Faculty of Resource Science and Technology

**Profiling of Total Fatty Acid Content During Jatropha Seed Development**

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Bachelor of Science with Honours
Biotechnology
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## Declaration

I want to declare that this report is originally written by me and this is a first submission to my supervisor and my co-supervisor. This report has not been sent to another university whether private or general or to other place in order to gain benefits for myself. The research or experiments which have been done are carried by myself under guidance of my comrades, lab assistants, supervisor and co-supervisor.

This report is sent in partial fulfillment for achieving the Bachelor Degree of Science, after completing three years study in UNIMAS.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACP | stearoyl-acyl carrier protein desaturase |
| ATP | Adenosine triphosphate |
| $B_o$ | external magnetic field |
| C16:0 | palmitic acid |
| C18:0 | stearic acid |
| C18:1 | oleic acid |
| C18:2 | linoleic acid |
| cDNA | complementary deoxyribonucleic acid |
| CN | cetane number |
| FAME | fatty acid methyl ester |
| FTIR | fourier transform infrared spectrometry |
| GC | gas chromatography |
| GTZ | Deutsche Gesellschaft für Technische Zusammenarbeit |
| H-NMR | proton nuclear magnetic resonance |
| IFEU | Institute for Energy and Environmental Research Heidelberg |
| JME | Jatropha methyl ester |
| JMV | Jatropha Mosaic Virus |
| JOMEs | Jatropha oil methyl esters |
| KOH | potassium hydroxide |
| MHz | megahertz |
| PNS | Philippine National Standard |
| ppm | part-per-million |
| RACE | rapid amplification of cDNA ends |

| | |
|---|---|
| rf | radio frequency |
| RFLP | restriction fragment length polymorphism |
| RNA | ribonucleic acid |
| RT-PCR | real time polymerase chain reaction |
| TMS | Tetramethylsilane |
| UNFCCC | United Nations Framework Convention on Climate Change |
| μ | magnetic moment |

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**Profiling of Total Fatty Acid Content During Jatropha Seed Deveopment**

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## ABSTRACT

*Jatropha curcas* is a potential source of vegetable oil in production of biodiesel. Enzyme such as stearoyl-acyl carrier protein desaturase (ACP) plays major role in catalyzing production of monounsaturated fatty acid that important for a good biodiesel characteristics. This experiment is conducted to investigate which period is the most valuable for the seed of *J. curcas* actively synthesizing fatty acids. This involves oil extraction of seeds from different intervals of seed development after anthesis and analysis with Proton Nuclear Magnetic Resonance (H-NMR) and Fourier Transform Infrared Spectrometry (FT-IR). Seed of yellow fruit has the highest oil content and most stages of fruit possessed unsaturated fatty acids. The highest concentration of unsaturated fatty acid is at stage IV (ripe yellow fruit). The presence of Fatty Acid Methyl Ester (FAME) can be detected at 3.81 ppm signal under H-NMR verification after transesterification with potassium hydroxide (KOH)-catalyzed methanolysis without reflux method with little modifications on original protocol. This is important for further analysis through Gas Chromatography (GC) to determine the percentage composition of fatty acid.

**Keyword: *Jatropha curcas*, seed development, fatty acid, H-NMR, FT-IR**

## ABSTRAK

*Jatropha curcas merupakan sumber minyak tumbuhan yang berpotensi untuk menghasilkan biodiesel. Enzim seperti stearoyl-acyl carrier protein desaturase (ACP) memainkan peranan utama dalam memangkin penghasilan lemak tak tepu ikatan ganda dua yang penting untuk penghasilan biodiesel yang baik. Eksperimen ini dijalankan untuk menentukan peringkat aktif biji buah J. curcas mensintesis asid lemak tak tepu ikatan ganda dua. Ini melibatkan pengekstrakan minyak biji dari peringkat berlainan selepas antesis dan analisis dengan Spektroskopi Resonan Magnet Nukleus (H-NMR) dan Spektrofotometer Inframerah Transformasi Fourier (FT-IR). Biji dari buah kuning mengandungi paling banyak minyak dan semua peringkat mempunyai komposisi asid lemak tak tepu. Peringkat paling banyak mempunyai asid lemak tak tepu ialah peringkat IV (buah kuning yang matang). Kehadiran Asid Lemak Metil Ester (FAME) dapat dikesan pada signal 3.81 ppm di bawah verifikasi H-NMR setelah transesterifikasi dengan kaedah pemangkinan metanol kalium hidroksida (KOH) tanpa refluks dengan sedikit pengubahsuaian pada protokol asal. Proses ini amat penting untuk analisis seterusnya, iaitu Gas Kromatografi (GC) bagi menentukan peratus komposisi asid lemak yang hadir.*

