Fresh Weight

As shown in Figure 4.7, the shoot fresh weight decreased as the concentrations (2%, 4%,6%, 8%, and 10%) increased for each of the aqueous leave extracts (Tulsi, Neem and combined). The greatest inhibitory effects were recorded by 10% extract where it has statistically significant inhibition was nearly 90% showed in Figure 4.7. The highest inhibition on shoot fresh weight of the plant was recorded by 10% of combined extract with 6.33% of where the inhibition was 93.66% as in Table 4.6. While 10% of Tulsi and 10% of Neem extracts held records of 10% and 6.67% respectively. This is evidence of high concentrations have a greater influence on plant growth (Sharma & Singh, 2003; Siddiqui et al., 2009). As previously mentioned, allelochemicals are capable of stunting the plant growth by interrupting the metabolic process of the plants. There was a significant range of different with the shoot fresh weight of control sample obtained when compared to all the test plants. Figure 4.7 clearly stated that the difference between the 0% extracts and 2% extracts was near to 60% by the combined extract. It is mentioned by Dilipkumar et al (2015), once the weed successfully emerged, it enjoys the same soil moisture conservation and other mulch benefits as does the established crop, resulting in increased of shoot fresh weight.

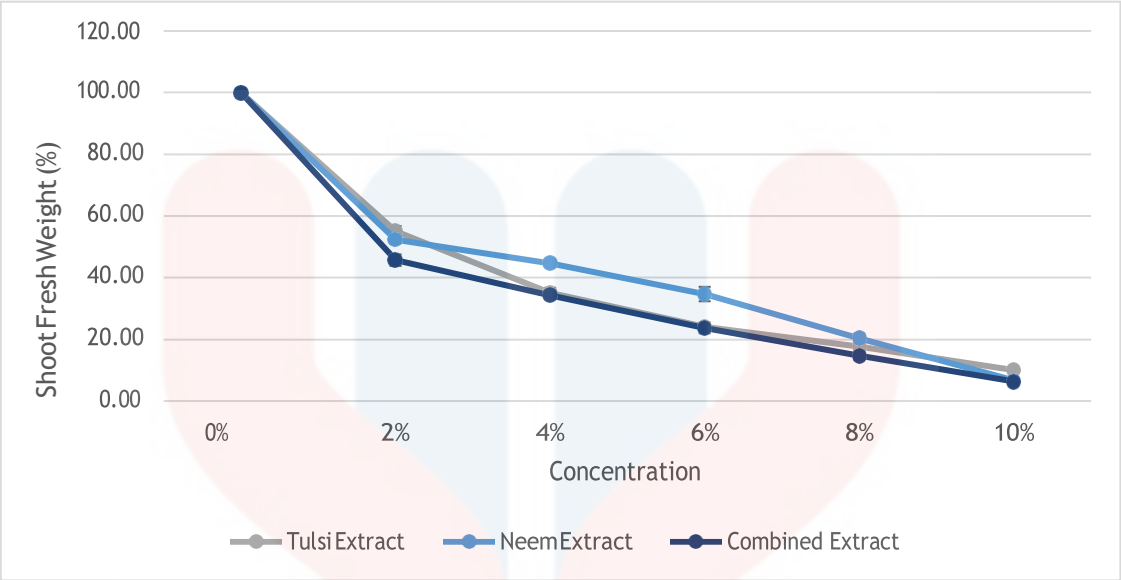


Figure 4.7. The effects of various extracts on goose grass seed in nursery conditions. (a) Tulsi extract, (b) Neem extract, (c) Combined extract on the shoot fresh weight (% of control) of *Eleusine indica* with different concentrations. Data are the means of three independent replicated with standard errors shown by vertical bar.

Table 4.4. Mean value of different concentration of aqueous Tulsi leaf extract treatments on seed emergence (% of control), shoot elongation (% of control), length of root (% of control) and shoot fresh weight (% of control) of *Eleusine indica* under nursery condition. (mean± standard error)

Concentration	Seed emergence (%/plant)	Shoot Elongation (mm/plant)	Length of Root (mm/plant)	Shoot Fresh Weight (%/ plant)
0%	100±0e	14.13±0.97d	14.63±0.95d	100±0.00f
2%	80.0±2.3d	8.27±0.44c	9.97±0.84c	55±1.73e
4%	65.3±3.5c	6.57±0.71bc	8.77±0.61bc	35±1.73d
6%	54.7±1.3b	5.5±0.25ab	5.83±0.20ab	24±1.16c
8%	40.0±2.3a	3.83±0.20a	4.13±0.98a	17.67±1.45b
10%	34.7±1.3a	4.23±0.22ab	3.97±0.09a	10±0.58a

Table 4.5. Mean value of different concentration of aqueous Neem leaf extract treatments on seed emergence (% of control), shoot elongation (% of control), length of root (% of control) and Shoot Fresh Weight (%of control) of *Eleusine indica* under nursery condition. (mean± standard error)

Concentration	Seed emergence (%/plant)	Shoot Elongation (mm/plant)	Length of Root (mm/plant)	Shoot Fresh Weight (%)
0%	100± 0.00e	14.13±0.97d	14.63±0.95c	100±0.00f
2%	68± 2.31d	8.1± 0.21c	7.5±0.85b	52.33±1.20e
4%	53.33± 1.33c	5.97±0.48ab	7.1±0.32ab	44.67±0.88d
6%	44±2.31b	5.6± 0.35a	6.6± 0.06ab	34.67±2.33c
8%	36±2.31b	3.7± 0.38a	5.07± 0.09ab	20.3±1.45b
10%	17.33±1.33a	3.9± 0.21a	4.87± 0.09a	6.67±0.67a

