Herbicidal Activities of N Fertilizer (Urea) Treated with Natural Plants Oil Herbicide on Control Barnyard grass (*Echinochloa crus-galli*) at the Vegetative Stage

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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 "Herbicidal Activities of N Fertilizer (Urea) Treated with Natural Plants Oil Herbicide on Control Barnyard grass (*Echinochloa crus-galli*) at the Vegetative Stage" by Fatin Nur Diyana Bt Mohd Zaini, matric number F18b0297 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agriculture Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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ABSTRACT

Introduction of new weed management in crops production are required since current weed control strategy is less effective and highly herbicide dependent, thus leading to development of herbicide resistance weeds as well as environmental and human health concerns. This study aimed to evaluate phytotoxic effects of N fertilizer (urea) treated with natural plant oil herbicide (lemon grass) for barnyard grass (*Echinochloa crus-galli*) control at the vegetative stage. The bioassay weed species was treated with a series application rate of urea (80, 160, and 240 kg ha^{-1}) and natural plant oil herbicide (0.01, 0.02, and 0.04 g/L). The results in current study have shown that natural plant oil herbicide at 0.04 g/L in combination with urea at rate of 80 kg ha^{-1} (**T7**) strongly inhibited *E. cruss galli* emergence by 90 %. Meanwhile, starting from 0.02 to 0.04 g/L natural plant oil herbicide with 80 to 240 kg ha-1 urea (**T4-T9**), the shoot fresh weight of *E. crus-galli* was significantly reduced by 67-94% inhibition. At these similar application rates, the root length of *E. crus-galli* was very susceptible to natural plant oil herbicide and urea fertilizer where the treated bioassay species shows 54-78% reduction in root length. The results of this study suggest that regardless of any application rate, the integration of natural plant oil herbicide provide effective weed control by increase the phytotoxicity of urea for weed inhibition.

Keywords: Natural Plants Oil Herbicide, Urea Fertilizer, Weeds

Aktiviti Herbisid Baja N (Urea) Dirawat dengan Herbisid Minyak Tumbuhan Semulajadi dalam Mengawal Barnyard grass (*Echinochloa crus-galli*) pada Peringkat Vegetatif

ABSTRAK

Pengenalan pengurusan rumpai baharu dalam pengeluaran tanaman diperlukan memandangkan strategi kawalan rumpai semasa adalah kurang efektif dan sangat bergantung kepada herbisid, seterusnya membawa kepada pembangunan kerintangan rumpai terhadap herbisid serta kebimbangan terhadap kesihatan alam sekitar dan manusia. Kajian ini bertujuan untuk menilai kesan fitotoksik baja N (urea) yang dirawat dengan herbisid minyak tumbuhan semulajadi (serai) untuk kawalan Barnyard grass (*Echinochloa crus-galli)* pada peringkat vegetatif. Spesies rumpai bioassai telah dirawat dengan satu siri kadar aplikasi urea (80, 160, dan 240 kg ha^{-1}) dan herbisid minyak tumbuhan semulajadi (0.01, 0.02, dan 0.04 g/L). Keputusan dalam kajian ini menunjukkan bahawa herbisid minyak tumbuhan semulajadi pada 0.04 g/L dan kombinasi dengan urea pada kadar 80 kg ha^{-1} (**T7**) menghalang kemunculan *E. cruss galli* dengan kuat sebanyak 90 %. Sementara itu, bermula dari 0.02 hingga 0.04 g/L herbisid minyak tumbuhan semulajadi dan 80 hingga 240 kg ha^{-1} urea (**T4-T9**), berat segar pucuk *E. crus-galli* telah dikurangkan dengan signifikan sebanyak 67-94% perencatan. Pada kadar aplikasi yang sama ini, panjang akar *E. crus-galli* sangat rentan kepada herbisid minyak tumbuhan semulajadi dan baja urea di mana spesies bioassai yang dirawat menunjukkan pengurangan 54-78% dalam panjang akar. Keputusan kajian ini mencadangkan bahawa tanpa mengira kadar aplikasi, integrasi herbisid minyak tumbuhan semulajadi memberikan kawalan rumpai yang berkesan dengan meningkatkan fitotoksisiti urea untuk perencatan rumpai.

Kata Kunci: Herbisid Minyak Tumbuhan Semulajadi, Baja Urea, Rumpai

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LIST OF SYMBOLS

N	Nitrogen
Ha	Hectares
KG	Kilogram
G	Gram
%	Percentage
Cm	Centimetre
g/L	Gram per litre
t ha-1	Tan per hectare
ml	Millilitre
mm	Millimetre
cm	Centimetre

LIST OF ABREVIATION

SPSS	Statistical Product and Service Solution
ANOVA	Analysis of variance
HSD	Honest Significance Different
Df	Degree of freedom
F	F-test
Sig	Significant
SD	Standard Deviation
ED50	Effective Dose for 50%

FYP FIAT

CHAPTER 1

INTRODUCTION

1.1Research Background

Barnyard grass (*Echinochloa crus-galli*) is a type of grass that originated in Europe and India. Barnyard grass (*Echinochloa crus-galli*) is widespread throughout the world's warm temperate and subtropical regions, extending into the tropics. Randall. (2017) had stated that *E.crus-galli* was remembered for the Global Compendium of Weeds and is viewed as one of the world's most noticeably awful weeds. The majority of this weed was discovered in Malaysian paddy fields. It is a common weed in irrigated crops grown on moist soils, such as rice, however, it is additionally a weed in numerous vegetable and field crops, like lettuce, tomato, and corn. Barnyard grass thrives in damp soil and can be found in plant nurseries or near water drainage areas. Weed plants, on the other hand, are undesirable because they compete with rice plants for nutrients, space, sunlight, and water. This can result in stunted paddy growth and poor paddy quality.

Fertilizer N (urea) is unquestionably an important nutrient that influences major functions such as photosynthesis, plant development, flowering, and senescence. There are some herbicides that work on crops as well as weeds, but the ones derived from urea work to a lesser the extent crop. There are also many examples, one of which is Soltani and Saeedipour (2015), who discovered a graminaceous herbicide effect and an important increase in wheat crop yields on silty clay soil.

For the past 10 years, Nichols et al. (2015) had discovered that N fertilizer can breaks seed dormancy and can influence density and structure of the weed. Azeez and Adetunji (2009) made an observation in maize crop where the best use of nitrogen has characterized genotypes with high uptake potential and 90 kg N ha-1 applied in urea form helped to increase the culture's grain yield and competitiveness against weeds.

In a farming systems, synthetic herbicides cannot be used to control weeds. As a result, hand hoeing, inter-row tillage, mulching, and ground covering are the most common weed control methods. However, these methods are expensive and may not be effective in controlling weeds. As a result, naturally occurring biologically active compounds from plants have gotten a lot of attention in recent years as a rich source of potential weed-control agents. Plants oil are a rich source of alternative and environmentally friendly weed control compounds. Oils from plant seeds, leaves, stems or flowers include plant oils. Plants oil are triglycerides that have three fatty acids esterified to the glycerine. They make up the majority of vegetable and animal fats.