**Kata kunci: *Jatropha curcas*, perkembangan benih, asid lemak, H-NMR, FT-IR**

## Chapter 1.0
## Introduction

Depletion of petroleum has raised awareness among the oil industries as well as the concern on other negative impacts like pollution of greenhouse gases and chemicals derived from the end-products of the diesel fuel (Akbar *et al*., 2009; Singh & Padhi, 2009). Hence, it is a good opportunity nowadays to find and explore the possibility of other fuel sources that are safe and be manufactured on massive scale for commercial utilization (Sayyar *et al*., 2009).

Vegetable oil is one of the materials which can be manipulated and enhanced for its ability, possesses advantages in term of renewable, environment friendly and grows easily in rural areas. Palm oil, soybean oil, sunflower oil, rapeseed oil and canola oil have become the sources for biodiesel production (Akbar *et al*., 2009). Many researches have been conducted and they found that vegetable oil-based products are the highest potential for boosting the rural economic development most of farmers (Akbar *et al*., 2009; Singh & Padhi, 2009). It is significant to use non-edible oil compared to edible oil after an observation is made on the cost and edible oil consumption (Singh & Padhi, 2009). This is because a factor affecting the supplied oil would be the availability and sustainability of the plants to compete in the different application whether as a food or biodiesel resource (Akbar *et al*., 2009). Non-edible oil is a potential low price biodiesel source compared to edible oil group which is usually subjected to higher cost production (Singh & Singh, 2009). Although biodiesel price is relatively higher than diesel fuel, it is worth enough for continuous supply to vehicles as well as safer and non-pollutant to the environment (Coltrain, 2002).

Jatropha plant is a suitable resource to access new oil replacing diesel. It has widely distributed in India and native to America (Agharkar Research Institute, n.d.) but the most popular and has potential to be utilized for its extract production is *Jatropha curcas* or physic nut, where overall, it is useful for potential feed stock, becoming pesticide, decorative plant, soil enrichment manure and alternative for biodiesel manufacturing (Sayyar *et al*., 2009). Malaysia has great potential to develop biodiesel from *J. curcas* because of high amount of monounsaturated fatty acids present in the seeds from *J. curcas* that grown in Malaysia. This has been proved with a research that found the plant seeds synthesized second highest monounsaturated fatty acid in comparison with those growing at Thailand and Indonesia (Akbar *et al*., 2010). Even though palm oil is one of Malaysia's potential sources to be applied in biodiesel manufacture; it is belong to edible oil category and may contribute to competition for the end-product either as cooking oil or biodiesel fuel. Furthermore, palm oil can give higher risk for greenhouse gases emission and environment impact (pollution problem) compared to *J. curcas* during processing and producing biodiesel fuel (Nazir & Setyaningsih, 2010). Thus, a right choice should be taken by Malaysia in order to choose the suitable resource for a safe and clean fuel.

Biodiesel is monoalkyl esters of fatty acids and it is processed by transesterification, performs best like petroleum diesel fuel, non-flammable, non-toxic and can reduce exhibition of noxious fumes and odors (Akbar *et al*., 2009). Fatty acid composition has been characterized into four most important group, - palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic acid (C18:2) (Heller, 1996). Fatty acid composition is a main factor that influencing the quality of fuel and the demand in market. Monounsaturated fatty acid is a major contributor for a production of good quality of biodiesel. This is because it gives better oxidation stability compared to polyunsaturated and saturated fatty acids. Hence, *J. curcas* is an alternative for a very promising source of non-edible oil to replace diesel fuel (Annarao *et al*., 2008).