Table 4.6. Mean value of different concentration of aqueous combine extract treatments on seed emergence (% of control), shoot elongation (mm/ plant), length of root (mm/ plant) and Shoot Fresh Weight (%of control) of *Eleusine indica* under nursery condition. (mean± standard error)

Concentration	Seed emergence (%/plant)	Shoot Elongation (mm/plant)	Length of root (mm/plant)	Shoot Fresh Weight (%/plant)
0%	100± 0.00e	14.13± 0.97c	14.63±0.95c	100±0.00f
2%	56± 2.31d	7.33± 0.67b	7.73± 0.15b	45.67± 1.86e
4%	40± 2.31c	5.5±0.59ab	6.73± 0.59ab	34.33± 1.20d
6%	28± 2.31b	5.6±0.27ab	7.17± 0.20b	23.67± 1.76c
8%	12± 2.31a	5.9± 0.20ab	6.73± 0.15ab	14.67± 1.45b
10%	13.33±1.33a	3.97± 0.46a	4.63± 0.22a	6.33± 0.88a

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Eleusine indica is a great problem in agricultural field especially paddy fields. Chemical herbicide is the only solution to suppress the weed growth which is also polluting the environment and causing harm to human and animals. Bioherbicide can be the alternative option to control weed. Thus, by using allelopathic plants such as Tulsi and Neem to turn as bioherbicide in order to manage the weed in eco-friendly way which was investigated in this study to determine the allelopathic effects of Tulsi and Neem leaves extract on seed germination and plant growth of Goose grass weed. Along with determining their influence towards the goose grass, the concentrations (2%,4%, 6%,8% and 10%) that influenced the plant growth was also measured. By taking three types of extracts and 5 different concentrations, the suitable extracts with best concentration was determined. In order to determine the extracts and concentration, data collection on parameters such as seed emergence, shoot elongation, length of root and shoot fresh weight was conducted in both laboratory and nursery conditions. After interpreting and data analysis, it can be concluded that the greater concentration gave strong weed control

effects. Through conducting this study in laboratory condition and field condition, the best extract of all the treatment was T16, the combined extract where Tulsi and Neem aqueous extracts were combined together. In wholesome, the combined extract possessed a significant inhibition of seed emergence, shoot elongation, length of root and shoot fresh weight. The inhibition was stronger as the concentration of the aqueous extracts increased, the 10% concentration of aqueous leaves extract of Tulsi, Neem and the combine gave a greater effect. This may due to the presence of allelochemical in the aqueous plant extracts from two different allelopathic plants which are responsible in suppressing the plant growth. This whole study strongly suggests that aqueous Tulsi, Neem and combine can be used for weed control management while the best to be said is combine extract. This can be used as source or reference to produce bioherbicide to protect our environment and human health.

5.2 Recommendation

Further research can be done to study the parts of the plants with higher concentrations such as 15%, Tulsi and Neem that contain higher amount of allelochemicals. This will help to commercialize the bioherbicide and increase the usage of bioherbicide. Besides, research on the allelochemicals that are responsible to the allelopathy in Tulsi and Neem could also be done. This will help to focus specifically on the allelochemicals which might found in other weeds. As the demand of bioherbicide in agriculture field is increasing this plant extracts could be a good solution or source for a best bioherbicide.

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APPENDIX A

Figure A.1. *Eleusine Indica* plant.

Figure A.2. Tulsi leaves into powder form.

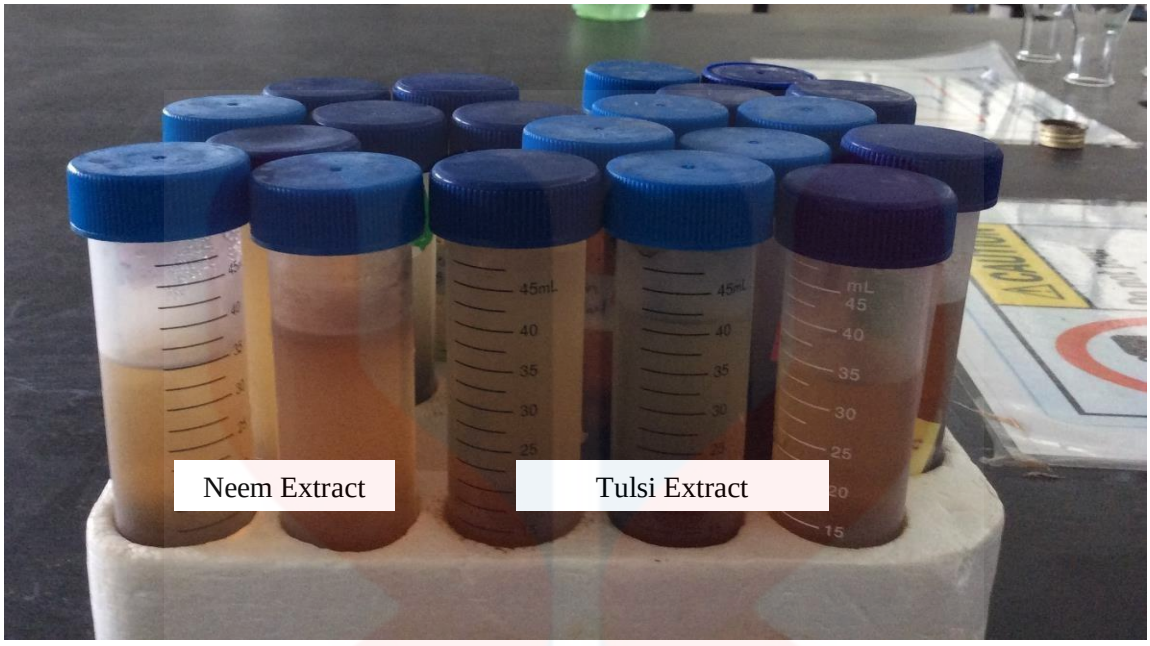


Figure A.3. Aqueous Tulsi, Neem and combine leaves extracts.