Fatty acids in naturally occurring triglycerides can have a variety of chain lengths, but the most common are 16, 18, and 20 carbons. Because of the way they are bio-synthesized from acetyl CoA, natural fatty acids found in plants and animals typically contain only even numbers of carbon atoms. According to Elshafie and Camele (2017), several studies have found that plant oil can be used to control weeds. The majority of natural plants oil are non-residual, safe for both people and wildlife, inexpensive, and beneficial. The advantage of utilizing such natural compounds is the rapid breakdown process into the environment and thus the potential application in sustainable agriculture such as organic farming.

1.1 Problem Statement

Globally, many herbicides have recently been criticized as chemical contaminants due to environmental concerns, and it has been suggested that their detrimental effects on the environment greatly outweighs any benefits that might be realized by their use. Most farmers in Malaysia still tend to rely on chemical herbicides as their sole strategy for weed control (Halimatunsadiah et al., 2016). There is evidence that adding fertilizers to the solution of herbicides can increase its activity in the control of annual weeds (El-Shahawy, 2007). According to Norhafizah et al. (2017), N fertilizer (urea) application alone, not enough to control the selected weed species. They reported that combination of commercial herbicide, propanil + thiobencarb and N fertilizer gave significant herbicidal activity in control Eleusine indica in dry seeded rice. However, to date, there is no study reported on the efficacy of urea fertilizer combined with the natural herbicides for weed control. Thus, natural plant oils would be a promising botanical herbicide to be combined with urea fertilizer to reduce the weed infestation, especially banyard grass *(Echinochloa crus-galli),* one of the most troublesome weed species in Malaysian rice field.

1.2 Hypothesis

H0 = N fertilizer (urea) and natural plants oil will not significantly inhibit the emergence and growth of barnyard grass (*Echinochloa crus-galli*).

HA= N fertilizer (urea) and natural plants oil will significantly inhibit the emergence and growth of barnyard grass (*Echinochloa crus-galli*).

1.3 Objective

1. To determine the effectiveness of N fertilizer (urea) treated with natural plants oil herbicide to control the emergence and growth of barnyard grass (*Echinochloa crus-galli*).

1.5 Scope of Study

The focus of the study is to determine the effect of N fertilizer (urea) treated with natural plants oil herbicide towards the barnyard grass (*Echinochloa crus-galli*) Seedlings. This research is also very important to obtain the suitable ratio of N fertilizer (urea) to inhibit the weed. The study's duration will be two months, depending on the amount of data collected and only quantitative information will measure the growth of the barnyard grass plant.

1.4 Significance of Study

Weed control is regarded as the most difficult challenge for farmers. Lower plant productivity in farming is primarily due to ineffective weed control. N fertilizer (urea) and natural plant oil herbicide will act to reduce herbicide usage rate. This research can help Malaysia to increase the rice yield by reducing the problem of barnyard grass in the paddy field.

CHAPTER 2

LITERATURE REVIEW

2.1 Barnyard Grass (*Echinochloa crus-galli*)

Figure 2.1: Barnyard Grass (*Echinochloa crus-galli*) at the rice field

2.1.1Characteristic and life cycle

Barnyard grass (*Echinochloa crus-galli*) is also called cockspur, barnyard millet, jajagoan and rumput padi burung. Barnyard grass (*Echinochloa crus-galli*) is one of the famous grass in the Malaysian paddy fields. (*E. crus-galli*) is a tough annual tufted grass with erect or geniculate culms that grow up to 1.5 feet tall and sometimes rooting and branching at the base. It has a vertical stem with height 1 m to 2 m, tapered leaves and pointed length 10 cm until 30 cm. The production of individual barnyard grass plants from 30 thousand seeds to over 200 000, with a mean of 100 000 seeds per plant according to environmental conditions, has been reported (Norris, 1992). Every year, barnyard grass is reproduced by caryopsis seeds, and the wall of the ovary is fused with a grain coat common to many grasses (Koo et al., 2000). The flower bouquet is usually 10 cm until 20 cm long and consists of 9 to 10 branches. It spikelet usually have long hairs while it will flowers throughout the year and reproduces through seeds. This grass is branched and has spikelet that grow singly. This type of weed usually has a round stem and tapered leaves. Plants can grow directly or directly along the ground depending on their habitat. The external layer of the stem (the sheath) is smooth and has a light shade of maroon to the base. The ripe, white and broad plant collar is smooth. From July to September, plants flourish. The root is fibrous and superficial.

Barnyard grass roots in nature are fibrous while the culm is smooth, thick, heavy and hairless. The side parts of farm grass and roots are created from the declining base, and the tallness of the plant is around 1.5 m. The opening on the ascent is smooth and rolling, while the completely evolved opening can have smooth or scant edges (Maun and Barret, 1986). Bloom is panicle; enormous quantities of little assessed flowers are annexed to rachis through little stalks (Chin, 2001). Awn, a hardened fibre developing from the ear of grasses, if present is variable; 0e1 cm long and can grow up to 3 cm sometimes. It has 54 chromosomes (2n) and is haploid with basic chromosome 9 (Maun and Barret, 1986).

2.1.2 Geographical distribution

Barnyard grass (*E. crus-galli*) has been distributed to 61 countries around the world and grows into weeds every day. It is found at a scope of 50 N to 40 S, and in certain nations like Canada, it additionally exists over 50 N scope. It is a plant in a hotter environment. It comprises 40 until 50 species all through the warm tropical and calm locales on the planet (Michael, 2003). *E. crus-galli* has spread broadly from one side of the planet to the other over the most recent thirty years. Its dispersion and presence have increased from 3.3% in the 1980s to 8.4 % in the 2000s (Lesson et al., 2005). According to Leeson et al. (2005), the region under its pervasion increased from 0.5 % to 1.8 %, while the thickness increased from 4.4 to 8.2 plants m from the 1980s to the 2000s.The weed has involved immense territories in North America, Canada, and Mexico (Hoste, 2004). Pratleyet al. (2008) found that it has been perceived as a significant weed of rice in Australia. The event on wet terrains of California, South Dakota, North Dakota, Kansas, Pennsylvania, Texas, and Arizona portrays its capacity to adapt assorted climatic and geological conditions (Van Devender et al., 1997).

2.1.3 Interference in Agriculture

Studies show that *E. crus-galli* for each square foot can decrease rice yields by about 25%, and 25 of *E. crus-galli* for every square meter can cause about half yield misfortune. The develop plant becomes higher than rice, with the goal that it seeks daylight, other than soil supplements. It is likewise a substitute host for tungro and rice yellow diminutive person infections. *E. crus-galli* can reduce corn yields by 20%. It is an alternate host for corn's pests. The emergence of *E. crus-galli* along with the emergence of crops provides more competition to crops than the late emergence of the barnyard grass. *E. crus-galli* was found to produce 39,000 seeds per plant when it was planted along with rice while seed production was reduced to 14,700 seeds per plant when controlled for up to 5 weeks (Bagavathiannan et al., 2012). When the weed was found in the 3-and 4-leaf phases of maize, 34,600 seeds of m 2 (10 m liters of a density) were produced, respectively (Bosnic and Swanton, 1997).

