UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF ROOT KNOT NEMATODES AT LADANG SAMARAKAN AND POTENTIAL OF BLACK PEPPER CULTIVATION IN HYDROPONICS FOR ROOTSTOCK

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PREVALENCE OF ROOT KNOT NEMATODES AT LADANG SAMARAKAN AND POTENTIAL OF BLACK PEPPER CULTIVATION IN HYDROPONICS FOR ROOTSTOCK

By

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ABSTRACT

Black pepper (*Piper nigrum* L.), the king of spice, holds high economic value as it is one of the most important and widely consumed spices in the world. The aim of this study is (1) to study the relationship between prevalence of root knot nematode and the soil properties; (2) to study the potential of black pepper rootstock cultivation in hydroponics; and (3) to characterize of black peppers growth in fully aerated hydroponics. At Ladang Samarakan, 10 completely random soil sampling points were done for each of the varieties of black peppers plants (Panniyur-1, Kuching, and Semongok Emas). Then, for each variety of the black pepper plants at Ladang Samarakan, 10 randomly selected black pepper plants were selected for survey of prevalence of root knot nematodes. There was 60% of Semongok Emas black peppers that was infected with root knot nematode compared to the 10% of Kuching and 10% of Panniyur-1 at Ladang Samarakan. Semongok Emas black peppers soil temperature was the lowest with 27.3 ± 0.945°C while Panniyur-1 with 28.7 ± 0.879°C and Kuching with the highest temperature of 29.5 ± 1.089°C. There was a negative correlation between soil temperature and the prevalence of root knot nematodes in black pepper plants at Ladang Samarakan at p 0.025 < 0.05. Then, potential environment for setting up hydroponics for black pepper rootstock cultivation was designed based on the aeration in the hydroponics (non-aerated, half-aerated and fully aerated). Fully aerated black pepper hydroponics shown 100% of survival and rooting with 75% of sprouting of black peppers. As for non-aerated and half aerated black pepper hydroponics, the black pepper cuttings could not grow and survive throughout the 12 weeks study period. The growth of black pepper cuttings in fully aerated hydroponics is recorded, showing 6.0 ± 4.60 cm increase in stem

length, 24.28 ± 3.10 cm increase in longest roots and 2.8 ± 1.2 total number of leaves throughout the 12 weeks study period. In conclusion, there was negative correlation between soil temperature and prevalence of root knot nematode at Ladang Samarakan and potential of growing black peppers in hydroponics.

ABSTRAK

Lada hitam (*Piper nigrum* L.), raja rempah-ratus, memegang nilai ekonomi tinggi kerana merupakan salah satu rempah yang paling penting dan digunakan secara meluas di dunia. Tujuan kajian ini adalah (1) untuk mengkaji hubungan antara peratusan nematode puru akar dengan sifat tanah; (2) untuk mengkaji potensi penanaman pokok lada hitam dalam hidroponik; dan (3) untuk mencirikan pertumbuhan lada hitam dalam hidroponik penuh pengudaraan. Di Ladang Samarakan, 10 titik pensampelan tanah secara rawak dilakukan untuk setiap jenis tanaman lada hitam (Panniyur-1, Kuching, dan Semongok Emas). Kemudian, untuk setiap jenis tanaman lada hitam di Ladang Samarakan, 10 pokok lada hitam dipilih secara rawak untuk membuat kaji selidik terhadap kelebihan nematod puru akar. Terdapat 60% lada hitam Semongok Emas yang dijangkiti nematod puru akar berbanding dengan 10% Kuching dan 10% Panniyur-1 di Ladang Samarakan. Pokok lada hitam Semongok Emas mempunyai suhu tanah yang paling rendah dengan suhu 27.3 ± 0.945°C manakala Panniyur-1 dengan 28.7 ± 0.879°C dan Kuching dengan suhu tertinggi 29.5 ± 1.089°C. Terdapat korelasi negatif antara suhu tanah dengan peratusan nematod puru akar dalam tanaman lada hitam di Ladang Samarakan. Kemudian, persekitaran yang berpotensi untuk penanaman pokok lada hitam dalam hydroponik direka berdasarkan pengudaraan dalam hidroponik (tanpa pengudaraan, separuh pengudaraan dan penuh pengudaraan). Hidroponik lada hitam yang penuh pengudaraan menunjukkan 100% survival dan 100% pengakaran dengan 75% percambahan untuk keratan lada hitam. Bagi hydroponik lada hitam yang tanpa pengudaraan dan separuh pengudaraan, keratan lada hitam tidak boleh bertumbuh dan bertahan hidup sepanjang tempoh kajian selama 12 minggu. Pertumbuhan

