THE INFLUENCE OF ... PARASITIC NEMATODES ON THE POSTHARVEST QUALITY OF KENAF (HIBISCUS CANNABINUS L.) PLANT

SITI NUR BAYA A. RAHMAN

FACULTY OF BIOTECHNOLOGY AND FOOD SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

THE INFLUENCE OF PLANT PARASITIC NEMATODES ON THE POST HARVEST QUALITY OF KENAF (*HIBISCUS CANNABINUS L.*) PLANT

By
Siti Nur Baya A.Rahman

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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

Signature : 

Name : Siti Nur Baya binti Che Abdul Rahman

Matric number : UK15695

Date : 3/5/2010

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ABSTRACT

Nematode is a pest which caused a serious problem to kenaf production in kenaf field at Telaga Papan, Merang, Terengganu. This problem caused high loss of kenaf production in pilot project under National Tobacco Board (LTN). In this study, the hypothesis that root-knot nematode was a constraint to the field production of kenaf was investigated. The natural population of root-knot nematodes was tested by assaying the infected kenaf field soil. The effects of plant post harvest physiology also being determined. By using the infected soil, kenaf plant was planted until their maturity stage to see the effect of their presence. The treated soil used as control soil to be as a comparison to infected soil. The growth rate was taken once every month. After three month, when the plant harvested the diameter of basal discs, moisture loss and microscopic observation have been done to the root. For the postharvest evaluation, the height of kenaf shows slightly differences where the infected soil is lower in height. The basal diameter of kenaf in infected soil is ununiformly form and high moisture loss which is more than 55% than control soil. The root of kenaf in soil infected by nematode is stunted with galls and egg mass on it but in treated soil, the root grows healthy. From the microscopic observation, the presence of *Meloidogyne spp.* had been determined based on their basic morphology.

ABSTRAK

Nematode adalah perosak yang menyebabkan masalah yang besar terhadap penghasilan kenaf di Telaga Papan, Merang ,Terengganu. Masalah ini membawa kepada kehilangan hasil kenaf dalam projek permulaan dibawah Lembaga Tembakau Negara. Dalam kajian ini, hipotesis bahawa nematod yang menyebabkan pembengkakan akar adalah penghalang yang mengurangkan hasil kenaf telah disiasat. Populasi semulajadi nematod dikaji dengan menguji tanah dari ladang yang telah diserang. Kesan nematod terhadap hasil lepas tuai kenaf juga dikenalpasti. Dengan menggunakan tanah yang telah dijangkiti, kenaf ditanam sehingga matang. Tanah yang telah dirawat digunakan sebagai kawalan untuk membuat perbandingan.kadar pertumbuhan pokok kenaf diambil sebulan sekali. Selepas tiga bulan,apabila pokok telah dituai,diameter batang,kadar kehilangan air dan pemerhatian terhadap akar kenaf dibuat. Dalam penilaian lepas tuai kenaf, ketinggian kenaf menunjukkan sedikit perbezaan dimana kenaf dalam tanah yang dijangkiti adalah lebih rendah. Diameter kenaf dalam tanah dijangkiti terbentuk secara tidak sekata dan peratus kehilangan air adalah tinggi sebanyak 55% berbanding kawalan. Akar kenaf dalam tanah dijangkiti nematod adalah terbantut dengan kehadiran bengkakan dan kantung telur manakala akar dalam tanah kawalan tumbuh dengan sihat. Daripada pemerhatian secara mikroskopik, kehadiran *Meloidogyne spp*. telah dikenalpasti.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

A fiber crop plant, kenaf (*Hibiscus cannabinus L.*) is originated from east central Africa and widely planted in many parts of the world as a source of fibers and food consumption (LeMahieu *et al.*, 2003). It is a cheaper source of material for high quality products such as charcoal, paper, non-woven matting in the automotive industry, animal bedding, textile, fuel, edible oil and fiber in new and recycled plastics (injected, moulded and extruded)(Webber and Bledsoe,1993).

Kenaf grown in Malaysia as a substitute crop replacing tobacco because of the effect of Asean free trade area (AFTA) expected in 2010 which will see Malaysia reducing the import duties on tobacco import making tobacco production in this country will be less competitive (Anon, 2009a). Kenaf can serve as important economic activities under the agriculture sector which will be the main trust of east coast economic region (ECER) which believed can provide higher socio-economic value to this country.

Kenaf is composed of two different fibers which are inner core fiber and outer bast fiber with a makeup of about 35% and 65% respectively (Abdul Khalil *et al*, 2006.). The outer bast fiber produces higher quality pulp than its core fiber. The two types of fiber, enourmously different properties and different commercial uses markets as a result. Recent studies have investigated the development of biodegradable composite material using natural fiber of kenaf. It is also high potential

plant because it can survive in harsh condition with low supply of water (Mat Daham *et al*, 2005).

According to this widely usage of kenaf, the research and development activities of kenaf was spearheaded by scientists since years 2000 and the technology of kenaf industries had been increased by producing new kenaf cultivar which will enhance the competitiveness in the production. Hence, research and development efforts in Malaysia Agriculture Research and Development Institute (MARDI) including developing varieties which are suitable for commercial planting for high fiber production in Malaysia. From all the varieties obtained, the kenaf V36 is the most highly production of stalks rather than other varieties (PS H'ng *et al*., 2009).

Under the National Tobacco Board, kenaf was planted and one of the potential area is in Telaga Papan, Merang, Terengganu. It was planted in bris soil with kenaf V36 cultivar. The expected yield is 15t/ha according to the research have been made on this cultivar. But, the yield obtained is not really satisfactory as expected because it is only 5t/ha. It is a big lost because only 30% from the expected yield have been collected.

The symptoms to the physiology of plant can be seen obviously in its roots which full of galls besides the stems stunted and lower yield. Kenaf also from family Malvaceae which is very susceptible to nematodes especially *Meloidogyne spp.* (Zhang and Noe,1996; Ibrahim *et al*,1982). Based on research by McSorley and Parrado, 1986 nematodes is a major pest in kenaf cultivating reducing up to 52% yield. The main problems in this plantation is believed caused by nematodes according to the above ground and underground symptoms identified from the kenaf plants physiology. The aim of this research is to evaluate effects of field production of root-knot nematode infection in kenaf field in Telaga Papan and the effect on the postharvest quality of kenaf.

1.1 Problem statement

The growth and yield of kenaf in the field at Telaga Papan, Merang, Terengganu, was seriously affected by plant parasitic nematodes. Plants were poorly infected, stunted and chlorotic. The yield obtained in the pilot project was less than 30% expected yield of 15 ton/hectare/crop season.

1.2 Significant of study

The significance of this study is to evaluate the natural field population level of Meloidogyne incognita to pre and postharvest yield and quality of kenaf plants. Results from this study will to determine if an alternative short term crop should be planted prior to kenaf to suppress the field population, for economic yield and quality of subsequent kenaf crop.

1.4 Objectives

1. To bioassay the natural field population of root-knot nematode, *Meloidogyne incognita*, in Telaga Papan, Merang, Terengganu.