2.1.4. Competitive features

Barnyard grass is a major weed in a variety of field crops, including rice (Chauhan and Johnson, 2011). Rao et al. (2007) have stated, under reduced rice soil conditions, that *E. crus-galli* is the largest plant. It continues to grow well and grow in humid, partly immersed conditions (Chauhan and Johnson, 2009). It is adapted by evolving essential physiological traits to a wide variety of environmental conditions. It can germinate in low oxygen potential in the soil. Glycolysis is not increasing in reduced soil conditions while the pentos is pathway increases supply of reduction agents, particularly in the first 40 to 50 hour of *E. crus galli's* emergence. (Honek et al., 1999). Barnyard grass (*E. crus-galli)* is morphologically similar to rice in the early stages of development, sometimes avoiding weeding operations. It may have made a significant loss of return by the time it is known in the field. Rice return losses are calculated to be about 35% by (*E. crus-galli)* rivalry worldwide (Oerke and Dhene, 2004).

2.1.5 Allelopathic potential

Allelopathy is an effective mechanism of invasion (Lorenzo et al., 2013). *E. crus-galli* is a dangerous weed with significant allelopathic potential. Its allelopathic activity was discovered through various bioassay studies in which the inhibitory effects on the growth of various plants were assessed (Rice, 1984). Using HPLC and 1H NMR, Li et al. (1992) identified p-hydroxybenzaldehyde and p-hydroxybenzoicacid as the key allelochemicals in the cultural solution produced by the roots of *E. crus-galli*. In addition, 3,4-dihydroxybenzoic acid, vanillic acid, and an unidentified in-hibitor were isolated from the solution. In another study, Yamamoto et al. (1999) isolated and identified the allelochemical p-hydroxymandelic acid from germinating *E. crus-galli* seeds growing near rice seedlings. They proposed it as a primary allelochemical, along with some other unknown secondary metabolites, responsible for E. crus-galli's inhibitory effects. A systematic research was carried out in 2006 to classify and extract 15 allelochemicals from *E. crus-galli* root exudates (Xuan et al, 2006).

2.1.6 Pest

Shah (1989) found that in Malaysia, barnyard grass (E. crus-galli) is an important alternative food plant for the coreid pest *Leptocorisa oratorius*, which during falow periods allows for survival of the pest in rice fields. In California, USA, according to Purcell and Frazier (1985) weed control was also suggested as a productive strategy for preventing diseases spread by the cicadellids *Draeculacephala minerva* and *Carneocephala fulgida*, vectors of Pierce's disease bacterium to grape and lucerne. Karl and Proeseler (1980) have found that another vector implicated in the spread of barley yellow dwarf luteovirus (BYDV) in barley is *Rhopalosiphum maidis,* which has been observed on *E. crus-galli*. Barnyard grass was found to be an important reservoir of the aphid and BYDV (Geissler and Karl, 1989).

E. crus-galli has also shown to be an alternate host to wheat streak mosaic rymo virus (WSMV) in Kansas, USA (Christian and Willis, 1993), *Ustilago trichophora* (Muller, 1985), *Pythium arrhenomanes* (Dissanayake et al., 1997) and Magnaporthe grisea (Du et al., 1997). As a host of plant parasitic nematodes, *E. crus-galli* has been observed heavily infested with *Meloidogyne graminicola* in rice fields in the Punjab, India (Kaul and Chhabra, 1989). Anwar et al. (1992) reported seven plant-parasitic nematode genera inhabiting the roots and rhizosphere soil of *E. crus-galli* growing in crop fields in Pakistan.

2.2 N Fertilizer (urea)

2.2.1 Benefit in agriculture

Nitrogen is a critical nutrient for all living organisms. Despite being the most abundant element in the atmosphere, N availability is the most important limiting factor for the productivity of terrestrial ecosystems, including agroecosystems. The existence of a billion-euro fertilizer industry attests to the fact that nitrogen is one of the most important inputs in crop production. Nitrogen (N) is a significant limiting nutrient in maintaining crop yields and quality. As a result, N fertilizer is commonly applied in large quantities to boost crop production around the world. The fertilizer based on urea or ammonium primarily yields ammonium, which is then converted to nitrate via a biologically mediated oxidation process. The use of nitrogen fertilizer as a source of nitrogen for plant growth is very common in Malaysia and throughout the world. The use of nitrogen fertilizers has increased crop yields and resulted in many developing countries achieving self-sufficiency in food production. Long-term application of ammonia-based N fertilizers, such as urea, has increased soil acidity, resulting in soil infertility where crops fail to respond to additional N fertilizer applications. Nitrogen application in excess of the crop's requirements can also result in yield loss.

2.2.2 Effect of urea on weed growth

Application of N fertilizer (urea) may affect weed germination, development and competitiveness. The competition for weed crops for nutrients, particularly nitrogen, is one of the most important problems because nitrogen availability is often the limiting factor in the crop, particularly with low additional capacities in soils. Nitrogen (N) is one of the major nutrients crucial for achieving high crop yield (Wang et al., 2016), and it can be an important component of integrated weed management, because N levels can remarkably affect crop-weed interference (Blackshaw and Brandt, 2008). Existing research shows that N addition increases, decreases or has few effects on weed competitiveness in crops as a consequence of inherent variations in soil N responsiveness. It has been suggested that the increased weed competition at higher N rates will be linked with an increased nutrient acquiring and weed use efficiency.

2.3 Plants oil

2.3.1 Types and benefit

There are two kinds of plants oil which come as vegetable oils and essential oils Plant oils come in the form of vegetable oils and essential oils. The primary kind is made by extricating oils from plant seeds and parts, for example, olive oil and sunflower oil. Orange oil, peppermint oil, and lavender oil are instances of the subsequent kind, which is removed from plants through refining. For centuries, plant oils or extracts have been used in alternative medicine, aromatherapy, as food favouring's, perfumes, preservatives and biological agents (Tak 2015).

For several thousands of years, vegetable oils and extracts have been used for various purposes. Various applications, such as raw and processed food preservation, pharmaceuticals, alternative treatments, and natural therapies, have utilized the antimicrobial properties of plant oils and extracts. All natural vegetable substances are found with plants oils that are thus considered to be potential products of biocontrol. There is a considerable interest in biocontrol products and particularly plant oil because bio-sourced products are considered more green and alternative solutions compared to synthetic pesticides that pose greater environmental and health risks for people.