keratan lada hitam dalam hidroponik penuh pengudaraan direkodkan, menunjukkan 6.0 ± 4.60 cm pertumbuhan tangkai keratan lada hitam, 24.28 ± 3.10 cm pertumbuhan pada akar terpanjang keratan lada hitam dan 2.8 ± 1.2 jumlah daun dalam tempoh 12 minggu kajian. Kesimpulannya, terdapat korelasi negatif antara suhu tanah dengan peratusan nematod puru akar dalam tanaman lada hitam di Ladang Samarakan dan tanaman lada hitam mempunyai potensi untuk tumbuh dalam hidroponik.

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APPROVAL SHEET

I certify that this research project report entitled "Prevalence of Root Knot Nematodes at Ladang Samarakan and Potential of Black Pepper Cultivation in Hydroponics for Rootstock" has been examined and approved as a partial fulfilment of the requirements for the Degree of Bachelor of Bioindustrial Science in the Faculty of Agriculture and Food Science, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATION

SAS	Statistical Analysis Software
Piper nigrum L.	*Piper nigrum* Linn.

CHAPTER 1

INTRODUCTION

Black pepper (*Piper nigrum)* of the family Piperaceae is a spice crop native to South India. Often nicknamed as "black gold" and "king of the spices", black pepper carries high economic value as it is one of the most important and widely consumed spices in the world. Black pepper accounts for about 20% of all spice imports around the world (Parthasarathy *et al.* 2008).

In Malaysia, Sarawak is the largest state producing about 14,903 tonnes of pepper, which is valued at RM 287.4 million in 2011. This ranks Malaysia as the fifth in the whole world in terms of producing pepper. About a third of Sarawak's agriculture export earnings is generated by pepper since the year 1990 (Olalere *et al.* 2016).

However, there are plenty of diseases and pests related to black pepper plants that can severely restrict the yield of black peppers (Shahnazi *et al.* 2014). Among the pests that are well known to target black pepper plants are the Root-knot nematode (RKN) which are plant-parasitic nematodes from the genus *Meloidogyne*. The few most prevalent species identified within the *Meloidogyne* genus is *Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria* and *Meloidogyne hapla.* Out of the four *Meloidogyne* sp., *M. incognita* is discovered to be more distributed in all the black peppers plantations in Sarawak (Ramana and Eapen 2000). *M. incognita* alone has been recorded to be responsible for 46% of loss in yield of black pepper worldwide (Reddy 2014).

It is difficult to research on root knot nematodes and other root diseases on black pepper plants due to the fact that observation of black pepper roots in the soil require uprooting completely and therefore may damage the rooting system. The soil itself is also not transparent for the researchers to observe directly on the roots. In addition, the soil itself is also a complex media containing a lot of microbes and other organisms (Young and Crawford 2004) that may have interactions or relationships with the root knot nematodes in the soil and also the roots of the black pepper plants.

Due to long-term of living in soils, a lot of soil variables such as soil temperature and soil pH can affect the growth of nematodes (Popovici and Ciobanu 2000). The life cycle of nematodes can be faster or slower depending on the soil temperature and even high heat of soil can kill the soil nematodes as seen from a process such as soil solarisation (Yücel *et al.* 2017).

These effect of soil towards the root knot nematodes can be reduced by using other techniques of planting such as tissue culture propagation or hydroponics. Tissue culture propagation is capable of producing plantlets rapidly using *in vitro* culture techniques (Jong 2011). The procedure of tissue culture requires higher techniques and skills involving aseptic techniques and also facilities to store the plantlets before acclimatization. Also, the plantlets are easy to undergo microbes contamination and also browning of the media which will result in the death of the black pepper plantlets (Hussain *et al.* 2011).