A similar research has been done to determine the fatty acid composition of phospholipid mixture isolated from this plant's oil and has found palmitic (21%), stearic

(12.2%), oleic (36.7%) and linoleic acid (30.0%) becoming the main component of fatty acid group (Rao & Chakrabarti, 2009). King *et al*. (2009) claimed that *J. curcas* has the potential to be developed in renewable oil source field and animal feed. This involves study the chance to increase the production of oil seed by alteration of genetics, and maybe to create new plant varieties in order to born a new biodiesel plant.

However, there is lack information on the period of high synthesis of total fatty acid during seed development in Sarawak soil climate, one of states of Malaysia. This is important to determine the suitable stage for RNA extraction to create cDNA molecules from active expression of gene regulating enzyme production that involved in the fatty acid synthesis. Hence, based on several researches that have been done to profile fatty acid in *J. curcas* seed, this study is performed to investigate the period of high synthesis of total fatty acid content during *J. curcas* seed development.

The objectives of this research are listed below:

(a) to extract the highest oil content among the stages of extracted *J. curcas* seed

(b) to verify the highest unsaturated fatty acid concentration among the stages of *J. curcas* seed by H-NMR

(c) to develop the simple and rapid way of preparing Fatty Acid Methyl Ester (FAME) for analysis through Gas Chromatography (GC).

# Chapter 2.0
# Literature Review

## 2.1 Energy crisis and global climate

As the oil demand is getting higher and higher, the world resources are more likely to shrink. The people have to think in other alternative instead depending on fossil fuels to move their vehicles and renewable energy has attracted their attention to try the possibilities. Any of these problems;- high fossil fuel price, risk of fossil fuel dependence and high emission of greenhouse gases can be counter if there is chance to find supply of reliable and affordable energy which is non-pollutant and safe for the environment. The current research is done on new energy sources like wind, solar, water and biomass (da Schio, 2010).

In developed and developing countries, vehicle productions are increase from year to year and it is predicted to be more drivers using the road in the future. So greenhouse gases emission will be a major problem that we have to face as it threatened the weather conditions and the health of living creatures. Particulate materials from burnt diesel fuel can cause irritation and produce carbon core that will bind heavy metal and organic compound. It has been reported that death has taken as more as 50 000 people per year in United States of America and 200 000 people per year in Europe countries due to inhaling this particulate matters (Ackland *et al*., 2008).

An alternative way to replace fossil fuel energy is by developing renewable energy that has its own benefit to biodiversity, water and soil reserve and human livelihood. United Nations Framework Convention on Climate Change (UNFCCC) has stated its support towards bioenergy like one of the "precautionary measures to anticipate, prevent or minimize the causes of climate change". First and Second generation of biofuel has taken attention most of researchers abroad. First generation biofuel is comprised mainly the crop feedstock like sugar cane and corn. Second generation biofuel is the non-derived food crops and forestry products like waste biomass and wood. Both of these generations of biofuel have their own impacts on the environment setting (da Schio, 2010). *J. curcas* is belonging to first generation biofuel feedstock and it has less pollution impacts to the ecology (Rutz & Janssen, 2007).

Life cycle assessment is a tool provided to evaluate the impacts by overview the different parameters and variables to choose the best solution on an environmental scale. This is usually done by taking various decisions and management actions on whole life cycle of specific subject (da Schio, 2010). Based on IFEU (2007), motorcar company of Daimler AG, Stuttgart has commissioned the Institute for Energy and Environmental Research Heidelberg, Germany to handle a project regarding screening life cycle assessment of jatropha biodiesel (Figure 2.1) which listing the pros and cons of jatropha biodiesel and conventional diesel. Under this project, the parameters that are taken into account are energy resources, greenhouse effect, acidification, eutrophication, summer smog and nitrous oxide. Other analyses are perform on cultivation scenario, decentralised and centralised conversion technologies and various utilization of main products and by-products where at the end, the obvious parameters will be highlighted in the result presentation. Several variations and sensitivity analyses in all life cycle stages and unit processes are crucial to detect the parameters with great influence on overall outcome as well as specific impacts on outcome in different edge conditions. This will provide knowledge to us about the potential developing jatropha biodiesel and chances for its optimization.