2.3.2 Effect of plants oil on weed growth

Plants are naturally capable of producing a wide range of molecules, particularly secondary metabolites, which are known to play a role in protecting plants from pathogens due to their biological properties. Therefore, there has been expanding revenue in weed control procedures utilizing common accumulates delivered by plants, called allelochemicals, in light of the fact that they have a short half-life as they are biodegradable, and are more secure than synthetic compound, with little harm to the climate (Topal and Kocac¸ alıs¸ kan 2006). Their viability in weed control comes from the joined activity of a wide range of held mixtures, the amount and industriousness of which in the climate might be lacking to restrain seed germination and plant development. Thus environmental friendly herbicides with new modes of action are in highly demand (Dayan and Duke, 2014). The phytotoxic effects of plants oils have increased the interest in exploring volatile oil from aromatic plants for potential weed management (Dayan et al., 2009). The scientists' investigations are important as they examine a wide range of problems, including environmental and human health issues that are caused by the chemical control, as well as increasing weed resistance, as a result of the increased use of synthetic herbicides.

CHAPTER 3

MATERIAL AND METHOD

3.1 Materials

3.1.1 Plant materials

Barnyard grass (*Echinochloa cruss-galli*) seeds were provided by the Lembaga Pertubuhan Peladang (LPP) Pokok Sena, Pulau Pinang, Malaysia. The seed that exhibit 80% - 90% of germination rate were used for this study.

3.1.2 Chemical

N fertilizer (urea) were purchased from Al Fateh Online Store at from Shoppe, meanwhile natural plant oil herbicide (lemon grass) was purchased from HiQiLi Official Malaysia at Shoppe.

3.1.3 Apparatus and Equipment

Table 3.1 shows list of the apparatus and equipment that were used in this study:

Activity	**Apparatus/ Equipment's**
1. Treatment preparation	- Micropipette - Beaker - Natural plant oil herbicide (lemon grass) - Fertilizer N (Urea)
2. Soil bioassay	- Seedling tray - Paper cup - Top soil
3. Data collection	- Digital scale (shoot fresh weight) - Ruler / measuring tape (root length)
4. Data analysis	SPSS SOFTWARE

3.2 Methods

3.2.1 Treatment Preparations

The natural plant oil herbicide was prepared by dissolving 0.05 g of natural plant oil in 1000 ml of distilled water to get the stock solution of 0.05 g/L. Then, the stock solution was diluted to a series of application rates as below:

Table 3.2: List of treatments

Treatment	Application Rate
To Control	
T0 (A)	Natural plant oil herbicide (0.0 g/L)+ 80 kg ha^{-1} urea
T0 (B)	Natural plant oil herbicide (0.0 g/L)+ 160 kg ha^{-1} urea
T0 (C)	Natural plant oil herbicide (0.0 g/L)+ 240 kg ha^{-1} urea
Treatment 1(T1)	Natural plant oil herbicide (0.01 g/L)+ 80 kg ha^{-1} Urea
Treatment 2(T2)	Natural plant oil herbicide (0.01 g/L)+ 160 kg ha^{-1} Urea
Treatment 3(T3)	Natural plant oil herbicide (0.01 g/L)+ 240 kg ha^{-1} Urea
Treatment 4(T4)	Natural plant oil herbicide (0.02 g/L)+ 80 kg ha^{-1} Urea
Treatment 5(T5)	Natural plant oil herbicide (0.02 g/L)+ 160 kg ha^{-1} Urea
Treatment 6(T6)	Natural plant oil herbicide (0.02 g/L)+ 240 kg ha^{-1} Urea
Treatment 7(T7)	Natural plant oil herbicide (0.04 g/L)+ 80 kg ha^{-1} Urea
Treatment 8(T8)	Natural plant oil herbicide (0.04 g/L)+ 160 kg ha^{-1} Urea
Treatment 9(T9)	Natural plant oil herbicide (0.04 g/L)+ 240 kg ha^{-1} Urea

3.2.2 Soil bioassay

The bioassay species was the *E. cruss-galli*, which were used to test the herbicidal activity of N fertilizer (urea) treated with natural plant oil. 140g of topsoil were placed into a paper cup with holes at the bottom. For proper rice seedling growth, the paper cup were placed in an 80-by-60-by-5-cm tray and water were applied from the bottom of the paper cup. The trays were placed in a nursery right away and kept at a relative humidity of 75 to 80% with a temperature of 25 to 30°C. As shown in Table 3.2.1, the urea treated with natural plant oil were prepared in three application rates: urea (80, 160, and 240 kg ha-1) and natural plant oil herbicide (0.01, 0.02, and 0.04 g/L). To provide a different level of N in the soil, urea fertilizer at each application rate were applied onto the soil surface within the paper cup. After one day 30 *E. cruss-galli* seeds were sown on the soil surface. The spraying volume for natural plant oil herbicide was 160 L/ha). Hence, based on the spraying volume, 0.05 mg/ml of natural plant oil herbicide were diluted into the distilled water and it were applied on the soil surface of each tested seedling according its respected concentration by using micropipette. Next day, a micropipette were used to apply natural plant oil herbicide at various rates to the soil surface. Control treatments will be *E. cruss-galli* seeds that have not treated.

3.2.3 Statistical Analysis

Each experiment will have three replications and were set up as a completely randomized design (CRD). One-way analysis of variance were used to analyze the data (ANOVA). To compare the means of the treatments, the Turkey HSD were used. When the p-values are less than 0.05 (P 0.05), the differences are considered significant.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effect of N fertilizer (urea) treated with natural plants oil herbicide on control barnyard grass (*Echinochloa crus-galli*) weeds under nursery conditions.

4.1.1 Weed emergence

Figure 4.1 shows the effect of N fertilizer (urea) treated with natural plants oil herbicide on weed emergence of barnyard grass (*Echinochloa crus-galli*). It was found that all treatments significantly inhibit the weed germination as compared to the control (Appendix B.3). At an application rate of **T7** [natural plant oil herbicide (0.04 g/L) + 80 kg ha^{-1} urea], the *E. crus-galli* was strongly inhibited by 90%. Similar trend was also observed when the weed species were treated with **T3** to **T9** with natural plant oil herbicide concentration ranging from 0.01 to 0.04 g/L with 80 to 240 kg ha^{-1} urea. At these application rate, the inhibition rate on weed emergence was from 76 to 87%. Furthermore, it was found that **T1** and **T2** with 0.01 g/L natural plant oil herbicide and 80 to 160 kg ha^{-1} urea exhibit less percentage on weed emergence with 60 to 65% inhibition (equal to 35 - 40, % of control).

There are limited findings from previous study regarding the effect of urea treated with plant natural oil herbicide for weed control. However, many studies have been conducted to determine the phytotoxic activity of single essential oil extracted from plants or nitrogen fertilizer application (urea) to inhibit the selected weed species.