Hydroponics is the method of planting plants in soilless culture with the use of nutrient solution that is necessary for optimal plant growth (Anderson *et al.* 2017). In hydroponics, plants are allowed to be grown while under controlled environment especially disease sources, nematodes, and insects that are always present in soil (Nhut *et al.* 2005). This system allows the growth of plants under controlled environment is best for researchers while also improving the yield and economic advantages over conventional planting system (Giurgiu *et al.* 2014). Hydroponics can also be used as an alternative for the production of rootstock for plants such as tomato plants and herb seedlings (Heim *et al.* 2001). It may have uses in the production of rootstock for black pepper plants.

This study will be able to provide the analysis of relationship between the soil properties and the prevalence of root knot nematodes of black pepper plants at Ladang Samarakan. This study also aims to provide information for further studies involving the use of hydroponics for black pepper plants and also for the rearing of root knot nematodes in hydroponics. The propagation of black peppers in hydroponics will be able to produce quality rootstocks that are suitable for the research on roots diseases of black pepper plants.

The objective of this study is to investigate the relationship between soil properties and the prevalence of root knot nematodes in black pepper plants at Ladang Samarakan and to study the potential of propagating Sarawak black pepper plant in hydroponics as rootstock cultivation.

CHAPTER 2

LITERATURE REVIEW

2.1 Black Pepper Plant (*Piper nigrum L.*)

Black pepper (*Piper nigrum* L.) of the family Piperaceae is cultivated mainly for its fruit. The fruit of black pepper is often dried and used as a food spice. Black and white pepper can be produced from the same fruit. Native to South India, black pepper is cultivated extensively and also to other tropical regions such as Malaysia. The peppercorn or dried black pepper fruit is a small 5 mm in diameter drupe. It is fully mature when it is dark red and it contains a single seed (Parthasarathy *et al.* 2008).

Dried ground pepper and the variants are known as one of the most common spices in European cuisine, as since old time it is known for both its flavour and usage as medicine. The black pepper contains a chemical called piperine that is what makes the black pepper spicy. Usually referred as 'pepper', ground black peppercorn may be found on nearly every dinner table in the world alongside with table salt. Also known as 'black gold' and the 'king of spices', black pepper is the most widely consumed and important spice in the world. When properly dried, black pepper can be stored in airtight containers for many years without losing taste and aroma compared to other spices. The words 'pepper' derived from Sanskrit pippali, via the Latin piper and Old English pipor. The Latin word is also the source of German pfeffer, French poivre, Dutch peper and other similar forms. Since the year 1840s 'pepper' meant 'spirit' or 'energy' in a figurative sense (Parthasarathy *et al.* 2008).

A lot of spices that have been used in food seasoning have a wide spectrum of antimicrobial activity. They also have antioxidant activity against lipid peroxidation that enhances the shelf life quality of the food. As for black pepper, aside from being a popular spice and flavouring substance, it is also used as drug in Indian and Chinese systems of medicine (Vijayan and Thampuran 2000). A naturally occurring alkaloid that is isolated from black pepper, piperine, is very helpful in the medical field. Recent studies have shown that piperine is very helpful in increasing absorption of nutrient such as Selenium, Vitamin B, and Beta-Carotene. Piperine also has a few of biological effect that is very promising in medical studies such as anti-inflammatory, antioxidant, antimutagenic and antitumor activities (Hussain *et al.* 2017).

In the early 17th century, black pepper was introduced into Malaysia probably by the European settlers. However, in the 14th century, the black pepper also would probably reach Malaysia through Zheng He, a Chinese trader, who organised series of expeditions to the Malabar Coast touching many sea coasts in between. Pepper cultivation in Malaysia is mainly done in Sarawak. The pepper cultivation is managed by the Chinese farmers and they had evolved an intensive production technology. This had led Sarawak to achieve high productivity levels. There is also pepper grown in Malaysia mainland especially in Johore (Ravindran 2000).

Annually, black pepper accounts for 35% of the world trade spices that are valued at US$1.5–2.5 billion, according to the International Trade Centre (ITC), Geneva (Ravindran 2000). An investigation by International Pepper Community has indicated that the worldwide supply of pepper is short of 43,500 tons. It is projected that the export of pepper for the year 2020 is estimated to be about 290,000 tonnes while the forecasted domestic consumption is about 270,000 tonnes (Olalere *et al.* 2016).