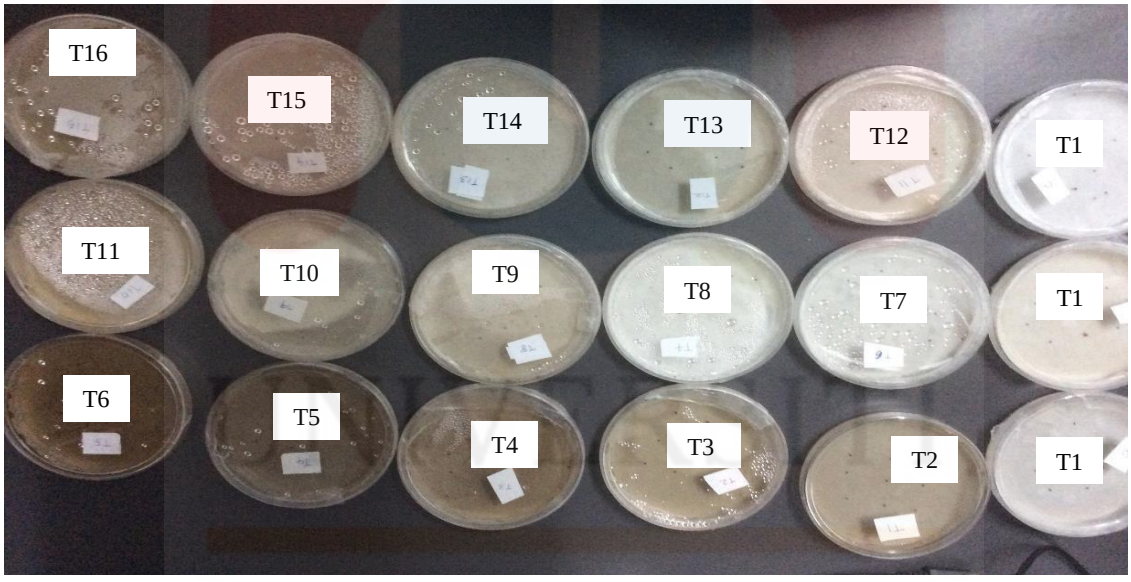


Figure A.4. Sample prepared and arranged according to concentration for seed emergence in laboratory conditions

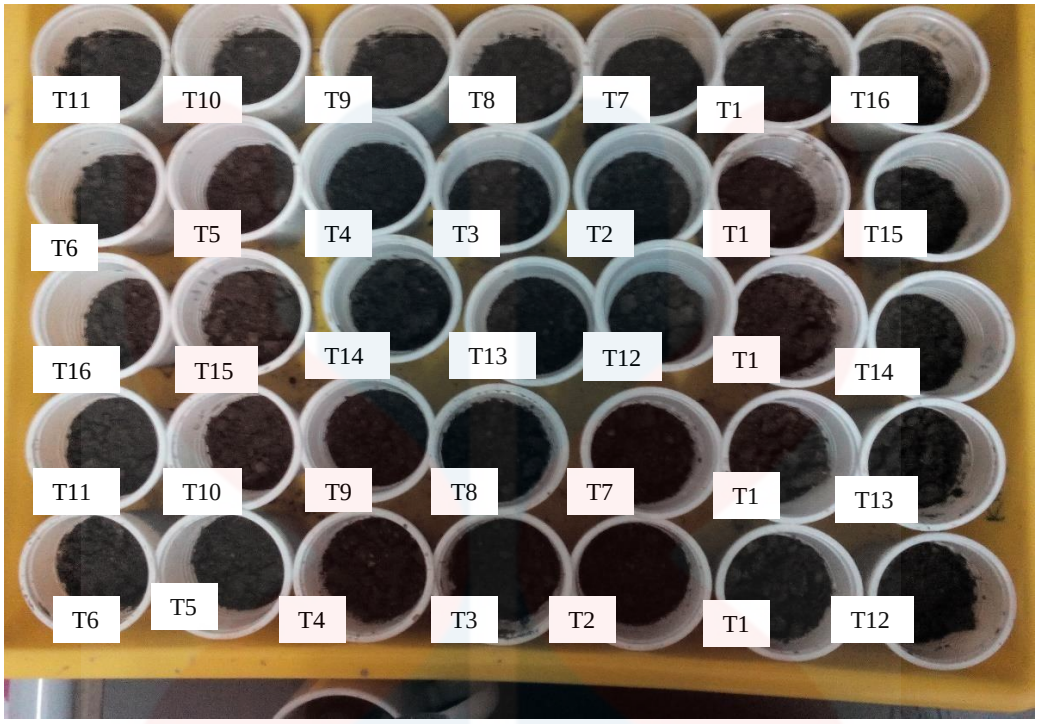


Figure A.5. Sample prepared after seed sowing and arranged according to concentration in nursery.