2. To study the effects presence of plant-parasitic nematodes on yield and post harvest quality of kenaf.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin of kenaf plant

Kenaf has been grown for thousand years in Africa (Western Sudan) until it was introduced into southern Asia in 1900, cultivation kenaf spread widely to India for the last 200 years, and Russia in 1902. In 1935, kenaf was introduced to China (Dempsey,1975). The kenaf production and research of in the United States started during World War II, the supplies of foreign fiber from Philippines for cordage material was interrupted by this war. Later, in 1950s and early 1960s, since kenaf was determined as a high potential crop there are many research initiated by USDA to increase the production of kenaf yield and products (Webber *et al*, 2002).

2.2 Characteristics of kenaf plant

Kenaf comes from Malvaceae family which is recognized as its economic and horticulture significance. The genus of *Hibiscus* is very large, comprising of 200 annual and perennial species and also related to okra, cotton, roselle and the hollyhocks (Dempsey,1975). Kenaf is short-day, annual herbaceous plant which is planted for its fiber, soft and bast fiber in its stems shows in the Figure 2.1.

The kenaf stalks always has a rounded, regular section. There is pith all along

the stalk tapering towards the top end.The whole stalk has two fibrous components - bast (bark) and core portions. The core can be separated from the bast fibers with a mechanical fiber separator. Kenaf leaves maybe completely whole or deeply lobed with serrated edge, it is highly variable in shape they can be whole that is like single leaf or palmate with leaf-stalk whole but deeply lobed. Kenaf well known as a good fiber quality produced from its stems but the whole parts of its plants actually have the potential uses.

In indusrial plantation, the kenaf stem is erect, straight with a cylindrical cross-section. It reaches height of over 4 meter. The stem is generally entirely green or green with reddish speckles or reddish or red depending on the cultivars or varieties. The plant on poor soil but the yield is influenced by day and night temperature, and suitable moisture condition. (Webber *et al*,2002)

Figure 2.1: The fibers in the kenaf plant cross sectional. a) high value bast fiber and b) core fibre which is lower quality than bast fibre.

Kenaf can adapt to relatively wide range of climate, soil from clay soil to sandy soil. However, it is more suitable sandy soil with high of nutrient content and have high water holding capacity. According to Dempsey (1975), one of most advantages as a crop is it can be successfully grown in a wide range of soil types from high organic peat soils to sandy desert soils. Although kenaf grows better on well-

drained, fertile soils with a neutral pH, the crop can withstand late season flooding, low soil fertility and a wide range of pH soil. Kenaf also shows excellent tolerance to drought condition. (Webber *et al*, 2002)

The suitable temperature for kenaf cultivation is about 25-28°C and rainfall about 240-490 mm per year with average of life span about 4-5 month of planting time. The optimum pH of soil for kenaf is about 6.0-7.0. This plant is very photosensitive. It starts to flower when day time is about 12.5 hours and plant growth decreases and eventually stop. In Malaysia, kenaf is recommended for planting during the rainy season to get high supply of water (Mat Daham *et al*., 2005).

The yield and quality of fiber and influenced by harvesting time. If the plant is harvested too early, fiber is still immature. Fiber yield will be low and destroyed during processing. If it is harvested very late, fiber yield will be high but percentage of lignin in the fiber increased. The fiber will be very rough and difficult to process. The best time to harvest the plant is during flowering (Figure 2.2). The actual cultivation time of kenaf is approximately from 90 to 150 day depending on the varieties (Mat Daham *et al*., 2005).

Figure 2.2 : The kenaf plantation in Telaga Papan, Merang , Terengganu which already can be harvested.

2.3 Potential and usage of kenaf

Kenaf is higher in cellulose fibre than wood. The main uses of kenaf fiber have been rope, twine, coarse cloth similar to that made from jute, and paper. Kenaf leaves are used as animal feed and also as human food in some sauces of African and Asian cuisine (Anon, 2009b).

Rising uses of kenaf fiber include engineered wood, insulation, and clothing-grade cloth, Panasonic has set up the plants in Malaysia to manufacture kenaf fiber boards and export them to Japan (Anon, 2010), as oil absorbent, soil-less potting mixes, animal bedding, packing material, organic filler for blending with plastics for injection molding (using the technology developed and patented by Fiber Packaging International, Inc.), as an additive for drilling muds, and various types of mats, such as seeded grass mats for instant lawns and moldable mats for manufactured parts and containers.

Kenaf seeds yield a vegetable oil that is edible and high in omega, antioxidants and protein content (Killinger,1969). The kenaf oil is also used for cosmetics, industrial lubricants and as bio-fuel. As the increasing uses potential of kenaf in the industry, there are many works being done to develop the production and uses of kenaf rapidly all around the world.

2.4 Research and development of kenaf in Malaysia

Kenaf was brought in Malaysia in the early 1970s and was highlighted as an alternative fiber crops in late 1990s because it can supply low cost of fiber. Malaysia Agriculture Research and Development Institute (MARDI) is the agency which play important role in development of new kenaf varieties in Malaysia. Since kenaf was exposed as a high potential plants in the future,many technology had been developed to find the high quality of kenaf to be planted.

The new variety of kenaf was produced by MARDI and institute of Tropical Forestry and Forest Product (INTROP). There are nine new varieties of kenaf which were identified as a potential for commercially planting namely, Q-Ping, KK60,V12 ,V19 ,V36 ,V132 , NS, V133 and TK have their own characteristic and differed in stalk production rate. Cultivar V36 has the highest stalks production and most suitable for commercial production. The cultivar reach a height over 3.0m with average basal stem diameter of 2.1cm and can be harvested after 3 months in the field. On BRIS soil, yield of dry stalk is about 15t/ha comprises of 4.0t/ha and 10.5t/ha bast and core fiber respectively. Based on this results V36 had been promoted for cultivation in the BRIS soil in Malaysia and utilized in Malaysia on BRIS soil (PS H'ng *et al.*, 2009).

2.5 Nematodes as a crop pest

Nematodes is an aquatic organism which only shows their symptoms on certain suitable plant host and belongs to the kingdom Animalia which is worm like in appearance but quite distinct taxonomically from the true worms. It is considered as a plant pest since the first discovery of *Anguina triciti* as plant parasitic nematode in 1743 by Needham. After that about 3000 species of plant parasitic nematodes have

been identified within 150 genera all around the world (Roland *et al*, 2006). Plant parasitic nematodes are small, 300 to 1000 micrometers, microscopic in size and cannot be seen with naked eye.

Several hundred species are known to feed on living plants getting their food with spears or stylets and causing a variety of losses. The annual estimated crop loss on the life-sustaining crops and most other economically important crops (vegetables, fruits, and non-edible field crops) are about 14% (George.N.A, 2005). Usually plant cells fed on by nematodes are not killed. Some of them are ecto-parasite and some are endo-parasite. Ecto-parasite are feed from the outside where as endo-parasite will feed from inside of the plant, both feeding types may be migratory or are sedentary and settle in some location permanently at their feeding site.