For example, previous study conducted by Amri et al. (2012) discovered that essential oils from *Thymbra capitata* L. *(Cav*), *Mentha x piperita* L., and *Santolina chamaecyparissus* L. had significant effects on *E. crus-galli* seedlings at the lowest concentration of 0.2 g/L. However, they found that only *T. capitata* and *M. piperita* essential oils inhibited total growth of *E. crus-galli* at a concentration of 2.0 g/L. At the same dose, the seedling growth of the bioassay species of *Eucalyptus camaldulensis*, *Eucalyptus torquata*, *Eucalyptus lesouefii*, *Santolina chamaecyparissus*, and *Eucalyptus occidentalis* were reduced by 67, 56, 54, 49, and 44%, respectively. Another study conducted by Dudai et al. (1999) investigated that essential oils from aromatic plants such as lemon basil (*Ocimum citriodorum* L.), oregano (*Origanum vulgare* L.), and sweet marjoram (*Origanum majorana* L.) inhibit the germination of Palmer amaranth (*Amaranthus palmeri* L.) by 50% respectively.

In this current study, it was noted that highest rate of the natural plants oil herbicide at 0.04 g/L treated with 80 kg ha-1 urea (T7) exhibit strong weed control by inhibiting 90% of *E. crus-galli* weed emergence. However, Gunavathy et al. (2017) found that the goosegrass (*Eleusine indica*) was found to be very sensitive to urea fertiliser at higher application rates ranging from 116 to 120 kg ha-1. Moreover, Norhafizah et al. (2017) stated that the application of N fertiliser at an application rate of 50, 100 and 150 kg ha^{-1} influences the soil environment and improve herbicide efficacy to suppress the emergence of *E. indica*. Thus, this current study suggests that natural plants oil herbicide and urea work synergistically to inhibit the emergence of *E. crus-galli*. This urea-natural herbicide application might influence the N soil environment by minimize the nutrient availability for the weed growth and may contribute to the long-term weed management.

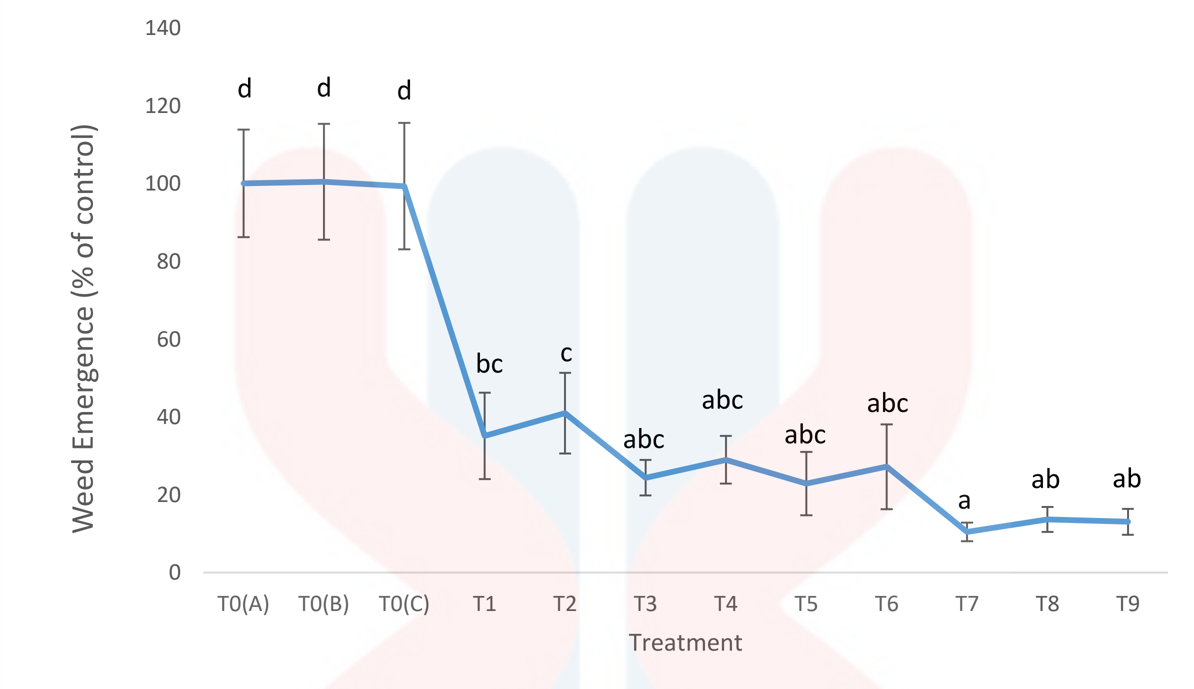

Figure 4.1: Effect of N fertilizer (urea) treated with natural plants oil herbicide on control of weed emergence of barnyard grass (*Echinochloa crus-galli*). Vertical bars represent standard deviation (SD) of the mean.

Legend

T0 (A): Natural plant oil herbicide (0.0 g/L) + 80 kg ha^{-1} Urea
T0 (B): Natural plant oil herbicide (0.0 g/L) + 160 kg ha^{-1} Urea
T0 (C): Natural plant oil herbicide (0.0 g/L) + 240 kg ha^{-1} Urea
(T1): Natural plant oil herbicide (0.01 g/L) + 80 kg ha^{-1} Urea
(T2): Natural plant oil herbicide (0.01 g/L) + 160 kg ha^{-1} Urea
(T3): Natural plant oil herbicide (0.01 g/L) + 240 kg ha^{-1} Urea
(T4): Natural plant oil herbicide (0.02 g/L) + 80 kg ha^{-1} Urea
(T5): Natural plant oil herbicide (0.02 g/L) + 160 kg ha^{-1} Urea
(T6): Natural plant oil herbicide (0.02 g/L) + 240 kg ha^{-1} Urea
(T7): Natural plant oil herbicide (0.04 g/L) + 80 kg ha^{-1} Urea
(T8): Natural plant oil herbicide (0.04 g/L) + 160 kg ha^{-1} Urea
(T9): Natural plant oil herbicide (0.04 g/L) + 240 kg ha^{-1} Urea

4.1.2 Shoot fresh weight

The effect of N fertilizer (urea) treated with natural plants oil herbicide on shoot fresh weight of *E. crus-galli* were shown in Figure 4.2. Similar to weed emergence, all treated bioassay species shows significant reduction in term of shoot fresh weight as compared to the control (Appendix B.4). The mean value of shoot fresh weight (% of control) shows a reducing trend as the concentration of natural plants oil herbicide increased. Starting from 0.02 to 0.04 g/L natural plant oil herbicide with 80 to 240 kg ha^{-1} urea (**T4-T9**), the shoot fresh weight of *E. crus-galli* was significantly reduced with the mean value, % of control; ranging from 6 to 33% (67-94% inhibition). It was interesting to note that at an application rate of 0.01 g/L natural plant oil herbicide + 80 kg ha^{-1} urea (**T1**), the shoot fresh weight of *E. crus-galli* was reduced by 50%. This application rate is likely the effective dose for 50% (ED50) of the shoot fresh weight inhibition.