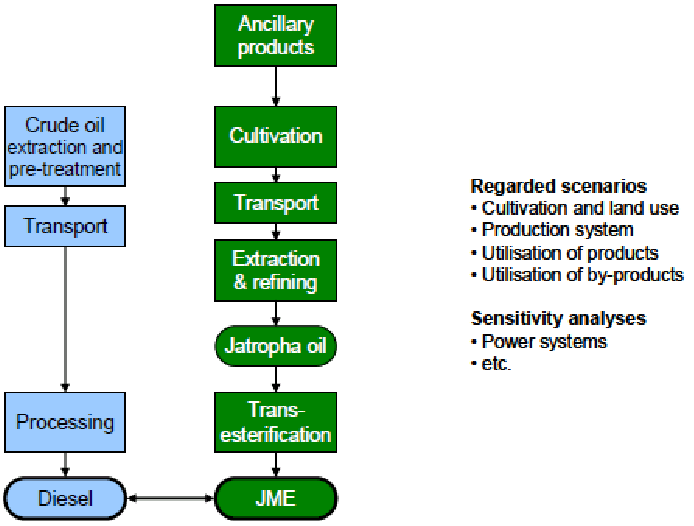



**Figure 2.1: Basic principle of life cycle comparison between jatropha biodiesel (JME) and fossil diesel fuel.** It is featured as production step "from well to wheel". This diagram is adapted from IFEU, 2007.

## 2.2 Jatropha Plant

*J. curcas* is first grown at Central America and later was distributed to Africa and Asia by the colonial country and seafarers, the Portuguese (Verma & Gaur, 2009). The genus of this plant, *Jatropha* has been taken from a Greek words, "Jatros" that means doctor and "trophe" that means food (Verma & Gaur, 2009). Jatropha has its own characterization which is different from other tree-borne oilseed species. It is a perennial crop, with deeper root system and has ability to maintain soil quality, store adequate carbon and regulate the essential materials very conventionally (King *et al.*, 2009). It is recognized to have shorter gestation period and allowed for harvesting about 37 days to 90 days after anthesis (Silip *et al*., 2010).

Cultivation of *J. curcas* can be made into four methods generally, that are direct seeding, pre-cultivation of seedling, transplanting of spontaneous wild plants and direct planting of cuttings (Kumar & Sharma, 2008). According to Leclear (2010), abundance of seed oil content and fatty acid composition as well as tolerance to harsh climates and fast growth characteristic have allocated this plant a suitable resource for biodiesel production. It is believed the quality of biodiesel that is manufactured is high enough though the crude seed oil contains high free fatty acid where it can negatively affect the transesterification process. The problem is however has been solved with proposal of new methods to reduce fatty acid, either chemically or biologically.

There are various types of jatropha;- *Jatropha gossypifolia, Jatropha multifida, Jatropha podagrica*, *Jatropha integerrima and Jatropha nana* (Agharkar Research Institute, n.d.). Among those jatropha plants, *J. curcas* has the potential benefit in its seed extraction for oil. It is a biodegradable drought-resistant perpetual plant (Oclarino, 2010) and can be manipulated for various purposes (Sayyar *et al*., 2009). Viscous oil contained from the seed is suitable to produce Jatropha Methyl Ester (JME) that potential to be utilized in manufacturing biodiesel, a safe and eco-friendly fuel. JME is also a renewable energy source and it is estimated that 99.6% of it shows the purest content and it is exceeding the percentage requirement of Philippine National Standard (PNS), a significant level for permission to certain fuel usage (Oclarino, 2010).

### 2.2.1 Growth and characteristics

*J. curcas* can achieve height up to 5 meter and has lifespan of more than 50 years (GTZ, 2000). Eight and ten meters heights are rare unless the environment is suited for its growth (Verma & Gaur, 2009). It has a look of small tree or large bush to the people. It has a toxic seeds, potentially dangerous to animals and usually planted as living fence. For generative propagation, this direct seeding is usually done in the opening of rainy season. The depth of soil during the sowing is 2-3 cm and at the end of third rainy season (after two years), new seeds would emerge from the plant. Along the ten days, germination process would be carried up in a suitable surrounding until the leaf is formed, where cotyledons are completely removed. Here, dormancy and complete ripening of harvested seed is crucial before seedlings can be achieved after the plant is at 30-40 cm height at three month age, when repellent smell substances, alkaloids are produced (Verma & Gaur, 2009).