Crops attacked by nematodes produce both above and below ground symptoms. Above ground symptoms related to roots destruction such as stunting, yellowing and wilting. Root symptoms may include galls, lesion, stunting, stubby appearance, excessive branching and a generalized darkening or rotting of the root tissues with highly number of nematodes (G.N.Agrios, 2005). Many plant parasitic nematodes enhance crop losses by forming disease complexes with other soil-borne pathogens such as fungi and bacteria, making it hard to make expectation exactly how much damage the nematodes are causing (J.P.Noe., 2008).

2.6 Root-knot nematodes as a plant parasite pest

The root-knot nematodes cause most of the damage reported on agricultural crops all around the world (Lamberti *et al*, 1979). They are distributed worldwide and parasitize for nearly most species of higher plants. Typically they reproduce and feed

within plant roots and form small to large galls or root-knots. Root-knot nematodes are often found in warmer climates and sandier soil.

This type of nematodes will hatch from their eggs when there are suitable condition. The second stage larvae, J2 hatches out of eggs and infect nearby galled roots or invade new roots. The attraction of J2 to plant roots depend on the concentration gradients of attractants emanating from the plant root. When the nematodes come in contact with plant roots, they often penetrate the root immediately behind the root tip. Several nematodes may invade the same area of the root. The root tip enlarges and root elongation stops. The galls are usually formed after 1-2 days after the nematode enter the root. The size of galls is depending on the host plant, number of J2 and nematode species.

The lifecycle of root-knot nematodes usually about 21days to 50days, depending on the environment factors, root-knot species and plant host (James.P.N., 2008).Temperature is one of the important factors in several stages of the nematodes development stages. As they are all poikilothermic animals, temperature influences distribution, survival, growth and reproduction (Roland *et al*, 2006). Soil texture, moisture and osmotic potential are relating factors that make it very difficult to find out the effects of each one separately. *Meloidogyne* species are active in soils with moisture levels at 40% to 60 % of field capacity. The activities of nematodes decrease as the moisture of soils decrease or dry.

Root-knot nematodes are commonly known as they have unbalance sex ratios which undergo cross-fertilization because in certain environmental condition, the male may be absence, rare or abundance. Root-knot nematode also exhibit sexual dimorphism at maturity or called hermaphrodite.

There are several species of *Meloidogyne*, and *Meloidogyne incognita* is the most common globally, possibly because it has a very wide host (James.P.N., 2008). Root knot nematodes damage plants by devitalizing root tips and causing the formation of swellings of the roots. When susceptible plants are infected at the seedling stage, losses are heavy and may result in complete destruction of the crop. Infection of older plants may have only slight effects on yield or many reduce yields considerably (George.N.A, 2005).

The above ground symptoms on nematode infected crops are similar damaged and un-functional roots. The symptoms is usually relate to the population of nematodes within the root tissue. Plant development is suppressed by infection that restrain the root growth during moisture pressure and avoiding the roots from extending into most soil (Roland *et al*, 2006).

2.7 Susceptibility of kenaf plant to root-knot nematode

Kenaf (*Hibiscus cannabinus* L.) is an important fiibre crop in several countries with tropical or subtropical climates. Kenaf is from malvaceae family such as roselle and okra which are proved to be very susceptible host to nematodes infection. *Melodogyne arenaria, Melodogyne incognita* and *Melodogyne javanica* are recognized pathogens of kenaf in nearly all production regions, causing substantial galling of the roots. Because of the widespread distribution of these species, they represent a potential hazard to kenaf wherever it is grown, especially in sandy soils (Ibrahim *et al*, 1982).

Table 2.1: Reaction of 15 malvaceous plant cultivar to *Meloidogyne incognita* (race2, race3 and race 4), *M. javanica* and *M.arenaria* (race 1).

Plant cultivar	M. incognita race 2		M. incognita race 3		M. incognita race 4		M. javanica		M. Arenaria race 1	
	gall	egg mass	gall	egg mass	gall	egg mass	gall	egg mass	gall	egg mass
cotton (*Gossypium barbadense L.)*										
Giza 67	0	0 R	4	3 S	3	3 S	2	0 R	0	0 R
Giza 68	0	0 R	3	2 R	3	1 R	2	0 R	0	0 R
Giza 69	0	0 R	5	5 S	4	4 S	3	3 S	0	0 R
Giza 70	0	0 R	3	2 R	3	2 R	2	0 R	0	0 R
Giza 75	0	0 R	3	2 R	3	1 R	2	0 R	0	0 R
Bahtim 110	0	0 R	4	4 S	4	3 S	2	0 R	0	0 R
cotton (*G. hirsutum L.*)										
Deltapin 16	0	0 R	4	3 S	3	3 S	2	0 R	0	0 R
Acala 4.42	0	0 R	3	0 R	2	0 R	2	0 R	0	0 R
Acala 67A	0	0 R	3	0 R	2	0 R	2	0 R	0	0 R
kenaf (*Hibiscus cannabinnus L.*)										
Giza 1	4	3 S	5	5 S	5	5 S	5	5 S	5	4 S
Giza 2	4	3 S	5	5 S	5	5 S	5	5 S	4	4 S
okra (*H. esculentus L.*)										
Clemson spineless	5	5 S	5	5 S	5	5 S	5	5 S	3	3 S
Baladi	4	3 S	5	5 S	5	5 S	5	5 S	4	4 S
Mallow (*Malva pavipora L.*)										
Baladi	5	4 S	5	5 S	5	4 S	4	4 S	4	4 S
Roselle (*H. sabdariffa L.*)										
Baladi	0	0 R	0	0 R	0	0 R	0	0 R	0	0 R

Index rating: 0=0,1=1-2,2=3-10,3=11-30,4=31-100,5 more than 100 galls or egg masses/root system.
Reaction: R= resistant; S= susceptible

Sources: Ibrahim *et al.* (1982)

Several studies have examined the relationship between initial nematode population densities and yield of kenaf. McSorley and Parrado (1986) were able to relate root galling due to M. incognita with the growth (height) using the Seinhorst model and observed a damage threshold of eight galls per root system. Root-knot nematode, *Meloidogyne* spp.,is one of the major problems associated with the successful cultivation of kenaf (Lawrence and Mclean,1992). From this research, it is indicate that the high population of root-knot nematodes will reduces the kenaf yield vastly.