Being the fifth largest pepper producer in the world, Malaysia has exported a total of 14,040 tonnes in 2010 that is valued at RM 190 million. In Malaysia, the major cultivation areas of black pepper are located at Sarawak, Johor, and Sabah. There are 14,000 hectares of pepper farm in Sarawak, of the 14,100 hectares in Malaysia. Therefore, pepper has been one of the important cash crops to support the Sarawak farmers. Pepper is usually planted in fertile hill slopes by smallholder farmers. The farms are mostly at Kuching, Samarahan, Sri Aman, Betong, and Sarikei. As Sarawak is the largest producer in Malaysia, contributing 95% of pepper production of the country, the trade name 'Sarawak Pepper' has been chosen to promote Malaysia peppers commercially. Under 'geographical indication', this brand name has been registered by the Malaysian Intellectual Property Organization. In Malaysia, this is the first agricultural product that has achieved this acknowledgment (Kamarulzaman *et al.* 2013).

Black pepper is usually grown in humid tropics mainly India, Indonesia, Malaysia, Sri Lanka, Thailand, and Vietnam. The climatic requirement for black pepper is high rainfall, uniform temperature, high relative humidity in which is typical climate condition for the tropical region (Sadanandan 2000). Black pepper plant requires 2000-3000 mm of rainfall throughout the year. In pepper cultivation, total rainfall and distribution play an important role in the productivity. The annual rainfall of 2000 mm with uniform distribution is the optimum rainfall for black pepper cultivation. During May – June, rainfall of 70 mm in 20 days should be sufficient to trigger flushing and flowering process of the plant. Once the process has started, continuous rain should happen until fruit ripening. Any dry spell for even a few days within this period will result in lower yield. In Malaysia, Sarawak, areas with pepper growing have an average annual rainfall of 3950 mm (Kandiannan *et al.* 2013).

2.2 Pest and Disease of Black Pepper Plant

Black pepper is affected by several diseases caused by fungi, bacteria, virus and mycoplasma, besides nutritional disorders. Crop losses due to diseases and pests are identified as major causes of low productivity of pepper. The major diseases of black pepper includes Phytophthora disease, Fusarium wilt, slow decline of pepper, anthracnose of pepper and stunted disease of black pepper (Anandaraj 2000).

Phytophthora disease or foot rot disease commonly known also as quick wilt disease is caused by *Phytophthora capsici*. It is one of the major limiting factors in black pepper production. The fungus is a soil borne pathogen can infect all parts of the plant. This disease occurs mainly from June to September and can result in sudden wilting, defoliation and death of vines (George *et al.* 2015). Fusarium wilt or root rot has caused the death of thousands of pepper trees. It is caused by the fungus *Nectria haematococca* f. sp. *piperis*, anamorph *Fusarium solani* f. sp. *piperis*. The fungus also inhabits the soil and survives both on the plant and soil organic matter. It infects the pepper plants through the root causing root to rot. If infected the aerial parts, the leaves will start to undergo yellowing and wilting of the leaves (Castro *et al.* 2016).

Anthracnose is a disease in black pepper that is caused by *Colletotrichum gloeosporioides*. *Colletotrichum gloeosporioides* is characterized by straight conidia, rounded or pointed ends, and is 12 to 10 mm or 5 to 35 mm long. This fungus infects the leaves and the spikes of black pepper and is becoming a major constraint in the production of black pepper (Vijayan *et al.* 2014). Slow decline disease of pepper affects the plant allowing the affected plants to survive for several years before the death of plants gradually occurs within 3 to 4 years. This disease was earlier referred to as "slow wilt" is known as slow decline in Malaysia. Aetiology studies on this disease have shown that the cause is due to feeder root damage by *Phytophthora capsici*, *Radopholus similis* and *Meloidogyne incognita* either alone or in different combinations (Anandaraj 2000).

2.3 Plant-Parasitic Nematode

In estimation of 5 to 15% of world's crop loss is due to plant-parasitic nematodes. There are even situations where higher losses sometimes occur and even having nematodes as one of the major limiting factors for the production of a particular crop (Stirling, 2014). Of all the plant-parasitic nematodes, there are 29 genera and 48 species are reported to attack black pepper plant (Sundararaju *et al.* 1979).