This plant is unique as it has resistant to factors which can interfere the growth such as drought and pests (Verma & Gaur, 2009). However, it still cannot escape to be infected in a less probability by Jatropha Mosaic Virus (JMV), fungus and insects (Department of Primary Industries and Fisheries, 2008). During vegetative propagation, the cutting is usually done on the plant older than one year, has lignified, in range of 60 to 120 cm long and the best planting is one to two months before the wet season comes (GTZ, 2000). However, this method produces lower longevity and lower drought and disease resistance compared to direct seeding way. This may due to pseudo-taproot produced from the cutting that penetrates less deep into the soil compared to true taproot (Kumar & Sharma, 2008). Sayyar *et al.* (2009) reported that the yield per hectare from this plant can be four times or ten times higher to soybean and corn. According to Verma & Gaur (2009), the plant branches contain latex, normally five roots are formed from seeds, one central (tap root) and four peripheral. It is a monoecious plant where the flowers having unisexual system, and this plant also called hermaphroditic.

While in the similar inflorescences, the male and female flowers will form with female parts positioned in the centre of the group of surrounding male flowers, giving ratio of female to male flowers for 1:29. This is one of strategy for a maximum pollination among female flowers (Raju & Ezradanam, 2002). For an inflorescence, there are productions until 1-5 female flowers and 25-93 male flowers. Male flowers begin to produce flower daily till the male buds are exhausted while female flowers produce flower only after the second day from inflorescence time. Male flower of *J. curcas* is described to have five petals and sepals with small and round shaped flower that is odorless. At the time of 5.30 am to 6.30 am, the flower will open every day exposing the yellow, dithecous and dorsifixed anthers (Raju & Ezradanam, 2002).

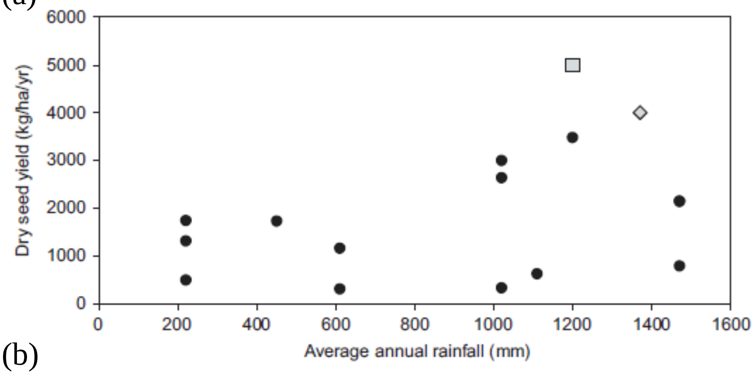
For a flower, it is calculated that production all amount of pollen is 655 and the pollen to ovule ratio is 6332:1. Pollination of flower mostly depends on the activities of insects. Bees, ants, thrips and flies are the usual visitors and come in daylight hour. They will forage mostly the male flowers to collect nectar as their source of food (Raju & Ezradanam, 2002). Female flower size is quite larger than male flower having three styles and stigmas. From the three carpels in its ovary, each has ovule and its flower usually open at exact time with opening male flower, exposing the receptive stigma. After pollination, sepals and petals will enlarge to protect the growing fruit until it matures (Raju & Ezradanam, 2002).

For planting jatropha tree, it is well suited to semi-arid land, but grows better in enough humidity condition to allow a maximum production of its oil. It can survive under a slight frost while during drought, it will shed most of its leaves in order to reduce transpiration process (Kumar & Sharma, 2008). Malaysia is a quite suitable place for planting jatropha because of its average temperature which is not too extreme or too low that can result damage on the plant. Malaysia has heavy rainfall (up to 2000 mm per annually) and covered by 2.7 million hectares of peat and organic soils, with 1.66 million hectares of them in Sarawak. This jatropha should avoid heavy clay soil so there is no risk of water logging that able to damage the roots and better aeration can be achieved. During planting and growing, at least 45 cm is the depth of soil and below 30 degree is the surface slope (Achten *et al.,* 2008).