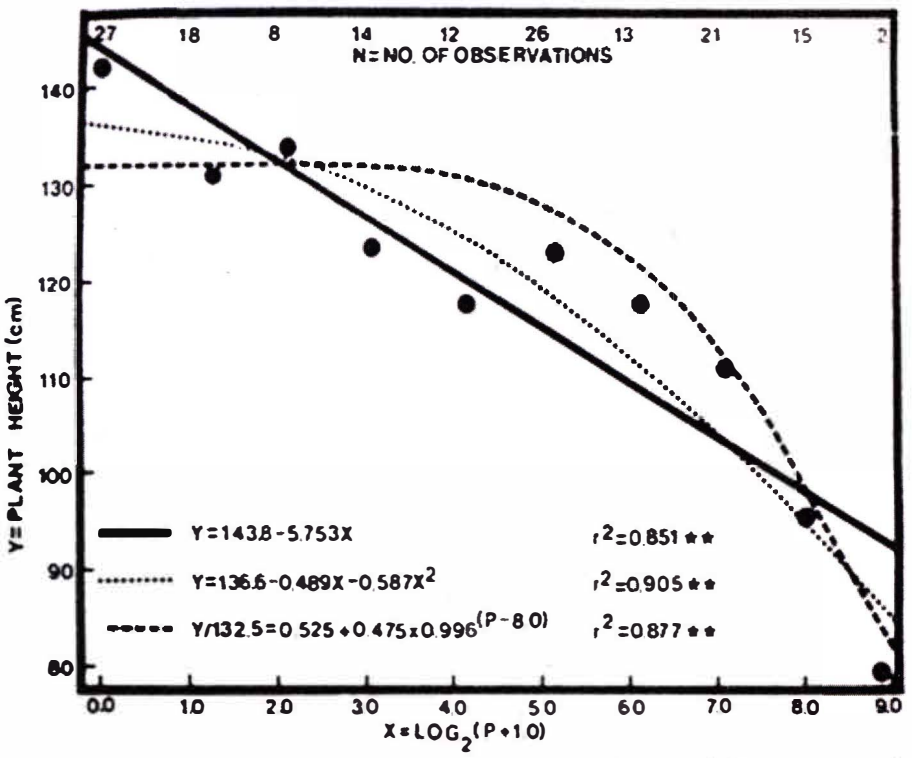

Figure 2.3: The relationship between height of kenaf plant with the galls and egg masses number the data set was not weighted by number of observation (N) per density class. Number of gall and egg masses per root systems.

Sources: McSorley and Parrado *et al.* (1986)

Root-knot nematodes have been reported to cause 20% to 60% yield losses on kenaf. Earlier research found out that kenaf is susceptible to three major root-knot nematode species: *M. incognita, M. javanica,* and *M. arenaria.* In greenhouse tests, kenaf was susceptible to all host races of M.*incognita*, to *M. arenaria,* and to M.*javanica* ((Veech 1989; 1992; Lawrence and Mclean, 1992). In field tests, severe galling and yield losses occurred when kenaf was infected by *M. incognita* (Wilson, 1966). Only at higher initial population densities of *M. Incognita* (5,000/500 cm ^{3} soil) were plant height, stalk diameter, root and stem weights significantly reduced (Barillas, 1993 et al.). From all the previous research being made we can see that there are highly potential of kenaf losses constraint by nematodes.

CHAPTER 3

MATERIALS AND METHODS

3.1. Sampling of soil

The soil which was infected by nematodes in kenaf field in Telaga Papan, Merang, Terengganu was collected according to the specific area. The area is A1.

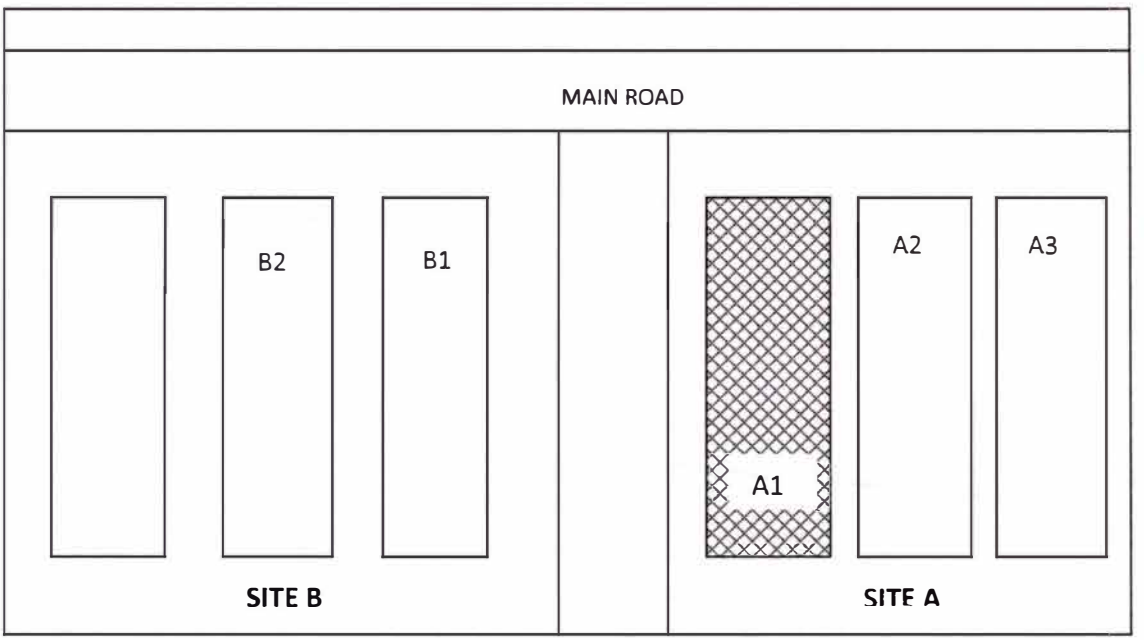

Figure 3.1: The soil sampling area with black area is the sampling place for the soil in this experiment.

3.2. Bioassaying of nematodes for root-knot nematodes only

Parts of the nematodes infected soil is planted with kenaf (*Hibiscus cannabinus* L.) and tomatoes (*Lycopersicon esculentum*) in 450ml polystyrene cups. The galls and egg masses of root-knot nematodes was observed and counted under dissecting microscope with phloxin B staining after 30 days planted. This method is

to ensure the presence of nematodes in the field area.

3.3 Planting the kenaf plant in infected and treated soil to determined the post harvest effects.

The infected soil from kenaf field was taken and mixed with organic soil and chicken dung in ratio 3:2:1, then it is placed into culvert. The control also use the same mixture and the infected soil placed with treated soil which have been treat by heat treatment under sunlight, this treatment is to kill the nematodes because it cannot live with absence of water. Kenaf variety V36 was used in this experiment. The plant transferred into culvert after 5 days germination process. The observation of the plant growth have been done for 3 month. The cultivation is according to the kenaf manual planting to ensure the trees get all the nutrient and water supply.

After three month, about 10 kenaf plants pulled out randomly to be observed. The postharvest physiology of kenaf was evaluated. The observation includes kenaf height, diameter, moisture loss and presence of galls and egg masses. In this experiment only the stalks is taken to account because it is the only value that is significant for fibers production in our country rather than other part of the plant. The stalk yields and stalk percentages are the source of the bark (bast) and core fiber.

3.3.1 The stem height

The height of kenaf stems was taken every month to see the rate of growth and the effects of nematodes to kenaf. This method had been done for both infected and control plants. The reading was taken for first, second and third month.

