



Faculty of Resource Science and Technology

**Effectiveness of Organic Compost Incorporated with *Burkholderia spp.* on the Growth Performance and Yield of Okra (*Abelmoschus esculentus*)**

**Rosediannah Binti Kotung**

**Bachelor of Science with Honours  
(Resource Biotechnology)  
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UNIVERSITI MALAYSIA SARAWAK

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Final Year Project Report

Masters

PhD

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Rosedianah KOTUNG 38510

Name of the student (Matric No.)

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Received for examination by: \_\_\_\_\_

Date: 30/6/2015

(Name of the supervisor)  
Dr. Mohd Effendi Wasli  
Head of Department  
Department of Plant Science & Environmental Ecology  
Faculty of Resource Science & Technology  
UNIVERSITI MALAYSIA SARAWAK  
94300 Kota Samarahan

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(Date)

Dr. Mohd Effendi Wasli  
Head of Department  
Faculty of Plant Science & Environmental Ecology  
UNIVERSITI MALAYSIA SARAWAK  
94300 Kota Samarahan

Current Address:

Kg Rasak Laut, P/S 747, 99108, Kota Marudu, Sabah.

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**EFFECTIVENESS OF ORGANIC COMPOST INCORPORATED WITH  
*Burkholderia Spp* ON THE GROWTH OF PERFORMANCE AND YIELD OKRA  
(*Abelmoschus esculentus*)**

**Rosedianah Binti Kotung**

This project is submitted in partial fulfilment of the requirements for the degree of  
Bachelor of Science with Honours'

**(Resource Biotechnology)**

**Supervisor: Dr Mohd Effendi Bin Wasli**

**Co Supervisor: PM Dr Awang Ahmad Sallehin Bin Awang Husaini**

Department of Molecular Biology

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2015

## APPROVAL SHEET

**Name of Candidate:** Rosediannah Binti Kotung

**Title of Dissertation:** Effectiveness of Organic Compost Incorporated with *Burkholderia spp.* on the Growth of Okra (*Abelmoschus esculentus*).

---

Dr. Mohd Effendi Bin Wasli

Supervisor

---

Associate Professor Dr. Awg Ahmad Sallehin Awg Husaini

Co-supervisor

---

Dr.Ho Wei Seng

Programme Coordinator

Department of Molecular Biology

Faculty of Resources Science and Technology

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I wish to submit my sincere thanks to my entire family members, my friends for their goods idea wishes for my success.

## DECLARATION

I hereby declare that this Final Year Project report 2015 is based on my original work expect for the quotations and citations which have been fully acknowledged also, declare that is has not been or concurrently submitted for any other degree or qualification at UNIMAS or other institution of higher learning.

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Rosediannah Binti Kotung

Biotechnology Resources

Department of Molecular Biology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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## LIST OF ABBREVIATIONS

PGPR	Plant Growth Promoting Rhizobacteria
SPP	Species
EFB	Empty Fruit Branches
SPSS	Statistical Package for the Science Social
CO <sub>2</sub>	Carbon dioxide
O <sub>2</sub>	Oxygen

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Programme of Resource Biotechnology  
Department of Molecular Biology  
Faculty of Resource Science and Technology  
Universiti Malaysia Sarawak

**ABSTRACT**

This study was conducted to evaluate the effectiveness of organic compost produced from Oil Palm Empty Fruit Branches (OPEFB) incorporated with locally isolated *Burkholderia spp.* on the growth of Okra (*Abelmoschus esculentus*). Preparation of the organic compost was conducted at Molecular Genetic Laboratory Universiti Malaysia Sarawak (UNIMAS), while the applications of compost were tested on *A. esculentus* at Greenhouse UNIMAS. The experiments were conducted seven treatments and each treatment consists of 20 replications. T0 is control only OPEFB compost without inoculation with *Burkholderia spp.*, T1 (2 weeks of composted + 10% volume of *Burkholderia spp.*), (2 weeks of composted + 20% volume of *Burkholderia spp.*), (2 weeks of composted + 30% volume of *Burkholderia spp.*), (4 weeks of composted + 10% volume of *Burkholderia spp.*), (4 weeks of composted + 20% volume of *Burkholderia spp.*) and T6 (weeks of composted + 30% volume of *Burkholderia spp.*). Parameter such as height, number of leaves per plants, root shoot ratio, dry weigh, fresh weight, dry weight, number of flower and yield were obtain. The data was collected from week 0 until week 12. From this study showed, *A. esculentus* planted in OPEFB compost inoculated with 20% of *Burkholdria spp.* at 2 weeks of curing period showed positive respond in term of survival rate, height, dry weight, flower and fruits production. Therefore it is recommended as bio fertilizer for the future use.