Over the globe, root knot nematodes (*Meloidogyne* spp.) are the most destructive nematodes parasites for black pepper plant. The few most prevalent species identified within the Meloidogyne genus is *Meloidogyne* incognita, *Meloidogyne javanica*, *Meloidogyne arenaria* and *Meloidogyne hapla*. At tropical, subtropical and warm temperate regions, *M. incognita* and *M. javanica* is more widely distributed (Ramana and Eapen 2000).

Of the four *Meloidogyne* spp. mentioned, three species which are *M. incognita*, *M. javanica* and *M. arenaria* were reported to be found on black pepper plant in Sarawak. However, *M. incognita* is discovered to be more distributed in all the plantations in Sarawak in the survey that was done in more recent years (Hajijah 1993).

2.4 Soil Characteristics and Nematodes

There are a lot of nematodes ranging in types that inhabit in the soil ranging from bacterial feeding, plant feeding, omnivorous group and hyphal feeding in nematode trophic structure. Due to close proximity with the soil environment, there are a lot of soil environment variables that can affect the nematodes in term of diversity and types within the soil such as humus content, total nitrogen, soil pH, exchangeable bases and soil type (Popovici and Ciobanu 2000).

Even the life-cycle of nematodes in soil depends on soil variables such as soil temperature to complete faster or slower depending on the season such as summer or winter (Rejendra and Swastik 2015).

2.5 Propagation Techniques for Black Pepper Plant

Conventional techniques for black pepper plants use the terminal shoot as planting material due to the ability to have laterals compared to using runners as planting material. If runners are used as planting materials, the laterals will take longer time to be produced, after 12 to 18 months of planting. These practices are common in Malaysia, Vietnam, and Indonesia (Nybe *et al.* 2016). The cuttings are kept protected in a shaded place to maintain cool and humid atmosphere. The cuttings, usually three nodes, will start sprouting within 3 to 4 weeks of planting and are ready for field planting after 2 months with 4 to 5 leaves and sufficient roots (Nybe *et al.* 2016).

Another type of propagation that is available for black pepper plants is the micropropagation using *in vitro* culture techniques. This is due to the ability to rapidly propagate black pepper and in mass scale of production (Hussain *et al.* 2011). And since the demand of quality and better planting material for black peppers is increasing, tissue culture is the most efficient as it can produce the genetically stable, disease free and identical progeny (Jong 2011). However, the downside to using tissue culture to propagate black pepper plants is the high rate of microbe contamination in the plantlets and the necrosis of the plantlet due to the browning of media caused by chemicals released by the black pepper plantlet itself (Philip *et al.* 1992).

Another alternative planting technique that is still not yet documented for propagation and production of new black pepper cutting that is fully rooted is the use of the hydroponic system. The wide range of hydroponic system that can accommodate to a lot of different types of plants from woody plants to herbs (Giurgiu *et al.* 2014) should yield positive results for black pepper planting materials.

2.6 Hydroponic System

Hydroponics is the soilless culture of plants in a nutrient solution that contains ions of all the necessary elements for good plant growth. Inert media such as perlite or rock wool are frequently utilized for support purposes and plants may be grown in a variety of ways such as deep water culture or via nutrient film techniques (Anderson *et al.* 2017).

Hydroponic techniques for cultivating crops began in the late 1920s and commercial scale hydroponic systems were developed in the 1940s. Currently, with the development of materials and equipment (e.g., media, tubes, connectors, valves, pots, water reservoirs or tanks, air or water pumps and electronic timers), various hydroponic systems have become available, notably: the gravel flow sub-irrigation, ebb and flow, drip irrigation, nutrient film, root mist, fog feed, aeroponic, raceways (deep flow), and aerated deep water culture (DWC). Most hydroponic systems operate automatically to control the amount of water, nutrients, and lighting time, based on the requirements of different plants. Likewise, different natural or artificial media can provide different particle sizes, shapes, and penetrability; each medium affects plants and roots differently by retaining water, supporting plants, and making pore space at different rates. The selection of a medium depends on the nature of the plants, cost, and the type of hydroponics that is employed (Lee and Lee 2015).