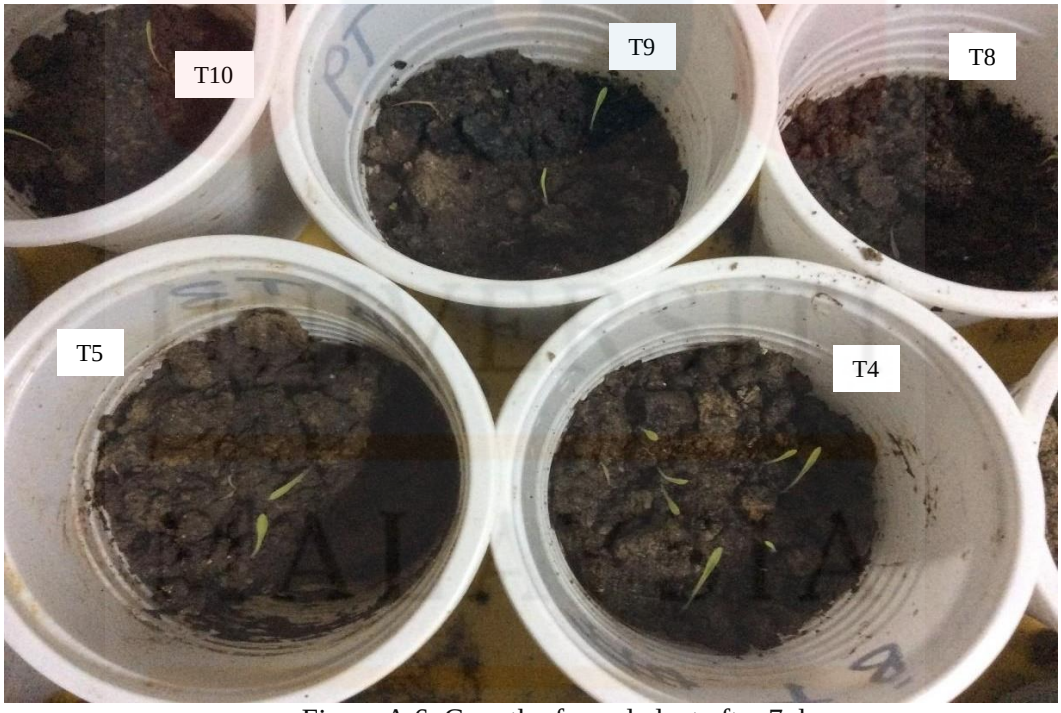


Figure A.6. Growth of weed plant after 7 days.