Keywords: Oil Palm Empty Fruit Branches, *Burkholderia spp.* and *Abelmoschus esculentus*.

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Universiti Malaysia Sarawak

**ABSTRAK**

*Kajian ini dijalankan untuk mengetahui keberkesanan kompos tandan buah kosong kelapa sawit (EFB) yang digaulakan bersama *Burkholderia spp.* dan diperam pada masa yang berlainan kemudian di uji pada tumbesaran Bendi (*Abelmoschus esculentus*). Penyediaan kompos OPEFB di jalankan di makmal Molekular Genetik Universiti Malaysia Sarawak UNIMAS sementara aplikasi kompos tersebut dijalankan di rumah hijau UNIMAS. Kajian ini mempunyai tujuh rawatan dan setiap rawatan mempunyai 20 replikasi. Rawatan kontrol hanya mengandungi kompos tanpa bakteria, manakala bagi (T1) (2 minggu EFB kompos +10% isipadu *Burkholderia spp.*), T2 (2 minggu EFB kompos +20% isipadu *Burkholderia spp.*), T3(2 minggu EFB kompos +30% isipadu *Burkholderia spp.*), T4, (4 minggu EFB kompos +10% isipadu *Burkholderia spp.*), T5(4 minggu EFB kompos +20% isipadu *Burkholderia spp.*) dan T6 (4 minggu EFB kompos +30% isipadu *Burkholderia spp.*). Parameter seperti tinggi, bilangan daun yang dihasilkan oleh setiap pokok, nisbah akar pucuk, berat bersih, berat kering, jumlah bunga serta jumlah buah yang dihasilkan akan di ambil. Data diambil bermula minggu 0 sehingga minggu ke 12. Hasil kajian ini menunjukkan kacang bendi yang ditanam menggunakan dua minggu kompos yang diperam bersama *Burkholderia spp.*, menunjukkan kesan positive terhadap peratusan kadar kematian, berat kering segar, penghasilan bunga serta buah. Oleh yang demikian ia dicadangkan sebagai baja organik untuk kegunaan pada masa hadapan.*

*Kata Kunci: Tandan buah kosongk kelapa sawit (EFB), *Burkholderia spp.* dan (*Abelmoschus esculentus*)*

## 1.0 INTRODUCTION

### 1.1 Background

Disposal of solid waste has become one of the major problems throughout the world. Thus, efforts in reutilizing solid waste into usable materials are essential in order to ensure sustainable use of resources from the environment. Composting is an effective approach for reducing the amount of disposed solid waste. Organic waste such materials that are environmentally friendly can be utilized as organic compost which is effective in promoting plant growth for the promotion of sustainable agriculture (Baya *et al.*, 2014).

According to Oviasogie *et al.* (2010), composting is a microbiological process which depends on the growth and activity of populations of bacteria, fungi or *actinomyces* to the waste which is composted. There are several methods in composting which are static piles, windrow composting, passively aerated windrow, enclosed or in-vessel composting and vermicomposting (Neher *et al.*, 2013, Recycled Organic Unit (ROU), 2007). The high quality soil produced from composting is humus. Humus is an organic material that decomposes as soil amendment or soil conditioner. This material is widely utilized in the agricultural sector as materials for supplementing the depletion of organic matter in permanent agricultural croplands.

Nowadays, production of compost for commercial purposes expanded to meet the demands for agricultural related industries. According to Ewan *et al.*, (2012), Malaysia is the world largest oil palm producer which has more than three million hectares of oil plantation and generated about 90 million tonnes of renewable biomass, apart from that about 8 million tonnes of Oil Palm Empty Fruit Branches (OPEFB) was left after oil extraction. In

recent years, disposal of OPEFB was via burning to generate thermal energy to the plantation factory or mills. However, it lead to environmental concern, Oviasogie *et al.* (2010) reported waste from OPEFB is a suitable as raw material for compost. This material is readily available due its large amount in quantity which derived from palm oil refinery factories.