In this current study, at the rate of 0.04 g/L natural plant oils and 240 kg ha^{-1} urea in T9, the rate was highly toxic to the bioassay species, where the highest rate resulted in a 78% reduction of weed shoot fresh weight. However, previous findings documented by Khanh et al. (2005) demonstrated that plant oils from nerium (*Nerium oleander* L.), purple passionflower (*Passiflora incarnate* L.), and Japanese pagoda (*Sophora japonica* L.) significantly reduced paddy weeds growth and fresh weight by 60–100% and 70–100% at 0.6 g/L, and 1.0 g/L, respectively. This result shows that the plant oils from nerium, purple passionflower and Japanese pagoda need more higher concentration to reduce the fresh weight of paddy weeds as compared to the natural plant oil herbicide (0.04 g/L) used in this current study, thus suggesting that the effect of essential oil or plant oil was different with different weed species.

Meanwhile, Awan et al. (2014) found that added N fertilizer favoured weed biomass by 82 to 160%, with the application of 50 to 150 kg N ha^{-1}, respectively. Conversely, in this current study, urea at higher rate of 240 kg ha-1 in T9 was strongly reduce the shoot fresh weight of *E. crus-galli* with the mean value of 22% (% of control). These contrary results demonstrated that weed species shows different response with N (urea) rates, therefore, manipulating the N environment could be effective in enhance herbicide efficacy.

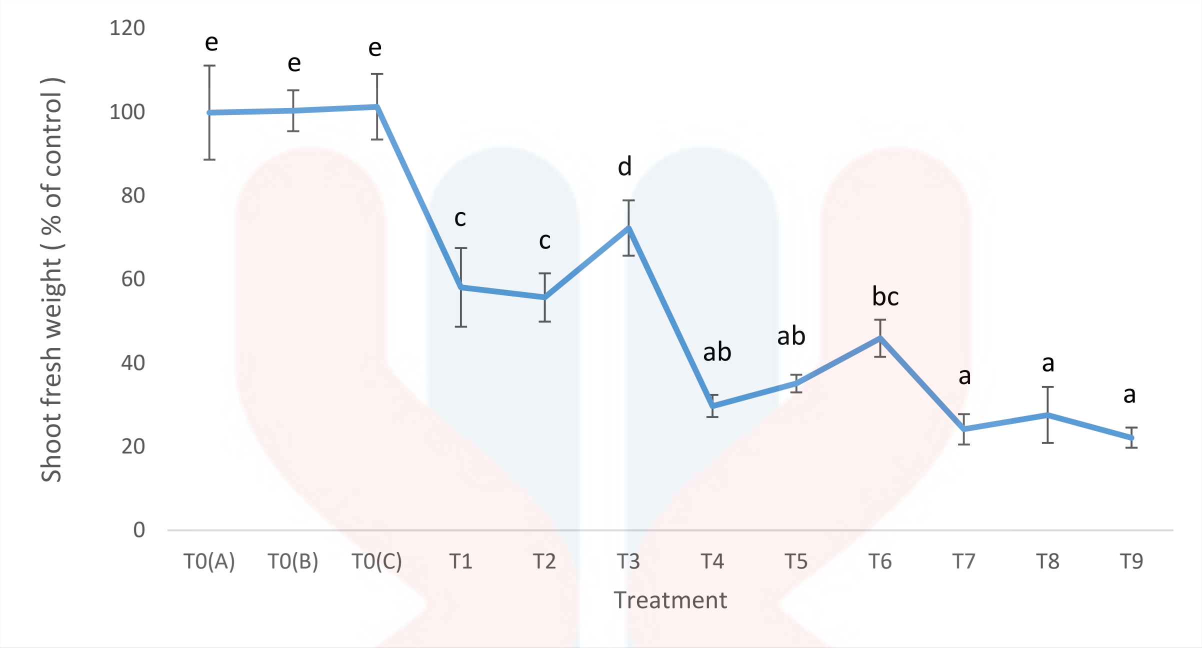

Figure 4.3: Effect of N fertilizer (urea) treated with natural plants oil herbicide on control of shoot fresh weight of barnyard grass (*Echinochloa crus-galli*). Vertical bars represent standard deviation (SD) of the mean.

Legend

T0 (A): Natural plant oil herbicide (0.0 g/L) + 80 kg ha^{-1} Urea
T0 (B): Natural plant oil herbicide (0.0 g/L) + 160 kg ha^{-1} Urea
T0 (C): Natural plant oil herbicide (0.0 g/L) + 240 kg ha^{-1} Urea
(T1): Natural plant oil herbicide (0.01 g/L) + 80 kg ha^{-1} Urea
(T2): Natural plant oil herbicide (0.01 g/L) + 160 kg ha^{-1} Urea
(T3): Natural plant oil herbicide (0.01 g/L) + 240 kg ha^{-1} Urea
(T4): Natural plant oil herbicide (0.02 g/L) + 80 kg ha^{-1} Urea
(T5): Natural plant oil herbicide (0.02 g/L) + 160 kg ha^{-1} Urea
(T6): Natural plant oil herbicide (0.02 g/L) + 240 kg ha-1 Urea
(T7): Natural plant oil herbicide (0.04 g/L) + 80 kg ha^{-1} Urea
(T8): Natural plant oil herbicide (0.04 g/L) + 160 kg ha^{-1} Urea
(T9): Natural plant oil herbicide (0.04 g/L) + 240 kg ha^{-1} Urea

4.1.3 Root length

Root length of *E. crus-galli* are very susceptible to natural plant oil herbicide and urea fertilizer (Figure 4.3). It was found that the root length of bioassay species shows significant reducing trend when treated with all treatments (Appendix B.5). Similar to shoot fresh weight, the reduction on the root length were more apparent at rates starting from 0.02 to 0.04 g/L natural plant oil herbicide with 80 to 240 kg ha^{-1} urea (**T4-T9**), with the mean value of 22- 46% reduction, % of control (equal to 54-78% inhibition). Conversely, the root length was moderately reduced at **T1** and **T2** with 0.01 g/L natural plant oil herbicide and 80-160 kg ha^{-1} urea. At these two application rates, the root length of *E. crus-galli* were inhibited by 58 and 56%, respectively.

According to (Wei et al., 2020), the root length of *Poa annua* was reduced to 49.75% and 59.72% after treated with *Onopordum acanthium* plant oil at 0.2 g/L and 2.5 g/L, respectively. In contrast, current study shows that natural plant oils herbicide (0.04 g/L) and urea (240 kg ha-1) in T7 strongly reduced the root growth of *E. crus galli* by 94%. These results indicate that the degree of susceptibility of root growth of the selected weed species toward plant oil or natural herbicide was different. Irrespective of plant oil herbicide and urea rates, the present study also found that the root growth of *E. crus galli* were decreases. This greater root growth reduction might be due to the influence of soil acidity created by the urea fertiliser. Fageria et al. (2011) reported that increasing rate of N fertiliser could increase the soil acidity which in turn will caused osmotic stress or salt toxicity to the weeds. In connection to this, the increasing of urea rate along with natural plant oil herbicide might influence the N environment which might increases the soil acidity that is lethal for *E. crus galli* weed. These results suggest that the weed control through manipulation of fertilization strategy and herbicide application was important by determining the correct doses of urea and herbicide.