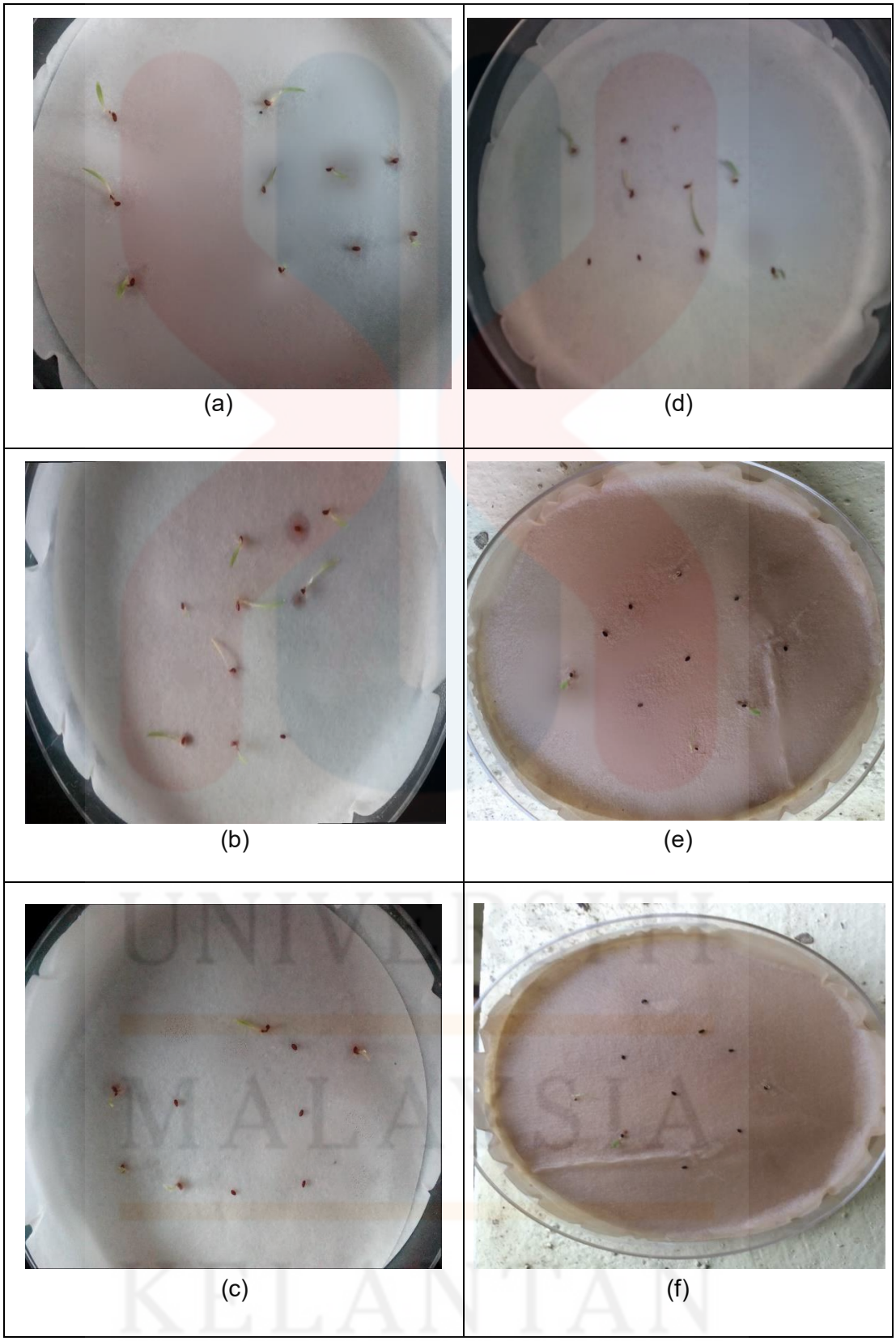


Figure A.7. Seed emergence of all six concentrations 0% (a), 2% (b), 4% (c), 6% (d), 8% (e) and 10% (f) (T1-T6) in aqueous Tulsi extract plate and kept in laboratory condition.

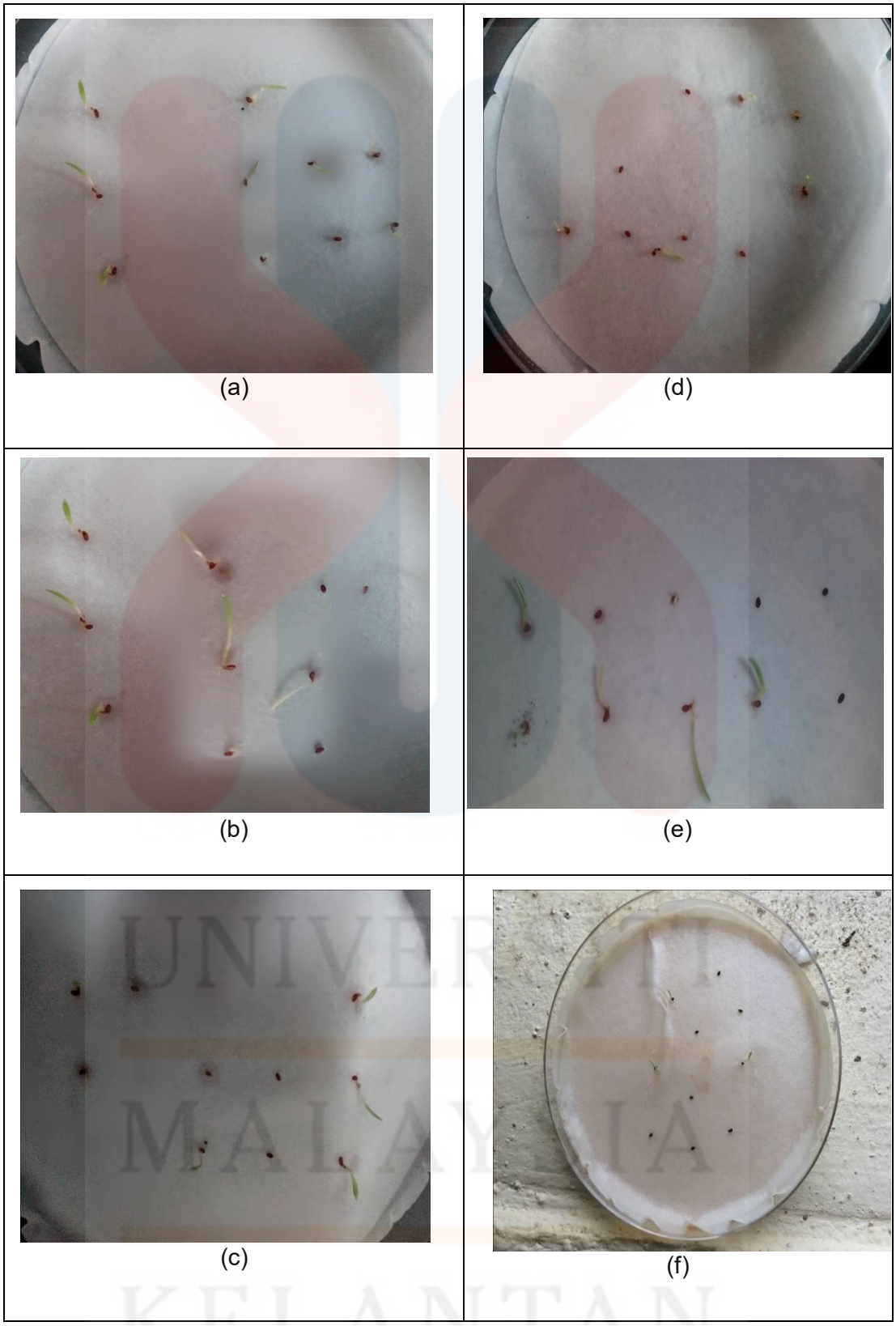


Figure A.8. Seed emergence of all six concentrations 0% (a), 2% (b), 4%, 6% (d), 8% (e) and 10% (f) (T7-T11) in aqueous Neem extract plate and kept in laboratory condition.