Recently, awareness among consumers towards food quality and safety is increased therefore many research try to find an alternative which can assist towards these problems. In order to increase the production of crops and fulfill the demand of increasing world population at the same time needs to minimize the effect towards the consumer an effective ways needs to implement. Organic Farming is one of the alternatives, this fields not also can helps to increased the crop production but also can reduce the environmental effect. Known as an environmental friendly form of agriculture practices, crops produced from Organic Farming is more safety compared to the conventional method because of the minimal use of agrochemicals such as chemical fertilizer, pesticides or herbicides to produced non-toxic crops. Moreover, it helps to prevents soil contamination (Assis and Ismail, 2011). From that research question were formulated

- Could the compost inoculated with beneficial bacteria promote better plants growth?
- Could the period of composting influence quality of compost?

Crop needs composts as a basis material for growth. Therefore, efficiency composting process which can produce compost in short of period times and better quality has received a lot attention from various researchers. Effective microorganism (EM) is use to accelerate the composting process under controlled condition (Baya *et al.*, 2014). One of the example EM groups of bacteria is Plants Growth Promoting Rhizobacteria (PGPR), this bacteria can



directly or indirectly enhance plants growth. There are several examples of PGPR such as *Bacillus*, *Pseudomonas*, *Azotobacter*, *Enterobacter*, *Azospirillum*, *Actinobacter*, *Burkholderia spp.*, and genera *Rizobium* (Hayat *et al.*, 2010).

Previous study on the effectiveness of organic compost produced from OPEFB after inoculation with *Bacillus sp.* showed that at certain concentration of PGPR at specific composting period, positive respond on the growth performance and yield of commercial crop (*Abemaschus esculentus*) was observed (Sarowani *et al.*, 2014). However, still limited information is available on the effectiveness of other PGPR as EM for organic compost production is available. Considering the statement above, a study was conducted in order to evaluate the growth respond and yield of selected commercial crops cultivated with organic compost produced from OPEFB inoculated with PGPR (*Burkholderia spp.*) was conducted.

The objective of this study were

- To determine the suitable ratio of bacteria (*Burkholderia spp.*) that promotes best plants response.
- To determine the best period of composting process that produce high quality of compost.

## 2.0 LITERATURE REVIEW

### 2.1. Definition of compost and composting

Compost is natural product made from biodegradable material that had been composted under managing condition. Humus is final product of composting process. Compost was dark brown with earthy smell appearance and safe used in the environment. Composts contain beneficial properties which can be applied in agriculture field (Paulin and Malley, 2008). According to Biernbaum and Fogiel (2005), rice straw, coffee husk, vegetable, olive husk, vegetable waste and animal manure such as sheep, horse, poultry, beef and chicken can be used as material of composts.

Recent years, the amounts of organic materials is increased which if it cannot manage in proper ways will cause environmental problem such as pollution (Singh *et al.*, 2010). Composting are one of the effective and eco-environments way to encounters this problems. According to Baharuddin *et al.* (2010), composting can be defined as controlled biological decompositions of organic substance whereby it carried out by the successive of microbial population on the combination of both mesospheric and thermopiles phase in order to breakdown the organic residues. Final product of composting process is humus. Humus is substance making excellent soil amendment (Cooperband, 2002).

There are several advantages of using composts such as can helps to improve the soil fertility increased the crop production, improved water holding capacity of soil and also can improved the biological richness in soil due to reducing of using pesticide or herbicide. Additionally, producing of composts is more cheeps, simple method to treat, stabilize and generated as organic fertilizer. In order to produced compost in large quantity with highly quality many factor is need to be accounts such as carbon nitrogen ratio,

moisture content, aeration (particle size, pile size and turning), temperature pH, organism present and time of composting (Biernbaum and Fogiel, 2005). Figure 1 show the diagram of composting process was applied the same principle which is raw material (for example EFB), oxygen ( $O_2$ ), carbon dioxide ( $CO_2$ ), water (process) and Product (compost).