Due to high requirement of jatropha on nitrogen and phosphorus for better growth and high biomass production, mycorrhiza is a suitable microbe to be added to assist up taking the micronutrients from the roots of jatropha. The soil also should be below pH 9 but calcium and magnesium elements can be helpful in lowering the acidity of extreme acidic soil. After digging a pit, a proper establishment to enhance plant growing is by filling it with mixture of local soil, sand and organic matter like compost fertilizer. Then, the convenient time to plant is during warm season, with enough supply of water that depends on local soil and climatic conditions (Achten *et al.*, 2008).

Water is an important element to assist photosynthesis processes and starch manufacturing in the leaves. However, this depends on the type of soil which the jatropha plants are growing because poor soil quality may affect the yield. Jatropha plant is also found to be helpful in improving soil structure and able to avoid the soil erosion together with carbon sequestration (Achten *et al.*, 2008). The production of seeds quantity will increase when there is high average annual rainfall following increasing age (Figure 2.2).

(a)




(b)

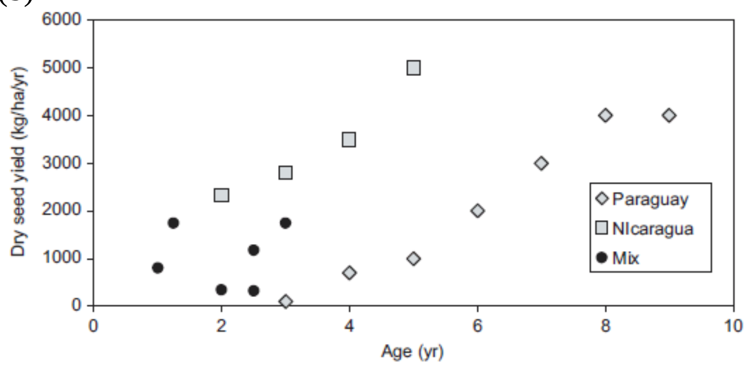



**Figure 2.2: Dry seed yield in kilogram per hectare per year depending on the average annual rainfall in millimeter and age in year**. (a) The plotted points mean mix of provenances, site conditions and plant age or average annual rainfall. (b) Under dry seed yield versus age of plant, Paraguay, Nicaragua and mix refer to the original site of the soil where mix means from both Paraguay and Nicaragua soils. These diagrams are adapted from Achten *et al.*, 2008.

High volume of water is required during harvesting of jatropha plant as the fruits and seeds are produced at this period and occurrence of the increase in rate of fruits growing (Table 2.1). The requirement for water is gradually decreasing as the plant is achieving to higher level of development. This table also shows that with minimum water requirements, jatropha plant can still survive and gives out better quality yield compared to other crops (Kheira & Atta, 2008). High irrigation water level gives plant characteristics like height, leaf number, stem diameter and leaf area to increase but it is also decreasing parameters of root lengths. This is because when the soil has low irrigation water level, then the roots start to elongate deeply into the soil (Azza *et al*., 2010).

**Table 2.1: Water requirements of *J. curcas* along the growing season;- initial development, flowering and harvesting**. Different amount of water should be supplied in unit of area (ha) to the plant for a successful growth and yield production. The table is adapted from Kheira and Atta, 2008

| Table 4 – Water requirements (L $tree^{-1}$) of jatropha trees throughout the growing seasons of 2005 | | | | | |
|---|---|---|---|---|---|
| Stages of growing season (day) | Growing stage | Water applied (L $tree^{-1}$) | | | |
| | | 125% of ETp | 100% of ETp | 75% of ETp | 50% of ETp |
| 43 days | Initial | 46.1 | 36.9 | 27.7 | 18.5 |
| 60 days | Development | 64.3 | 51.4 | 38.5 | 25.6 |
| 30 days | Flowering | 32.2 | 25.7 | 19.2 | 12.8 |
| 75 days | Harvest | 80.4 | 64.3 | 48.2 | 32.2 |
| Total water applied (L $tree^{-1}$) | | 223.0 | 178.3 | 133.6 | 89.1 |
| Total water applied ($m^3$ $ha^{-1}$)[a] | | 557.4 | 445.5 | 333.9 | 222.8 |
| Hectare = 2.38 feddan | | | | | |
| Jatropha tree dropped down its leaves during dormancy stage (01/10/2005 31/10/2005) | | | | | |