One of the hydroponic systems is deep water culture (DWC). This system uses nutrient that is pre-mixed in water where the roots are immersed in the solution. This ensures the root of the plant will absorb the nutrient to grow (Saaid *et al.* 2015). The benefits of using DWC system is that it contains highly oxygenated nutrient solution, uses less fertilizer and has a low maintenance cost and monitoring time when compared to other systems of hydroponics (Saaid *et al.* 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 Soil Sampling

The soil used in this study was sampled at depth of 0 to 25 cm at Ladang Samarakan (3°00'40.6"N 113°01'32.1"E) (Figure 3.1). The sampling area is 33.7 km away from Universiti Putra Malaysia, Bintulu Campus (Figure 3.2). This soil is used for cultivation of black pepper plants. The sampling points were done according to the variety of black pepper plants on the farm. A total of 3 black pepper varieties (Panniyur-1, Kuching and Semongok Emas) with 10 sampling points for each variety.

Figure 3.1 Aerial view of Ladang Samarakan where the soil was sampled for this study.

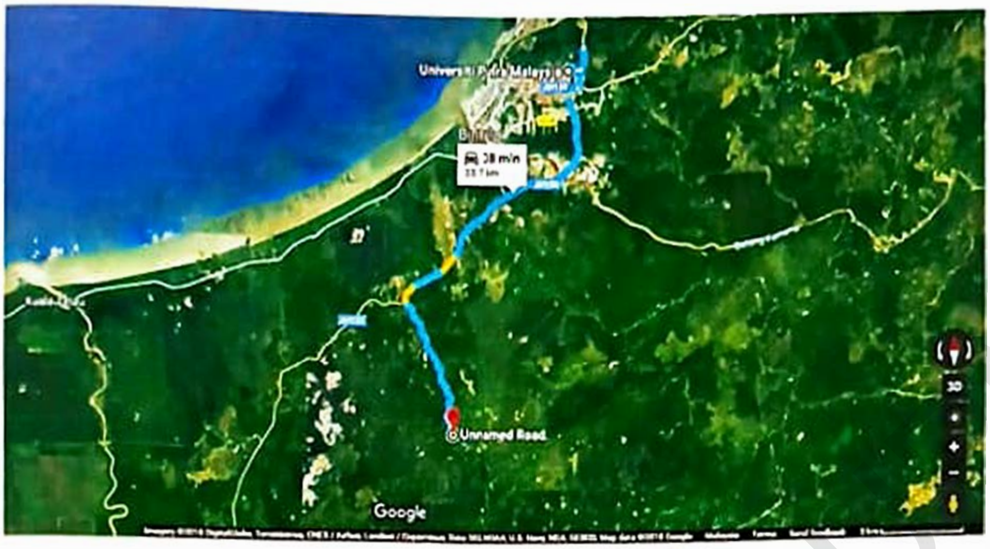

Figure 3.2 The distance of the farm from Universiti Putra Malaysia Bintulu Campus.

3.1.1 Soil Texture Analysis

Wet and moist soil samples were squeezed between fingers. If the soil contains clay, the soil feels sticky when wet and the moist sample slicks out to form a thin ribbon. For sand, its presence was felt when the soil sample was gritty. In the case of silt, its presence in the soil sample was felt when it produces a slick, a soapy feeling. The general guideline was followed using a flow diagram (Thien 1979). Multiple points of the farm was sampled and the soil texture was analysed accordingly in order to get a uniform reading of the whole farm.

3.1.2 Soil Colour Determination

Multiple sampling points of the soil on the farm has the soil colour was determined using Munsell® Soil Colour Chart and the range was recorded.

3.1.3 Soil Temperature Analysis

A 10 random sampling point for each variety was taken for the soil temperature using HI 145 T-shaped thermistor thermometer resulting in a total of 30 sampling points. Then a total of 3 time period (8 a.m., 12 p.m. and 5 p.m.) was taken for the day of soil sampling resulting in 3 sets of soil temperature according to varieties. Then the value was averaged and used for further analysis in SAS (Ramakrishna *et al.* 2006) under Completely Randomized Design (CRD).

3.1.4 Survey of Prevalence of Root Knot Nematodes in Black Pepper Plant

The black pepper plants were selected randomly, 10 plants for each variety, and the surface of the soil was removed. The feeder roots were then collected and observed for formation of root knots. The root knots that were identified was collected back to the laboratory and then the root knots was cut in half using scalpels. The roots were then observed under imaging microscope (LEICA DM500) and the nematodes images were taken using LAS-EZ software.