3.3.2 Diameter of basal stems

The discs from each portion of the (top, middle, base) was used for measurement of the core and bast fiber.

3.3.3 The moisture loss

This is the usual methods used to determine the quality of good trees and market value loss of crops especially in kenaf. The higher the loss of moisture, the lower the quality of plants. In this study the direct methods according to Tsoumis (1991), for determination of moisture content being used by drying and weighing. The aerated oven had been used to keep constant temperature at 100°C. The weight of kenaf taken until its weight becomes constant. The specimen allowed to cool down before weight being measured to ensure no false result. Then the percentage of total loss of each plant measured. According to this method, moisture content relationship is based on this formula:

$$Y = \frac{M_X - M_0}{M_0} \times 100$$

or

$$Y = \left[\frac{M_X - 1}{M_0}\right] \times 100$$

Y = moisture content (%)
M_X = initial mass (weight) of kenaf (g)
M_0 = oven dry mass (g)

3.3.4 The observation of galls and egg masses on the roots.

Observation had been done by staining the kenaf root with phloxine B and observed under dissecting microscope. The phloxine B is red acid dye which is a potent modulator of the cystic fibrosis trans-membrane conductance regulator which will stain the egg mass of nematode. (Zhiwei C. and David N.S., 2002)

The other root which is planted in nematode infected soil was cut and put into boiled acid fuschin laptoglycerol for 2 to 3 minutes by covered with muslin cloth. After that the root soaked into clear laptoglycerol (100ml lactic acid+ 100ml glycerol + 200ml distilled water) and let it for 2 to 3 weeks. The transparency of the root will increase and the nematode can be easier to be observed (Mireille *et al*, 2006).

The next root will be observed under scanning micron electroscope. Firstly, it is fixed with DESS solution, washed with buffer (sodium cacodylate for 30minutes) ,post fix with 0.1 osmium tetroxide and then hydrated with ethanol (30% + 50% + 70% + 95% + 100% + 100%) for every 30 minutes. The specimen then dried using critical drying point by using liquid carbon dioxide. After that, it is mounted on the aluminum stub to ensure the nematode in the root become more visible. The specimen then splutter coated with gold and then observed under scanning electron microscope.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Planting the kenaf plant in infected and treated soil to determined the post harvest effects.

As this bioassays carried out to conform the presence of nematodes in the kenaf field. So there are presence of nematodes in the root of kenaf (*Hibiscus cannabinus*) and tomato (*Lycopersicon esculentum*) plants. All the galls and egg masses of the nematodes can be observed after stained with phloxin B. The egg masses was colored in red. Between these two types of crop, the number of galls and egg masses in kenaf root is more than the number found in tomato roots. So the kenaf plant can be said to be more susceptible to root-knot nematodes rather than tomatoes. As stated in the Table 4.1 the number of galls and egg masses is decrease after the soil cultivated with kenaf. This is resulted because of the production of nematode has reduced due to kenaf cultivation which may be suppressed by another agent in the soil or the absence of host plant.

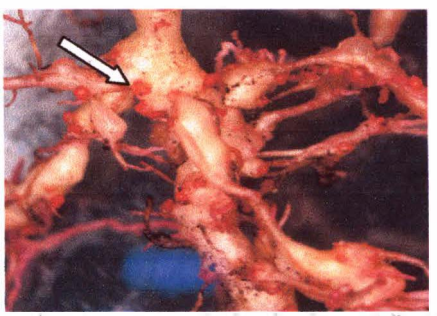

Figure 4.1: The presence of root-knot nematode in the kenaf field. The red color is egg masses of root-knot nematodes stained with Phloxin B on kenaf bioassays.

Table 4.1: The number of galls and egg masses of root-knot nematodes before and after planting of kenaf in the infected soil.

		before planting				after planting			
		kenaf		tomato		kenaf		tomato	
		Galls	Egg mass	Galls	Egg mass	Gall	Egg mass	Gall	Egg mass
plants	1	3	1	1	0	1	0		
	2	2	0	3	1	2	0		
	3	1	1	5	0	2	1		
	4	3	0	4	1	0	0		
	5	2	0	1	0	0	0		
	6	4	0	3	0	2	0		
	7	6	1	0	0	1	0		
	8	1	1	0	0	1	0		
	9	1	0	0	0	0	1		

For the second bioassays, there are no results for tomato because tomato is not planted due to technical problems.

4.2 Observation of post harvest effects on kenaf cultivated in infected and treated soil.

4.2.1 height of kenaf plant

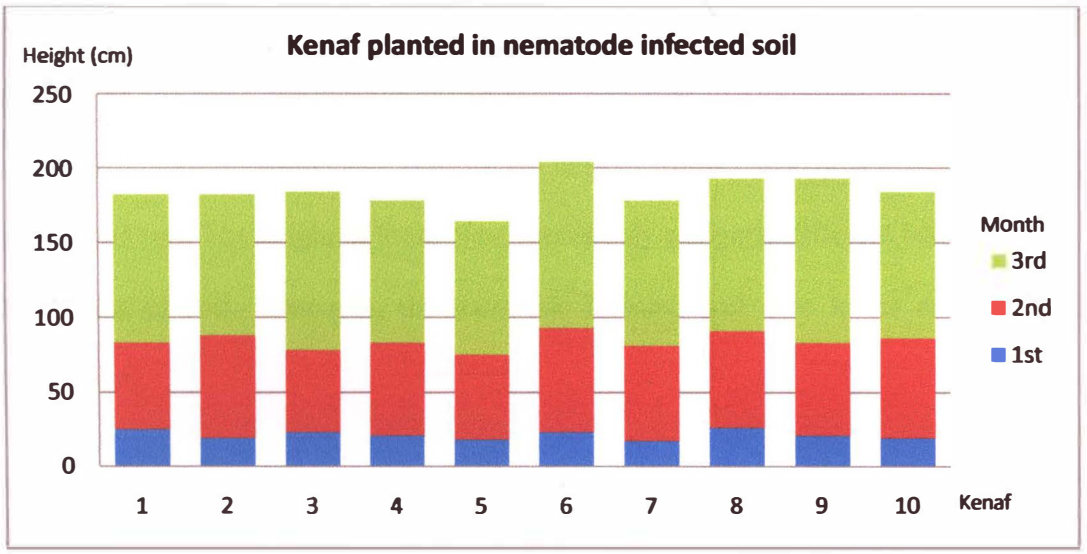

Figure 4.2: The height of kenaf plant in nematode infected soil for 3 month period.