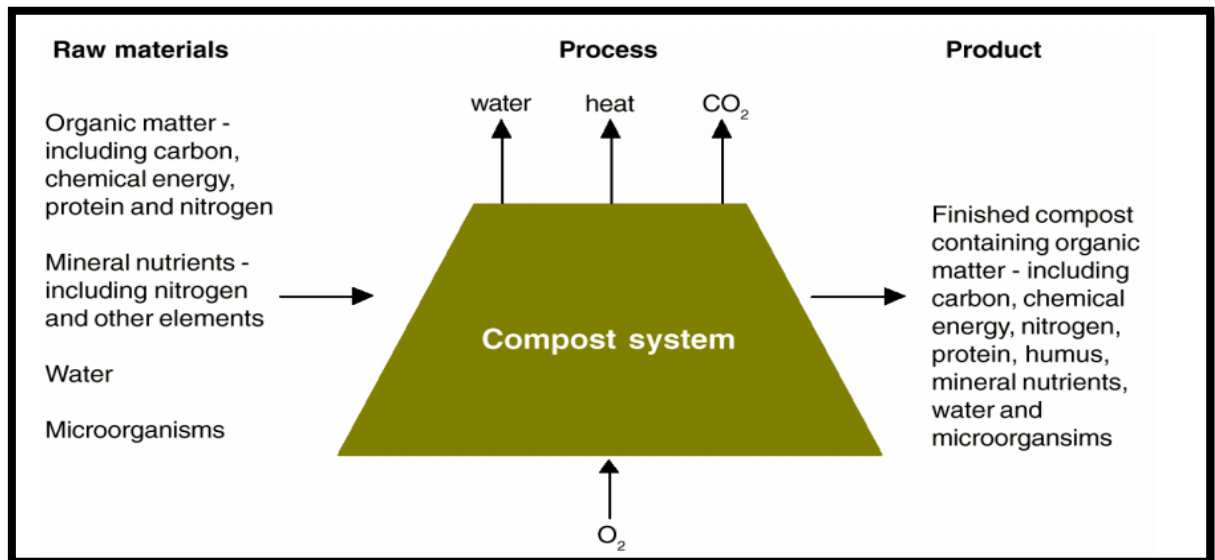


Figure 1: Diagram of process composting. All composting processes are based on the same principle (Recycled Organic Unit (ROU), 2007).

## 2.2 Phase in composting.

There are three phases in composting which is first mesophilic phase. These phases will initially the process of composting and the temperature in this phase is approximately ambient condition is between  $10^{\circ}C$  to  $40^{\circ}C$ . Additionally during this phase the number of population of microorganism will be increased and became more active then later it follows by thermophilic phase.

Thermophilic phase is most importance phase on composting whereby almost all the organic matter being converted into humus. According to Brinton (1994), there are plenty of thermophilic bacteria such as *Bacillus spp.*, *Clostradium spp.*, *Escherichia spp.*, *Salmonela typhimurium* and *Pseudomonas aeruginosa* which contributed in generating the heat which it can helps the organic material being composted. The level of temperature in

this phase was high whereby it can be exceeding up 71°C and at these points the microbial activity can be stop. This because bacterium needs oxygen to perform their activity, mixing or turning the pile will helps to increase the size and number of pore which then helps provided air to the pile (bacteria). Pores were serves as passage for  $O_2$  enter to the pile. Therefore weekly mixing and monitoring was importance on this stage (Brinton, 1994).

Curing or maturation is the last stage for composting process. In this stage fungi and actinomycetes become more predominant. The composting process will be continuing to helps degrade the organic matter such as chitin, lignin and cellulose which is less degradable to fully degrade. Temperatures of pile during this stage are slowly falling down because of the population of bacteria was decreased. These due to lacking of source of their metabolism such as carbon and nitrogen which is fully utilized, therefore many bacteria dose not survived on this phase (Biernbaum and Fogiel, 2005). Figure 2 shows the phase and temperature development during aerobic composting.