3.1.5 Soil Moisture Analysis

The soil samples were collected in airtight seals for soil moisture analysis. 10 g of the soil samples were weighed in crucibles before heating in the oven at 105°C. After drying, the crucible is removed from the oven and let it cool before weighing. The soil moisture content was then calculated using the following formula:

$$MC\% = \frac{W_2 - W_3}{W_3 - W_1} \times 100$$

Where;

W_1 = Weight of crucible (g)

W_2 = Weight of moist soil + crucible (g)

W_3 = Weight of dried soil + crucible (g)

The soil moisture was then analyzed using SAS under Completely Randomized Design (CRD).

3.2 Soil Samples Preparation and Analysis

The soil samples were air-dried, crushed manually and sieved to pass a 5-mm sieve. Then, the soil is tested for soil pH in water.

3.2.1 Determination of Soil pH in Water

Soil pH was determined using the potentiometric method with a ratio of 1:2.5 (soil: distilled water) suspension using a digital pH meter. A 12.5 mL of distilled water was added to 5 g of air-dried soil in a plastic vial at a ratio of 1:2.5. The samples were shaken for 15 minutes at 180 rpm. Afterward, the samples were left to equilibrate for 24 hours before using pH meter (SevenEasy pH, Mettler-Toledo GmbH, Switzerland) for pH determination. The soil pH was then analyzed using SAS under Completely Randomized Design (CRD).

3.3 Deep Water Culture (DWC) Hydroponics System

A plastic container with lightproof coat was selected to be used for the hydroponics. The top lid was made sure to be able to retain position even under moderate physical weight pressure to make sure the lid does not collapse when the plant cuttings grow up. Four holes were drilled on the top of the lid to enable four netting pot to be placed on the lid. The holes were made sure to be able to fit with the netting pot so that the netting pot does not drop into the container. On one side of the container, drill a small hole to allow only one air tube to pass through the container itself. The location of the hole must be higher than the water level of the nutrient solution in the hydroponics. Inside the container, the air tube was interconnected with three aquarium air stone to ensure air bubble can be produced inside the hydroponics. The other end of the air tube was connected to an aquarium air pump (Appendix, Plate A.1).

3.3.1 Preparation of Nutrient Solution

The preparation of sterile nutrient solution for hydroponic cultures was done by mixing 5 mL of City Farm Stock Nutrient solution A and B each into 2.0 L of distilled water which was then poured into the hydroponics. The distilled water was autoclaved prior to mixing with nutrient solution to ensure clean and sterile environment for the rooting zone of the cutting. The water level was determined by estimating the height of water level required to be touching the tip of the cutting area but not completely immersing the whole cutting to allow air spaces inside the hydroponics. The nutrient solution was replaced every 2 weeks throughout the study period.

3.3.2 Black Pepper Plant (*Piper nigrum L.*) Cuttings

Black pepper plant samples (Kuching variety) was gathered at University Putra Malaysia Bintulu Campus. Nodes of the black pepper plants is identified and proceed to cut at 45 degrees at the stem 5.0 cm below the nodes. Single nodes of black pepper were used for the hydroponics.

3.3.3 Black Pepper Cuttings Propagation in Hydroponics

Rooting hormone powder (SADEX 1) was made wet using a few drops of distilled water before stirred uniformly. The rooting hormone was then applied directly onto the cuttings near the cutting area in order to ensure proper rooting area can be achieved for each of the plants. The cutting was the wrapped with 2 small sized sponges cut into cube-shaped for ease of wrapping the plant. The plant was then immersed into the hydroponics. The end of the plant cutting, around 1 cm, was made sure to have been immersed in the water to ensure cuttings do not dry up too fast before rooting starts.

3.4 Experimental Design of Black Pepper Hydroponics

Three treatment of hydroponics black pepper was set up according to the aeration in hydroponics. It was separated into 3 treatments which are non-aerated hydroponics, half-aerated hydroponics, and fully aerated hydroponics. Each of the hydroponics will have 4 replicates of black pepper cuttings. For non-aerated hydroponics, there was no air pump attached to the hydroponics. For half-aerated hydroponics, the air pump was attached to the hydroponics. It will be at full power setting air pump from 8 a.m. to 5 p.m. every day, half power setting air pump from 5 p.m. to 8 a.m. throughout the study period. As for the fully aerated hydroponics, the hydroponics will have air pump attached to it with full setting air pump throughout the whole day for the study period. The percentage of survival, rooting and sprouting of the black pepper plants was recorded.