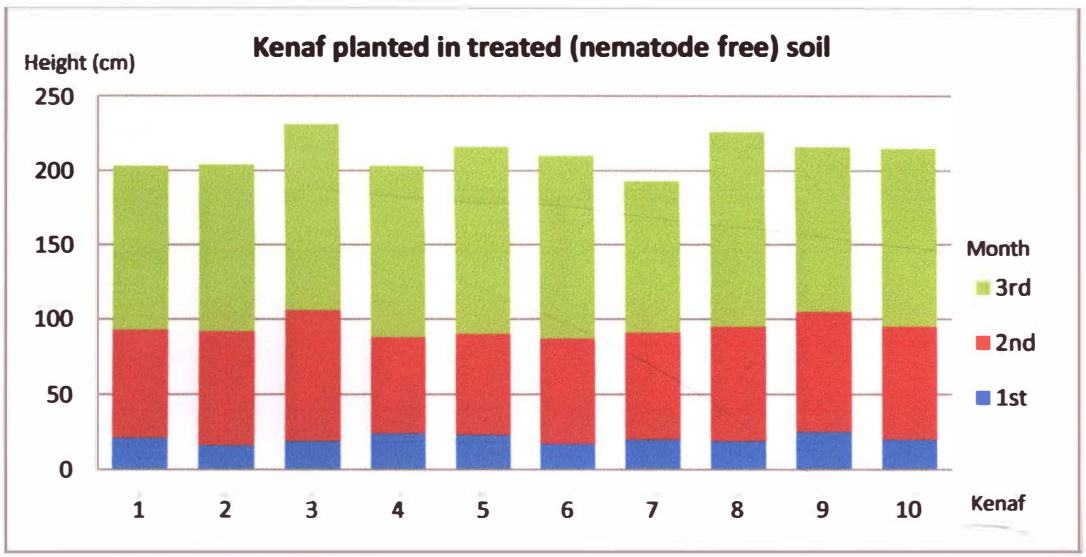

Figure 4.3: The height of kenaf plant in nematode treated soil for 3 month period.

The height of kenaf plant which taken out randomly from the soil shows the differences between kenaf cultivated in nematode infected and treated soil. Kenaf in nematode infected soil is 21.2cm for the first month in average, 62.9cm and 100.1cm averagely for the second and third month. For the kenaf planted in treated soil the height is 20.4cm ,73.8cm and 117.5cm in average for the first, second and third month. At the early growth development for the first month kenaf in infected soil grow faster than in treated soil. But, for the next month the growth is slowly decrease in infected soil. The height of these plant shows only a slightly different between these two types of soil. Generally, the nematode infected soil give lower reading than treated soil.

4.2.2 Diameter of basal stem of kenaf

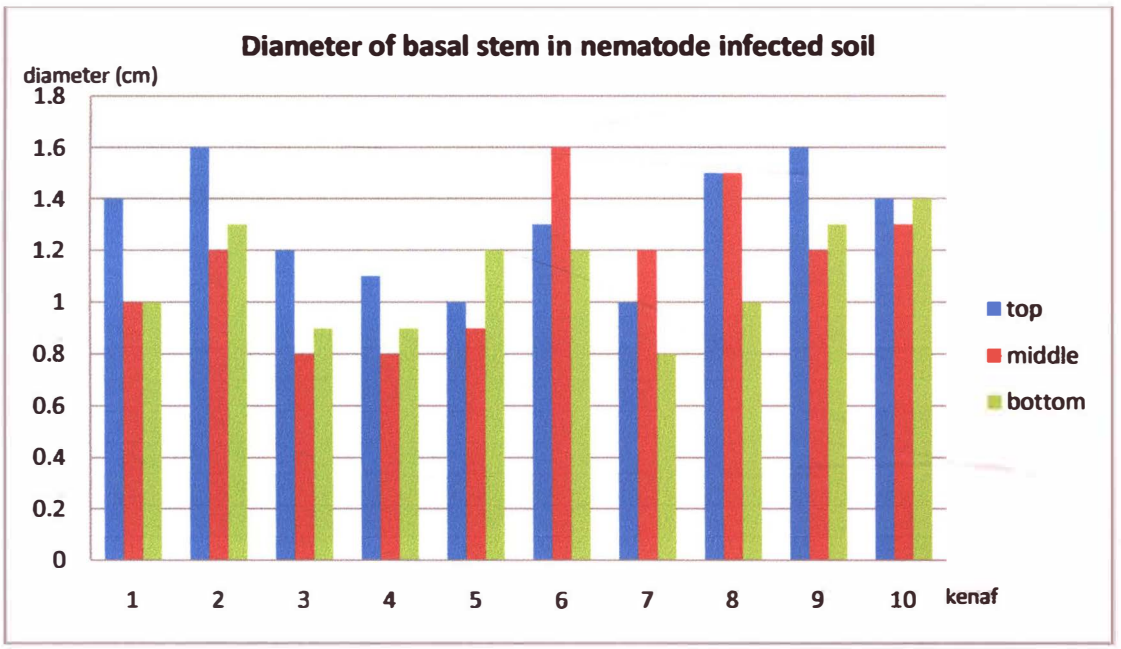

Figure 4.4: The diameter of basal stem for kenaf planted in infected soil

4.2.4 Observation of the roots

4.2.4.1Observation under dissecting microscope

From the observation of the kenaf root in both nematode infected soil and treated soil, there are different appearance of kenaf physiology. Kenaf planted in infected soil have galls and shows the red in color when stained with phloxin B which indicate the presence of nematode egg masses. The root also stunted and short in comparison with kenaf root planted in treated soil. There is no galls and egg masses in kenaf cultivated in treated soil. The plant root also grows healthy.

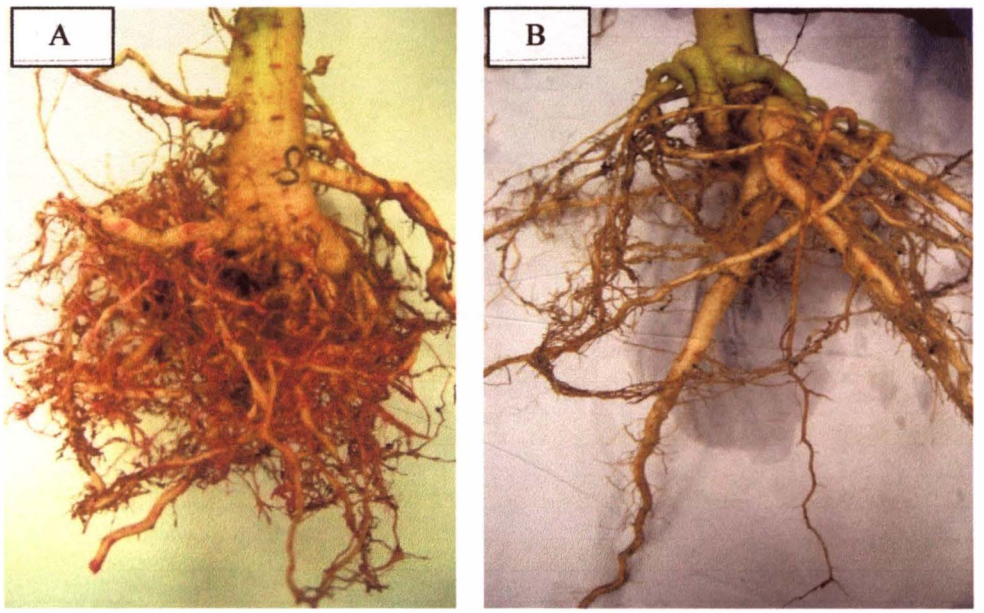

Figure 4.7: The roots of kenaf planted in infected and treated soil. (A) kenaf root planted in infected soil and (B) kenaf planted in treated soil.