ETp = evotranspiration

### 2.2.2 Seed development and fruit maturation

It has been reported that within 85 to 98 days, the first bud of young *J. curcas* plant will be clearly emerged after seeding and continued to flower after 7 to 18 days within whole of the period 93 to 124 days. After 1 to 8 days, fruit will slowly to form where it starts to become mature green stage after 21 to 35 days. Ripening will be 2 to 4 days after that until it reaches fully yellow and continued to fully black within 3 to 9 days (Silip *et al*., 2010). The amount of fruits at the end of branches usually about 5 to 20 bunches and each has shape more likely to "American football" (van der Putten *et al.*, 2010).

According to Effendi *et al.* (2009), there has been a system to evaluate the ripeness of the *J. curcas* by distinguishing the color properties, showing raw, ripe and over ripe fruit. Color grading is a main indicator to differentiate the ripeness of the fruit as well as the quality. It is hoped to assist the farmers in determining and selecting the grades of this plant. One fact that handlers need to know when collecting this plant seeds is the presence of accumulated toxicity (Kumar & Sharma, 2008). The toxic curcin protein is able to inhibit the protein synthesis *in vitro* and the main agent of toxicity, phorbol esters is found in high concentration in jatropha seed. Phorbol ester is heat stable so high temperature treatment may not be effective but more suitable to undergo chemical treatment. Most accidental consumption had caused sickness like giddiness, vomiting, diarrhea and eventually death (Kumar & Sharma, 2008).

As the seed is processed, usually having 30 % to 40 % non-edible oil (Veny, 2009), the oil is suitable for standard diesel engine and the residue produced is an important element of biomass. Those seeds can vary in weight; about 0.4 to 10 kg plant per year depending on the age of plant and soil or environment conditions (Verma & Gaur, 2009). With black bean appearance, the seed has 18 mm long and 12 mm wide and 10 mm thick but these parameters may change according to the growth of plant. Hard shell constitutes about 37 % weight of seed while soft white kernel constitutes 63 % of it. Usually most oil present in kernel (van der Putten *et al.*, 2010). The fruit maturity development has been determined in 7 stages. Stage 1 is small young immature fruit, stage 2 is mature green, stage 3 is half green and half yellow fruit coat, stage 4 is completely yellow fruit coat, stage 5 is half yellow and half black fruit coat, stage 6 is a black wet fruit and finally stage 7 is black dry fruit (Silip *et al*., 2010). Referring to Annarao *et al*., (2008), the synthesis of lipid beginning nearly three weeks after the jatropha ovule fertilization and after 17 days from fertilization, the free fatty acid content has started to decrease.

It has been reported that there would be a problem during harvesting process because of large distribution of maturity and heterogeneity of the fruits (Silip *et al*., 2010). This means their maturity stages are not uniform for a tree and a study has shown that this jatropha is an indeterminate plant where the fruit matured at bottom of stem while the buds are slowly grew at top of it. Hence, it needs a careful harvest and post-harvest handling so we can maximize the yield (Silip *et al.*, 2010).

The period of drying and brown colored fruit of jatropha should indicate the maturation of its seeds and they can be collected with hand picking for small tree or using long stick for large one (Verma & Gaur, 2009). To assist in selecting the specific stages of jatropha, pattern recognition is important by depending on human abilities to characterize according to observations. There are four approaches for pattern recognition based upon technology help, like template matching, statistical classification, structural recognition and artificial neural networks (Effendi *et al.*, 2009). Fresh *J. curcas* seeds possessed high level of viability though they also have low level of germination. It has been reported that when air dried seeds is stored in unrestrained room conditions for 12 months, the level or rate of germination is higher than 93 %. This happened in Zimbabwe. It is also reported that seed germination is highest in soil with pH 8 but after 112 days kept in 27 $^{o}$C – 28 $^{o}$C, germination rate of seed will decrease (Department of Primary Industries and Fisheries, 2008).