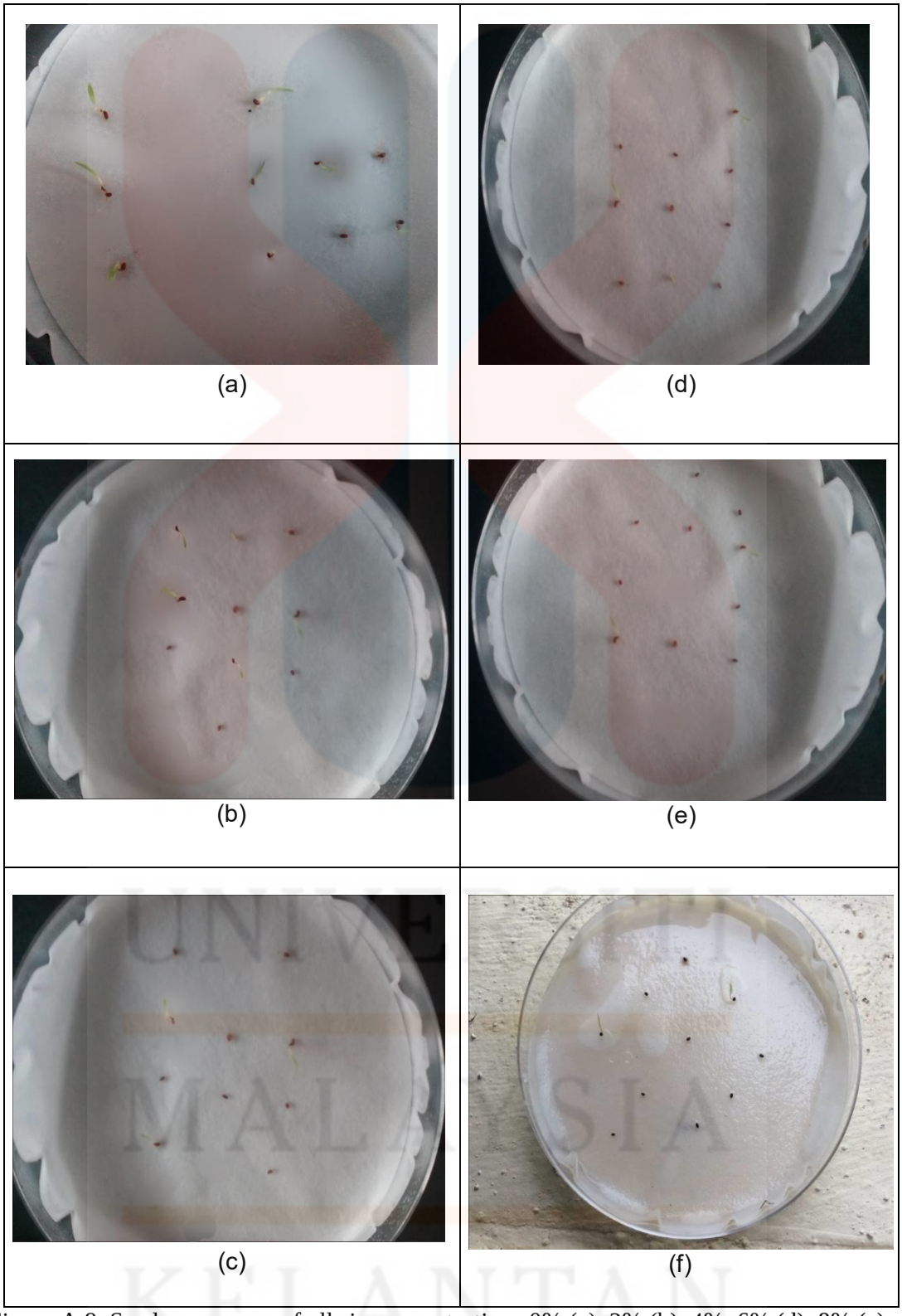
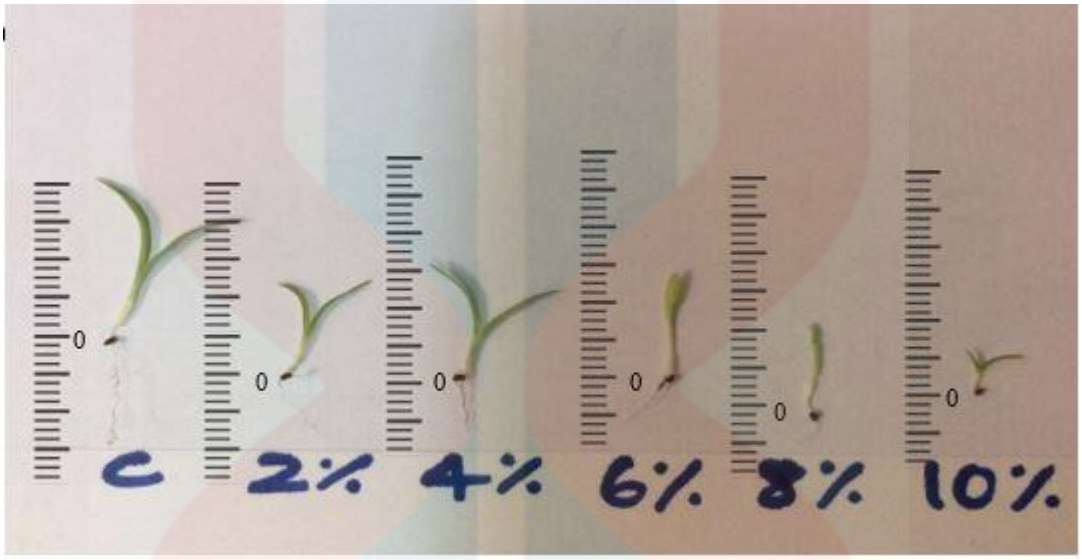
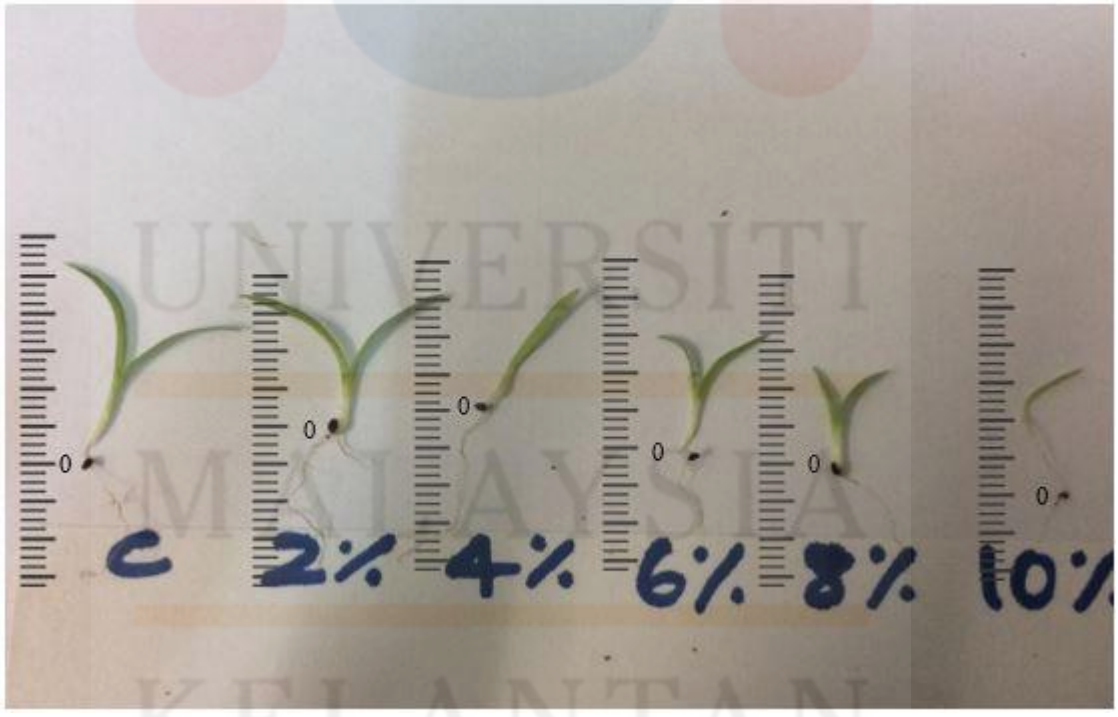