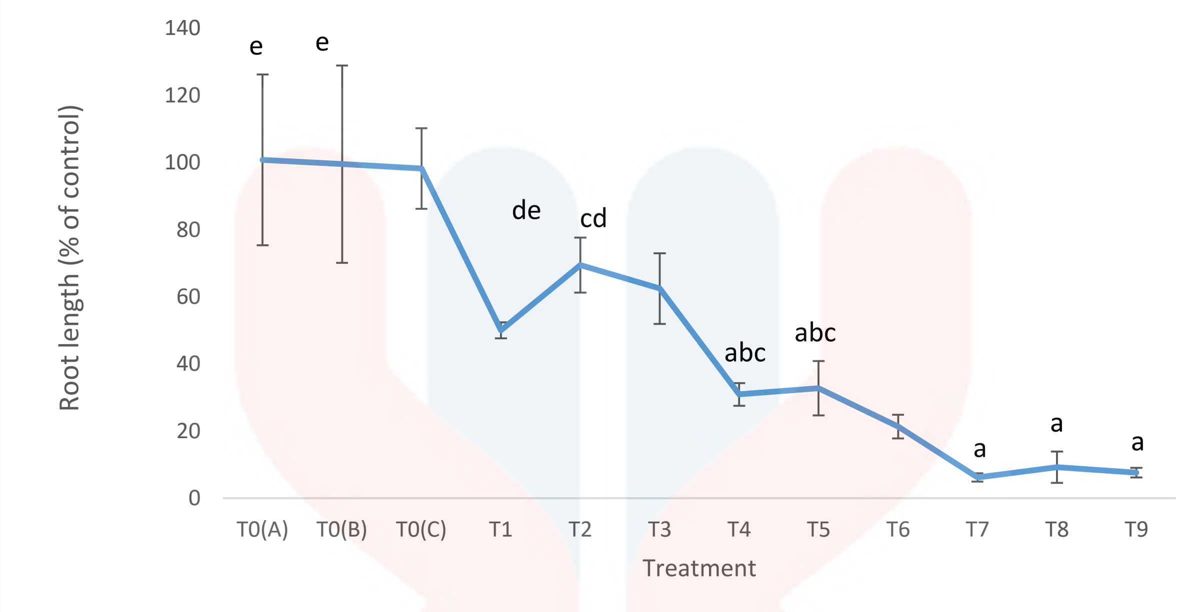

Figure 4.3: Effect of N fertilizer (Urea) treated with natural plants oil herbicide on control of root length of barnyard grass (*Echinochloa crus-galli*). Vertical bars represent standard deviation (SD) of the mean.

Legend

T0 (A): Natural plant oil herbicide (0.0 g/L) + 80 kg ha^{-1} Urea
T0 (B): Natural plant oil herbicide (0.0 g/L) + 160 kg ha^{-1} Urea
T0 (C): Natural plant oil herbicide (0.0 g/L) + 240 kg ha^{-1} Urea
(T1): Natural plant oil herbicide (0.01 g/L) + 80 kg ha^{-1} Urea
(T2): Natural plant oil herbicide (0.01 g/L) + 160 kg ha^{-1} Urea
(T3): Natural plant oil herbicide (0.01 g/L) + 240 kg ha^{-1} Urea
(T4): Natural plant oil herbicide (0.02 g/L) + 80 kg ha^{-1} Urea
(T5): Natural plant oil herbicide (0.02 g/L) + 160 kg ha^{-1} Urea
(T6): Natural plant oil herbicide (0.02 g/L) + 240 kg ha^{-1} Urea
(T7): Natural plant oil herbicide (0.04 g/L) + 80 kg ha^{-1} Urea
(T8): Natural plant oil herbicide (0.04 g/L) + 160 kg ha^{-1} Urea
(T9): Natural plant oil herbicide (0.04 g/L) + 240 kg ha^{-1} Urea

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Soil bioassay was conducted to combine chemical (urea) and natural (plant oil herbicide) weed control methods by treating urea fertilizer with a series concentration of natural plant oil herbicide and applied on the soil surface before the emergence of *E.cruss galli*. It is interesting to note that natural plant oil herbicide is compatible with urea fertilizer because urea-treated natural plant oil herbicide gave excellent control of *E.cruss galli*. Natural plant oil herbicide at 0.04 g/L in combination with urea at rate of 80 kg ha^{-1} **(T7)** strongly inhibited *E. cruss galli* emergence by 90%. Integrated application of natural plant oil herbicide and urea at 0.02 to 0.04 g/L with 80 to 240 kg ha^{-1} urea, respectively were required to achieve 67-94% reduction on the shoot fresh weight. Furthermore, it was found that the root length of *E. crus-galli* was very susceptible to natural plant oil herbicide and urea fertilizer at these similar rates where the treated bioassay species shows 54-78% reduction in root length, implying that regardless of any plant oil herbicide concentration, the emergence and growth of *E. crus-galli* has achieve an optimum control. These results clearly shows that an integrated weed management by manipulation of fertilization strategy by using urea treated with natural plant oil herbicide is effective to provide broad spectrum of weed control.

5.2 Recommendation

Further research is required to determine the effectiveness of urea-treated natural plant oil herbicide application for weed control in field condition since the present study was only conducted at vegetative stage under nursery condition. It will also be necessary to further examine effects of different soil types on efficacy of this treatment for suppression of weeds particularly in the rice field.

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APPENDIX A

Figure A.1. N (urea) fertilizer that had been used in this study

Figure A.2. Paper cup that had been used in this study

Figure A.3. Natural plant oils that had been used in this study

Figure A.4 .top soil that had been used in this study

Figure A.5. Sample of seeds in nursery before application of treatment

Figure A.6. Sample of seeds in nursery before application of treatment

A.7. Sample of weeds in nursery after application of treatment

A.8. Sample of weeds after application of treatment

Figure A.9. Barnyard grass (*Echinochloa crus-galli*) at
0.0 g/L natural plant oil herbicide and 80 kg ha^{-1} urea after 11 days

Figure A.10. Barnyard grass (*Echinochloa crus-galli*) at
0.0 g/L natural plant oil herbicide and 160 kg ha^{-1} urea after 11 days

Figure A.11. Barnyard grass (*Echinochloa crus-galli*) at
0.0 g/L natural plant oil herbicide and 240 kg ha^{-1} urea after 11 days

Figure A.12. Barnyard grass (*Echinochloa crus-galli*) at
0.01 g/L natural plant oil herbicide and 80 kg ha^{-1} urea after 11 days

Figure A.13. Barnyard grass (*Echinochloa crus-galli*) at
0.01 g/L natural plant oil herbicide and 160 kg ha^{-1} urea after 11 days

Figure A.14. Barnyard grass (*Echinochloa crus-galli*) at
0.01 g/L natural plant oil herbicide and 240 kg ha^{-1} urea after 11 days

Figure A.15. Barnyard grass (*Echinochloa crus-galli*) at
0.02 g/L natural plant oil herbicide and 80 kg ha^{-1} urea after 11 days