4.2.4.2 Observation under compound microscope

The observation had been done in kenaf root planted in infected soils. The root stained with acid fuschin laptogylcerol and clear laptoglycerol to increase their transparency. The Figure 4.8 shows the presence of nematodes egg in the egg mass attached to the root. The egg can be seen through clearly in the egg mass.

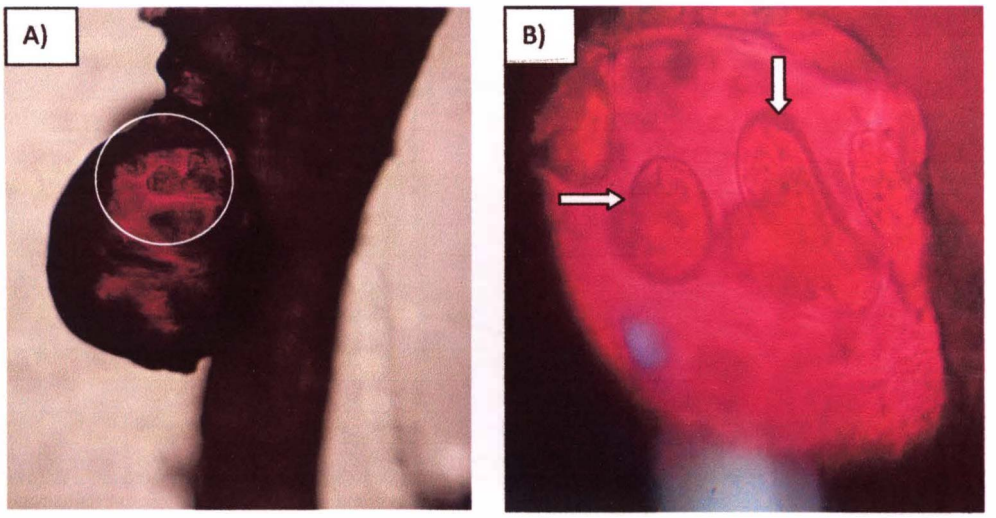

Figure 4.8 shows the nematode egg in the egg mass. A) root of kenaf attached with nematode egg mass. B) nematode eggs within the egg mass.

4.2.4.3 Observation under Scanning Electron Microscope (SEM)

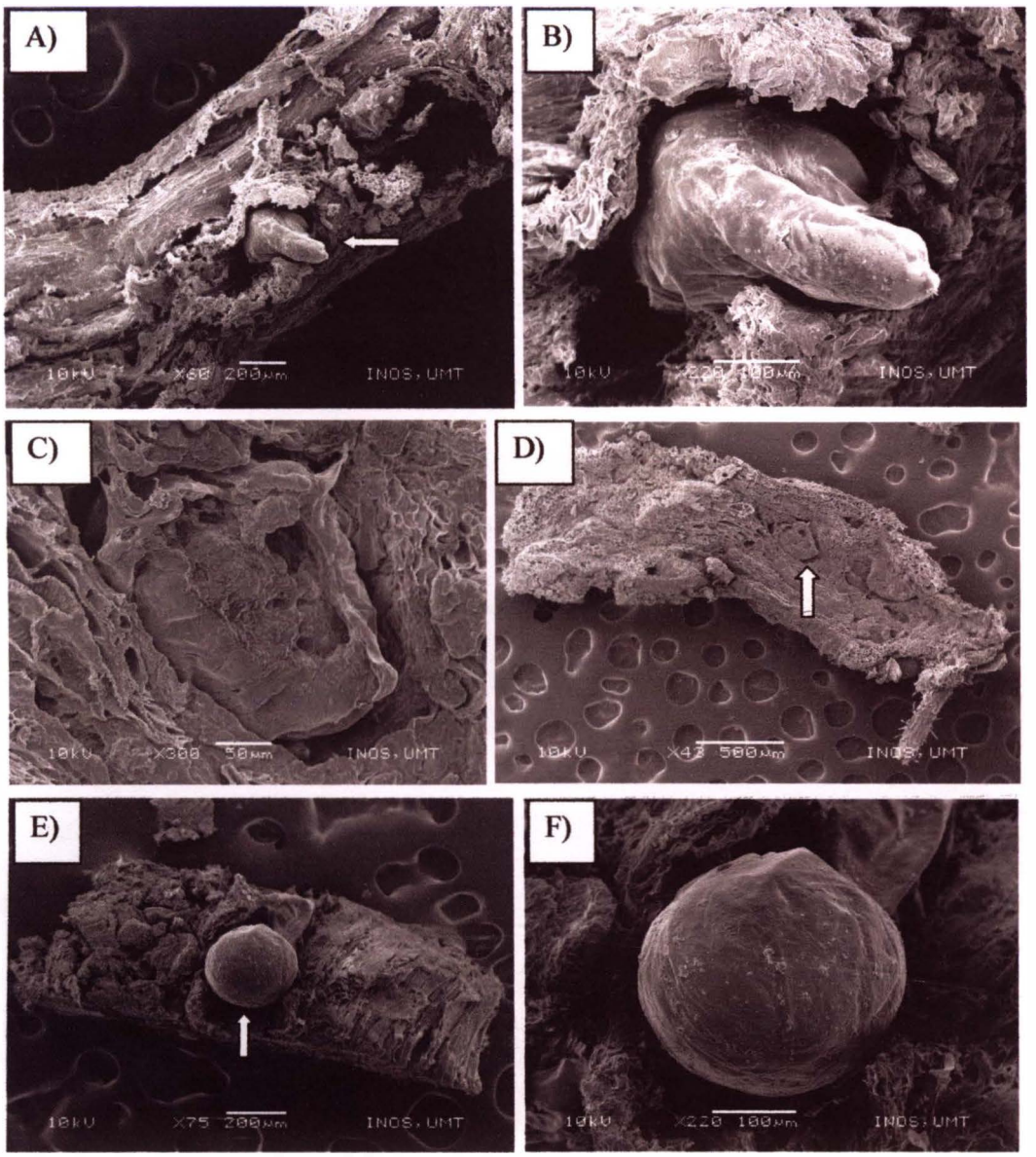

Figure 4.9: The nematodes in the kenaf root under observation of scanning electron microscope. A) nematode presence in the kenaf root, B) female nematode is presence in kenaf root with their anterior part, C) cross section of egg mass. D) cross section of egg mass in kenaf root, E) female nematode laid in the root cells and F) female nematode.

Root-knot nematode is one of several nematode genera which can positively diagnose from observation of the roots which may be the reason why it is the most common plant-parasitic nematode. First stage juveniles of this nematode undergo one molt while still in the egg.