Figure A.9. Seed emergence of all six concentrations 0% (a), 2% (b), 4%, 6% (d), 8% (e) and 10% (f) (T12-T16) in aqueous combined extract plate and kept in laboratory condition.



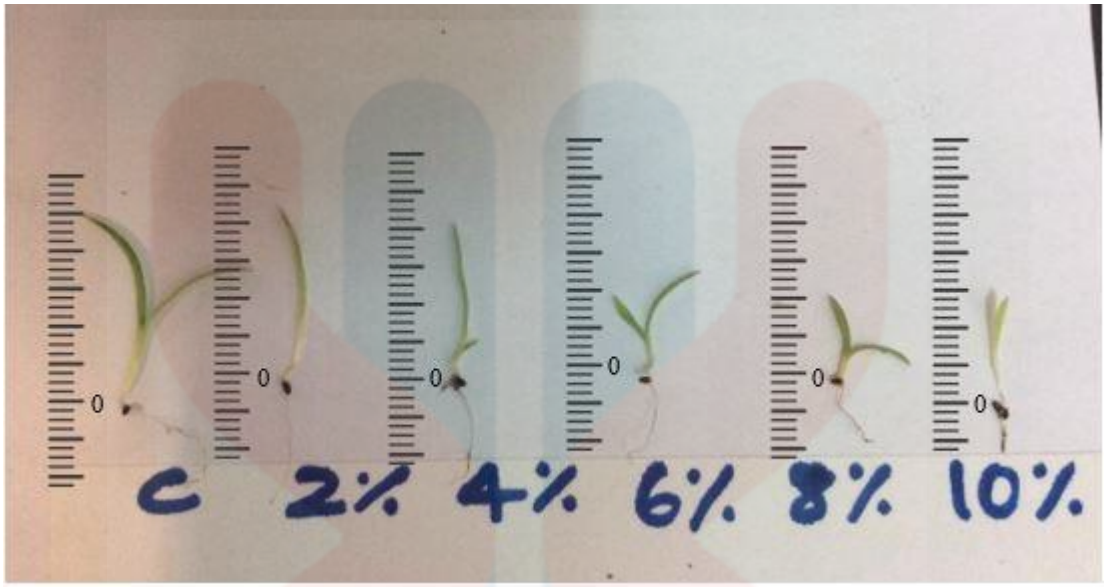
*C= 0% concentration of the aqueous extract

Figure A.10. The difference in shoot elongation and length of root of the weeds for all 6 concentrations including control of Tulsi extract in comparison.