Figure A.16. Barnyard grass (*Echinochloa crus-galli*) at
0.02 g/L natural plant oil herbicide and 160 kg ha^{-1} urea after 11 days

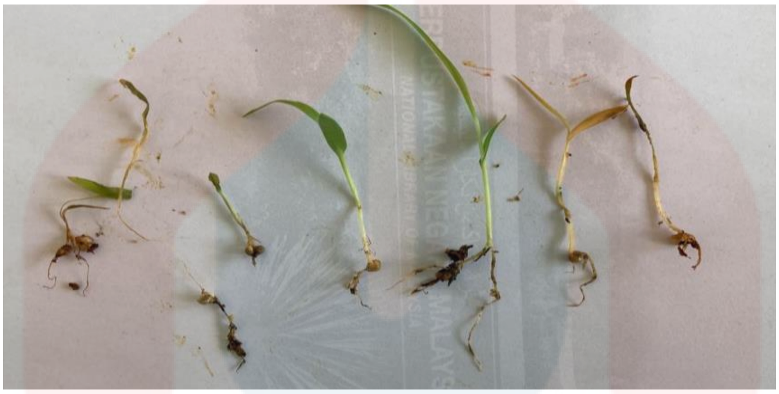

Figure A.17. Barnyard grass (*Echinochloa crus-galli*) at
0.02 g/L natural plant oil herbicide and 240 kg ha^{-1} urea after 11 days

Figure A.18. Barnyard grass (Echinochloa crus-galli) at

0.04 g/L natural plant oil herbicide and 80 kg ha^{-1} urea after 11 days

Figure A.19. Barnyard grass (*Echinochloa crus-galli*) at
0.04 g/L natural plant oil herbicide and 160 kg ha^{-1} urea after 11 days

Figure A.20. Barnyard grass (*Echinochloa crus-galli*) at
0.04 g/L natural plant oil herbicide and 240 kg ha^{-1} urea after 11 days

APPENDIX B

Table B.1. Mean value of different rate of N fertilizer (urea) treated with different concentration of natural plant oil herbicide and control treatment on weed emergence(%), shoot fresh weight (% of control) and root length (% of control) of Barnyard grass *(Echinochloa crus-galli)* under nursery condition. (Mean ± standard deviation).

TREATMENTS **Natural plant oil herbicide (g/L) + Urea (kg ha-1)**	**No of weed emergence**	**Shoot fresh weight (mg/plant)**	**Root length (mm/plant)**
Natural plant oil herbicide (0.0 g/L) + 80 kg ha-1 Urea	100 ± 14	100 ± 11	101 ± 25
Natural plant oil herbicide (0.0 g/L) + 160 kg ha^{-1} Urea	101 ± 15	100 ± 5	100 ± 29
Natural plant oil herbicide (0.0 g/L) + 240 kg ha^{-1} Urea	99 ± 16	101 ± 8	98 ± 12
Natural plant oil herbicide (0.01 g/L) + 80 kg ha-1 Urea	35 ± 11	58 ± 9	50 ± 2
Natural plant oil herbicide (0.01 g/L) + 160 kg ha-1 Urea	41 ± 10	56 ± 6	69 ± 8
Natural plant oil herbicide (0.01 g/L) + 240 kg ha-1 Urea	24 ± 5	72 ± 7	63 ± 11
Natural plant oil herbicide (0.02 g/L) + 80 kg ha-1 Urea	23 ± 8	30 ± 3	31 ± 3
Natural plant oil herbicide (0.02 g/L) + 160 kg ha-1 Urea	27 ± 11	35 ± 2	33 ± 8
Natural plant oil herbicide (0.02 g/L) + 240 kg ha-1 Urea	10 ± 2	46 ± 4	21 ± 4
Natural plant oil herbicide (0.04 g/L) + 80 kg ha-1 Urea	14 ± 3	24 ± 4	6 ± 1
Natural plant oil herbicide (0.04 g/L) + 160 kg ha-1 Urea	13 ± 3	28 ± 7	9 ± 5
Natural plant oil herbicide (0.04 g/L) + 240 kg ha-1 Urea	15 ± 14	22± 2	8 ± 1

Table B.2. ANOVA test results for weed emergence shoot, root length and shoot fresh weight for Barnyard grass (*Echinochloa crus-galli*) under nursery condition.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
WE	Between Groups	73649.676	11	6695.425	63.875	.000
	Within Groups	5241.033	50	104.821		
	Total	78890.710	61			
RL	Between Groups	68754.988	11	6250.453	28.867	.000
	Within Groups	9526.994	44	216.523		
	Total	78281.982	55			
SFW	Between Groups	54985.036	11	4998.640	117.532	.000
	Within Groups	2466.736	58	42.530		
	Total	57451.771	69			

*WE = Weed emergence, RL = Root length, SFW = Shoot fresh weight

Table B.3. Turkey HSD test for weed emergence of Barnyard grass (*Echinochloa crus-galli*).

Weed emergence

Tukey HSDa,b

TREATMENT	N	Subset for alpha = 0.05			
		1	2	3	4
9	4	10.2500			
11	3	13.0000	13.0000		
10	5	13.4000	13.4000		
7	6	22.8333	22.8333	22.8333	
5	6	24.5000	24.5000	24.5000	
8	6	27.3333	27.3333	27.3333	
6	5	28.8000	28.8000	28.8000	
3	4		35.2500	35.2500	
4	6			41.0000	
2	5				99.6000
0	6				100.3333
1	6				100.3333
Sig.		.193	.052	.217	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.932.

b. The group sizes are unequal. The harmonic mean of the group sizes is used.

Type I error levels are not guaranteed.

Table B.4. Turkey HSD test for root length of Barnyard grass (*Echinochloa crus-galli*).

Root length

Tukey HSDa,b

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
9	3	6.3333				
11	3	7.6667				
10	4	9.2500				
8	4	21.5000	21.5000			
6	4	31.2500	31.2500	31.2500		
7	7	32.8571	32.8571	32.8571		
3	3		50.0000	50.0000	50.0000	
5	6			62.6667	62.6667	
4	4				69.5000	69.5000
2	7					98.2857
1	8					99.3750
0	3					100.6667
Sig.		.317	.223	.124	.751	.131

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 4.123

b. The group sizes are unequal. The harmonic mean of the group sizes is used.

Type I error levels are not guaranteed.

Table B.5. Turkey HSD test for shoot fresh weight of Barnyard grass (*Echinochloa crus-galli*).

Shoot fresh weight

Tukey HSDa,b

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
11	3	22.3333				
9	5	24.2000				
10	5	27.4000				
7	6	35.1667	35.1667			
6	5	35.6000	35.6000			
8	6		46.0000	46.0000		
4	7			55.7143		
3	7			57.8571		
5	8				72.3750	
0	5					99.8000
1	5					100.4000
2	8					101.3750
Sig.		.057	.233	.135	1.000	1.000

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 5.449.

b. The group sizes are unequal. The harmonic mean of the group sizes is used.

Type I error levels are not guaranteed.