The second stage is the destructive stage of this nematode as it juvenile move out from the egg and juveniles migrate to the nearest cell near the endodermis to begin the feeding when found the suitable host roots at the root tips. The nearby cells will be injected with the stylet and secretion of their esophagus glands. The cells serves as feeding cells for the whole nematodes life cycle and become a giant cells as it is enlarge and multinucleate because of nematode feeding activities. The giant cells function as a tube which deflect the downward flow of plant nutrient from the phloem to feeding the nematode. The galls will swiftly develop around the feeding juvenile as the cells continue to divide and enlarge.

The third and fourth stage of nematode is the shorter stage which the female will enlarge and spherical in shape called pyriform. The male nematodes molt to a large vermiform shape and migrate out of the root. The posterior end of the adult will protrudes from the root gall surface which usually produces about 300 to 500 eggs. The egg will survive as it is protected by egg-mass matrix and host plant debris.

The raised of metabolic activity of giant cells stimulates mobility of photosynthetic product from all the plant parts, in particular to the giant cells where they are eradicate and exploit by the feeding nematodes. The substance accumulation and mobilization is maximum when the matured female start to lay the eggs and then it will decline. The root infected by *Meloidogyne* will decrease the photosynthesis in plant leaves as the nematode interfere with the production of root-deduce factors which regulate photosynthesis. *Meloidogyne* act as a metabolic descend in infected

plant. The infection caused the abnormal root growth results in condensed root surface area and restraint the root capacity system to explore the soil. As a result from the infection of root-knot nematode, the essential cause of reduced nutrient uptake and suppressed growth of infected plants which may be related to shortened root growth.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In this study, there are presence of nematode had been determined in the kenaf field in Telaga Papan, Merang, Terengganu. Nematode is a plant parasite organism which had been a constraint to the kenaf production. Kenaf plant is very susceptible to root-knot nematode and a very good host, so the loss is very high. The post-harvest physiology of kenaf affected by nematode shows the symptom such as low growth rate and plant stun- ted. The moisture lost of kenaf is more than 55% which is high economic value loss. But in this study, the environmental factor influenced the results of the kenaf growth rate caused by climate changes. From the observation under the microscopic, the nematode from *Meloidogyne spp.* is determined based on their general morphology. So the nematode is proved as a pathogen to kenaf plant and it give negative effects to the production of kenaf as a new alternative crops in Malaysia.

5.2 Suggestion for future study

The research on plant parasitic nematode should be carried out for more as there are lack of expert in this field in our country because nematode can be considered as a very destructive pest to certain crop.

More development of nematode pest management for infected soil should be established as the controlling methods available is crop rotation by using unsusceptible host plant and nematicides which is very high in cost and cannot be afforded by most of farmers in our country as agriculture is still in low level in our country. So, the cheaper and affordable controlling method should be utilized.

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APPENDICES

A: the height of kenaf plants in 3 month planting period

Kenaf plant		Length (cm)/month		
		1st	2nd	3rd
Infected soil	1	25	58	99
	2	19	69	94
	3	23	55	106
	4	21	62	95
	5	18	57	89
	6	23	70	111
	7	17	64	97
	8	26	65	102
	9	21	62	110
	10	19	67	98
Average		21.2	62.9	100.1
Treated soil	1	21	72	110
	2	16	76	112
	3	19	87	125
	4	24	64	115
	5	23	67	126
	6	17	70	123
	7	20	71	102
	8	19	76	131
	9	25	80	111
	10	20	75	120
Average		20.4	73.8	117.5

B: the diameter of basal stem of kenaf from top,middle and bottom part.

Kenaf plant		Diameter (cm)		
		top	middle	bottom
Infected soil	1	1.4	1	1
	2	1.6	1.2	1.3
	3	1.2	0.8	0.9
	4	1.1	0.8	0.9
	5	1	0.9	1.2
	6	1.3	1.6	1.2
	7	1	1.2	0.8
	8	1.5	1.5	1
	9	1.6	1.2	1.3
	10	1.4	1.3	1.4
Treated soil	1	2	1.5	0.9
	2	2	1.1	0.8
	3	2	1.1	1
	4	2.2	1.6	1.2
	5	2	1.2	1.3
	6	1.1	1.5	2.3
	7	0.8	1.2	1.7
	8	1.4	2	2.2
	9	0.8	1.2	2.1
	10	1.1	1.6	1.8

C: the percentage of moisture loss in kenaf

Kenaf plant		Wet mass (g)	Dry mass (g)	Moisture loss (%)
Infected soil	1	87.7	34.4	60.8
	2	82	35.7	56.5
	3	101	44.4	56
	4	79.9	28.9	64
	5	99.3	32.9	66.9
	6	114.9	46.6	59.4
	7	86.2	31.7	63.2
	8	99.1	44.9	54.7
	9	95.3	33.7	64.6
	10	90	35	61.1
Treated soil	1	89.1	71.4	19.9
	2	92.3	76.3	17.3
	3	130	92.1	29.2
	4	99.3	63.3	36.3
	5	104.3	64.7	38
	6	112.2	75.7	32.5
	7	99.4	74.6	24.9
	8	99.1	63.9	35.5
	9	121.7	73.7	39.4
	10	119.3	83.8	29.8

CURRICULUM VITAE (CV)

Name : Siti Nur Baya binti Che Abdul Rahman

Permanent Address: 1202 Jalan Sultanah Zainab, 15050 Kota Bharu, Kelantan.

Telephone Number: 013-9165519

Email : baya7078@gmail.com

Date of birth : 01 July 1988

Place of birth : Tumpat, Kelantan

Nationality : Malaysian

Race : Malay

Gender : Female

Religion : Muslim

Educational Background

2007-2010 Universiti Malaysia Terengganu

2006-2007 Kolej Matrikulasi Perak

2004-2005 Maktab Rendah Sains Mara Muadzam Shah, Pahang.

2001-2003 Sek.Men.Keb. Ismail Petra,Kota Bharu.

Skills and Experiences

- Fluent on Bahasa Malaysia and English
- Fast learner and love to try new things
- Experiences as a trainer in Deparment of Agriculture in Kota Bharu during internship
- Can be a good team player and like to work in groups.

- Microscopic handling
- Skillful in managing Microsoft Power Point, Microsoft Word, Microsoft Excel and Adobe Photoshop.
- Love to work in the field

Co-curricular Activities

- Participating in Gunung Ledang Trekking 2010
- Attending pre-intership workshop of Faculty of Agrotechnology and Food Science.
- Participant in Permuafakatan Assyura Programs 2009
- Participant in Halal Slaughtering Workshop 2008
- Participant in Rakan-muda wawasan desa IPT 2008
- Committee member of Rugby Tournament MASUM 2008
- Committee member of Pertandingan Dikir Barat IPTA MAKUM 2007
- Participant in Malaysia book of records for making the longest palm print jalur gemilang,08/2007

THE INFLUENCE OF PLANT PARASITIC NEMATODES ON THE POST HARVEST QUALITY OF KENAF (HIBISCUS CANNABINUS L.) PLANT - SITI NUR BAYA A. RAHMAN