*C= 0% concentration of the aqueous extract

Figure A.11. The difference in shoot elongation and length of root of the weeds for all 6 concentrations including control of Neem extract in comparison.



*C= 0% concentration of the aqueous extract

Figure A.12. The difference in shoot elongation and length of root of the weeds for all 6 concentrations including control of combined extract in comparison.

APPENDIX B

Table B.1. ANOVA test results of Tulsi extract for germination percentage, shoot elongation, length of root and shoot fresh weight on *Eleusine indica* under laboratory condition.

		Sum of Squares	df	Mean Square	F	Sig.
SE	Between Groups	11977.778	5	2395.556	86.240	.000
	Within Groups	333.333	12	27.778		
	Total	12311.111	17			
SEL	Between Groups	238.223	5	47.645	171.520	.000
	Within Groups	3.333	12	.278		
	Total	241.556	17			
SFW	Between Groups	12523.833	5	2504.767	495.448	.000
	Within Groups	60.667	12	5.056		
	Total	12584.500	17			

**SE= Seed Emergence, SEL= Shoot Elongation, SFW= Shoot Fresh Weight*

Table B.2. ANOVA test results of Neem extract for germination percentage, shoot elongation, length of root and shoot fresh weight on *Eleusine indica* under laboratory condition.

		Sum of Squares	df	Mean Square	F	Sig.
SE	Between Groups	9827.778	5	1965.556	70.760	.000
	Within Groups	333.333	12	27.778		
	Total	10161.111	17			
SEL	Between Groups	196.651	5	39.330	37.537	.000
	Within Groups	12.573	12	1.048		
	Total	209.224	17			
SFW	Between Groups	15304.444	5	3060.889	550.960	.000
	Within Groups	66.667	12	5.556		
	Total	15371.111	17			

**SE= Seed Emergence, SEL= Shoot Elongation, SF W= Shoot Fresh Weight*

Table B.3. ANOVA test results of combine extract for germination percentage, shoot elongation, length of root and shoot fresh weight on *Eleusine indica* under laboratory condition.

		Sum of Squares	df	Mean Square	F	Sig.
SE	Between Groups	11761.111	5	2352.222	105.850	.000
	Within Groups	266.667	12	22.222		
	Total	12027.778	17			
SEL	Between Groups	270.038	5	54.008	381.230	.000
	Within Groups	1.700	12	.142		
	Total	271.738	17			
SFW	Between Groups	15843.611	5	3168.722	704.160	.000
	Within Groups	54.000	12	4.500		
	Total	15897.611	17			

**SE= Seed Emergence, SEL= Shoot Elongation, SFW= Shoot Fresh Weight*

Table B.4. ANOVA test results of Tulsi extract for seed emergence, shoot elongation, length of root and shoot fresh weight on *Eleusine indica* under nursery condition.

		Sum of Squares	df	Mean Square	F	Sig.
SE	Between Groups	9188.444	5	1837.689	137.827	.000
	Within Groups	160.000	12	13.333		
	Total	9348.444	17			
SEL	Between Groups	217.684	5	43.537	48.766	.000
	Within Groups	10.713	12	.893		
	Total	228.398	17			
LR	Between Groups	252.865	5	50.573	33.678	.000
	Within Groups	18.020	12	1.502		
	Total	270.885	17			
SFW	Between Groups	16512.944	5	3302.589	675.530	.000
	Within Groups	58.667	12	4.889		
	Total	16571.611	17			

**SE= Seed Emergence, SEL= Shoot Elongation, LR= Length of Root, SFW= Shoot Fresh Weight*

Table B.5. ANOVA test results of Neem extract for seed emergence, shoot elongation, length of root and shoot fresh weight on *Eleusine indica* under nursery condition.

		Sum of Squares	df	Mean Square	F	Sig.
SE	Between Groups	12228.444	5	2445.689	250.127	.000
	Within Groups	117.333	12	9.778		
	Total	12345.778	17			
SEL	Between Groups	226.687	5	45.337	59.567	.000
	Within Groups	9.133	12	.761		
	Total	235.820	17			
LR	Between Groups	193.836	5	38.767	44.054	.000
	Within Groups	10.560	12	.880		
	Total	204.396	17			
SFW	Between Groups	15726.444	5	3145.289	615.383	.000
	Within Groups	61.333	12	5.111		
	Total	15787.778	17			

**SE= Seed Emergence, SEL= Shoot Elongation, LR= Length of Root, SFW= Shoot Fresh Weight*

Table B.6. ANOVA test results of combined extract for seed emergence, shoot elongation, length of root and shoot fresh weight on *Eleusine indica* under nursery condition.

		Sum of Squares	df	Mean Square	F	Sig.
SE	Between Groups	16441.778	5	3288.356	284.569	.000
	Within Groups	138.667	12	11.556		
	Total	16580.444	17			
SEL	Between Groups	196.993	5	39.399	38.292	.000
	Within Groups	12.347	12	1.029		
	Total	209.340	17			
LR	Between Groups	177.863	5	35.573	51.307	.000
	Within Groups	8.320	12	.693		
	Total	186.183	17			
SFW	Between Groups	17001.111	5	3400.222	624.531	.000
	Within Groups	65.333	12	5.444		
	Total	17066.444	17			

**SE= Seed Emergence, SEL= Shoot Elongation, LR= Length of Root, SFW= Shoot Fresh Weight*