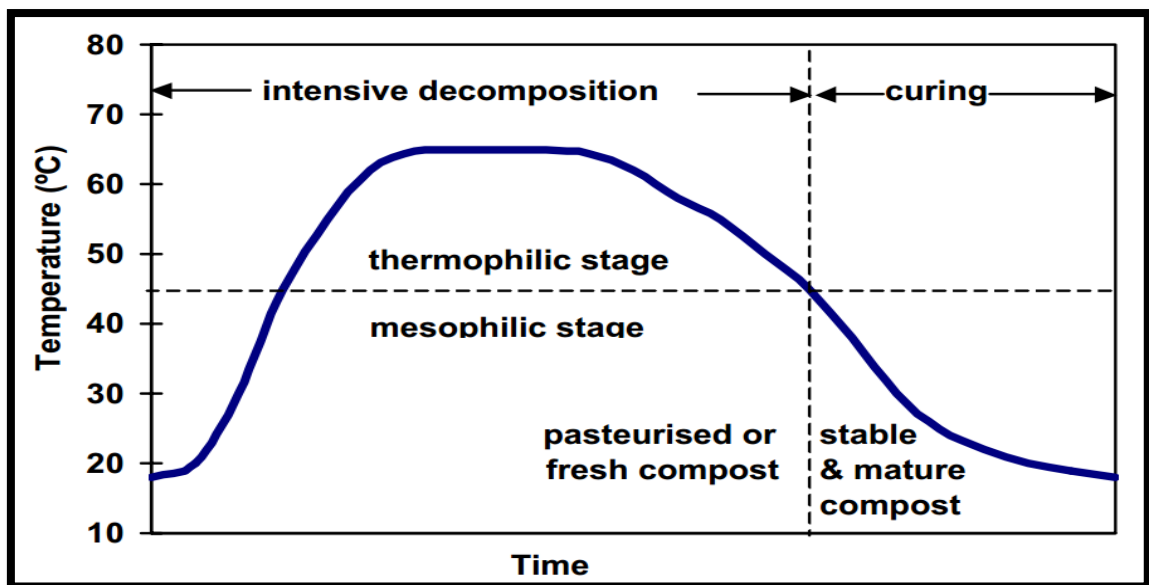


Figure 2: The phase and temperature development during aerobic composting (Recycled Organic Unit (ROU), 2007).

### **2.3 Oil Palm Empty Fruit Branches (OFEFB)**

Oil palm fruit generated two components product which is crude palm oil (CPO) was obtain from mesocarp and crude palm kernel oil (CPKO) from endosperm (kernel). During extraction process, it generated by-products and waste and know as Empty Fruit Bunch (EFB), Palm oil mill effluent (POME), palm fibres and palm kernel shell. EFB contains 25% lignin, 50% cellulose and 25% hemicelluloses (Kavitha *et al.*, 2013).

EFB were highly lignocellulostic residues make its suitable for many applications such as boiler fuel for steam turbines which used to produce steam for generation electric and also for sterilization food. Additionally EFB also used in production of bio-plastic and source of animal feed. However, demands reused of EFB in industrial scale still limited, therefore, EFB are used as composts (Yeoh *et al.*, 2011). The characteristic of EFB which contain high of porosity, water holding capacity and also nutrients holding capacity making is suitable used as raw material of aerobic composting. By composting it can reduce the bulk of organic material, eliminating risk of spreading pathogen, weeds or parasite.

Moreover it can be obtain in large quantities because are localized area. According to Zahim and Asis (2010), the enhancement of the composting process can be archived by adding amendment material such as animal waste chicken manure, goat manure and Palm oil mill effluent (POME). Hence utilizing of EFB into valuable products which is compost, therefore it can reduce approximately 50% of volume also transportation cost (Oviasogie *et al.*, 2010).

## **2.4 *Burkholderia spp* as microbial agent for plants growth.**

*Burkholderia spp.* is a genus of environmental bacteria also known as soil saprotrophic. These bacteria are found widely on soil, water (including marine water), rhizosphere, animals and human. *Burkholderia spp.* are rod shaped, motile, Gram-negative bacteria which capable of being facultative pathogens on humans and plants and generally aerobic. This species also is originally classified as member of the genus *Pseudomonas*, whereby it shares common characteristics with genus *Pseudomonas*. This genus was first described in 1950 by Walter Burkholder and it then reclassified into the genus *Burkholderia* because there distinct and separated in term of the basis of their 16s rRNA sequence and also fatty acid composition (Stoyanova *et al.*, 2014).

There are several species of *Burkholderia* was identified which contributed on agricultural field whereby it helps on increased the production of crops either direct or indirect mechanisms. Firstly is as bio control agents of many plants-pathogenic fungi such as *Fusarium sp*, *Phytophthora capsici* and *Rhizoctonia solani*. Moreover have strain was belong on these bacteria were able to inhibit growth of other bacteria, pathogenic yeast also protozoa. The ability of these strain suppressed some plants diseased was observes on many different type of crops such as corn, sweet cotton, tomato and pepper whereby its helps in increasing the crop yield (Devi *et al.*, 2012).

Secondly the advantages of these bacteria is it can be applied on the agricultural field were they help on the plants growth promotion and biological nitrogen fixation. The biological nitrogen  $N_2$  fixation was common process that associated with *Burkholderia spp.* The ability to fix atmospheric nitrogen was observed on *B. vietnamiensis*, *B. kururiensis*, *B. unname* and *B. tropica*, Moreover some strain such as *B. tuberum*, *B. phymatum* and *B. Caribinsis* had ability to modulated tropical legume plants beside associated with nitrogen

fixation (Santos et al, 2001). There are several research was experimentally conducted by researcher shows that this bacteria have potential on agricultural especially contribution on increasing yield crops. For instance, in Vietnam the inoculation of seeds rice with *B. vietnamiensis* showed the result that up 22% increased on the grain yield. Other research at Brazil also shown that endophytic *Burkholderia spp.* lead to increased approximately 69% of rice biomass also it shown onto other crops such as sugarcane and maize (Coenye and Vandamme, 2003).

Moreover, this strain not only contributed on nitrogen fixation, they also have ability to synthesis phytohormones and vitamins, make this bacteria exclude as Plants Growth Promoting Rhizobacteria (PGPR). For example, *B. vietnamiensis* strain TVV75 was able to produced indole acetic acid (IAA) whereby it shows a significant increased in plants growth under the nursery stage. Other strain such as *B. ambifaria* also contributed on the increasing of growth maize when the seeds are inoculated with these bacteria as seed treatments. In addition strain PsJN from *B. phyofirmans* which inoculated on plants also show a more developed in term of root system (Glick, 2012).

Recently there are several example of soil bacteria are used to increase the yield of crop production. These free living soil bacteria not only performed symbiotic with the plants and give benefit to the plants, also being consider as an alternative to reduce the use of chemical fertilizer, pesticide, herbicide or fungicide on the agricultural (Compant *et al.*, 2014). There are example of commercialization PGPR bacteria which huge contributed on the agriculture field which is *Azospirillum spp.*, *Enterobacter spp.*, *Klebsiella spp.*, *bacillus spp.*, *Actinobacter spp.*, *Pseudomonas spp.*, *Burkholdera spp.*, *Rhizobium spp.*, *Serratia spp.* and *Agrobacterium spp.* (Compant *et al.*, 2005, Hayat *et al.*, 2010)

## 2.5 Agronomic information of Okra (*Abelmoschus esculentus*)

Okra (*A. esculentus*) is grown at tropical and sub-tropical, recently become economically importance crop. It's commercially planted in India, Pakistan, Africa, Bangladesh, Burma, Japan, Malaysia, Thailand, Brazil and Shorthand United State. Different countries okra well know with different name for example Lady's finger in England, *Gumbo* in United State of America and France (Kumar *et al.*, 2010) and *Bhendi* in India. This plant is popular among farmer because, easy to growth and can adapt in varying moisture condition. There are almost 50 species of okra was identified by taxonomist and from family of Malvaceae (Kumar *et al.*, 2010).

Okra is annual plants and propagates by seeding in duration 90 to 100 days. These plants are highly dependent on biotic and abiotic stress. Temperature between 24°C to 28°C and long warm and humid are more preferred for normal growth of okra. For seed germination optimum soil moisture and the temperature around 25°C to 35°C in more preferred, beyond this range germination will be delayed and may not germinate. Another research shown, in order to increase the percent of germination, seeds need to soak in water for overnight. Sowing depth around 1-3 cm will enhance the emergence seedling (Odeleye *et al.*, 2005).

Okra can be grown on sandy or clay soils which the tap root system developed well. The optimal growth of okra can be increased when the soil having good drained and supply with organic matter. pH around 6.0 to 6.8 is ideal for optimal growth of okra. These plants are very susceptible infected by root-knot nematode (*Meloidogynes spp.*) and sting nematode (*Belonolaimus spp.*).

Okra can be growth up to 3 to 6 ft (0.9 – 1.8 m) and form deeply penetrating roots up to 18 inches (46cm) in soil (Lamont, 1999). Okra fruit colour appearance is yellowish