3.5 Observation of Black Pepper Plant Growth in Hydroponics

The increase in stem length, the length of longest root and number of leaves of black pepper plants in the hydroponics was observed and recorded for the entire study period. The plant with its root exposed was placed on a piece of paper to record the longest root length. The data collected was then analysed using SAS under Completely Randomized Design (CRD).

CHAPTER 4

RESULTS

4.1 Relationship between Prevalence of Root Knot Nematode and The Soil Properties at Ladang Samarakan

At Ladang Samarakan, for Semongok Emas variety, 60% of the black pepper plants were infected with root knot nematodes while only 10% of Kuching and 10% of Panniyur-1 was infected with root knot nematodes. There were no significant differences in the soil pH under Tukey's HSD test ($p > 0.05$) (Appendix C.3). The soil pH for Semongok Emas was 3.89 ± 0.164, Kuching was 3.92 ± 0.180 and Panniyur-1 was 3.81 ± 0.069. As for soil temperatures, there were significant differences under Tukey's HSD test ($p > 0.05$) between all three varieties, Semongok Emas, Kuching and Panniyur-1 which was represented by different letter after the means ± SD (Appendix C.4). Semongok Emas soil temperature was the lowest with 27.3 ± 0.945°C while Panniyur-1 with 28.7 ± 0.879°C and Kuching with the highest temperature of 29.5 ± 1.089°C. For soil moisture, there were significant differences under Tukey's HSD test ($p > 0.05$) between Semongok Emas and Panniyur-1 (Appendix C.2). The soil moisture for Semongok Emas was 28.09 ± 1.515%, for Kuching was 27.35 ± 2.832% and Panniyur-1 was 25.10 ± 2.55%. Soil temperature showed correlation with the percentage of black pepper plants infected with nematodes with $p = 0.025 < \alpha = 0.05$ (Appendices C.1).

As for the soil colour evaluation, the majority of the soil colour was 10YR 6/8 with a few of the soil colour ranging from 10YR 4/4 to 10YR 6/8 according to Munsell® Soil Colour Charts. Based on *in situ* direct determination of soil texture, the soil texture of the farm was sandy clay loam. The soil texture was uniform across the farm based on multiple sampling points.

For field observation, the prevalence of root knot nematodes in black pepper plants at Ladang Samarakan was determined by the formation of root knots in the feeder roots. The root knots (Appendix, Plate A.5) was then collected back to the laboratory for further processing and observation under imaging microscope (Appendix, Plate A.2 and Plate A.6). The observation of canopies of the black pepper plants between infected and not infected with root knot nematodes, there were no differences in the canopies (Appendix, Plate A.3 and Plate A.4). In terms of yellowing of leaves and wilting of leaves, there was also no observable differences between infected and non-infected black pepper plants. As for the sunlight reaching the surface of soil, there were also no differences between infected and non-infected black pepper plants.

There were observable differences between the three varieties which is Panniyur-1, Kuching and Semongok Emas in terms of canopies. Panniyur-1 and Kuching had lesser leaves and canopies compared to Semongok Emas (Appendix, Plate A.7 and Plate A.8). Therefore, more sunlight was capable of reaching the surface of the soil at Kuching and Panniyur-1 compared to Semongok Emas.

Table 4.1 Soil parameter and percentage of plants infected with root knot nematodes based on different varieties of black peppers at Ladang Samarakan.

Parameter	Variety		
	Semongok Emas	Kuching	Panniyur-1
Soil pH	3.89 ± 0.164a	3.92 ± 0.180a	3.81 ± 0.069a
Soil Temperature (°C)	27.3 ± 0.945a	29.5 ± 1.089b	28.7 ± 0.879c
Soil Moisture (%)	28.09 ± 1.515a	27.35 ± 2.832ab	25.10 ± 2.55b
Percentage of plants infected with root knot nematodes (%)	60	10	10

Values are means ± SD. The values followed by the same letter do not differ significantly ($P > 0.05$, Tukey's HSD test)

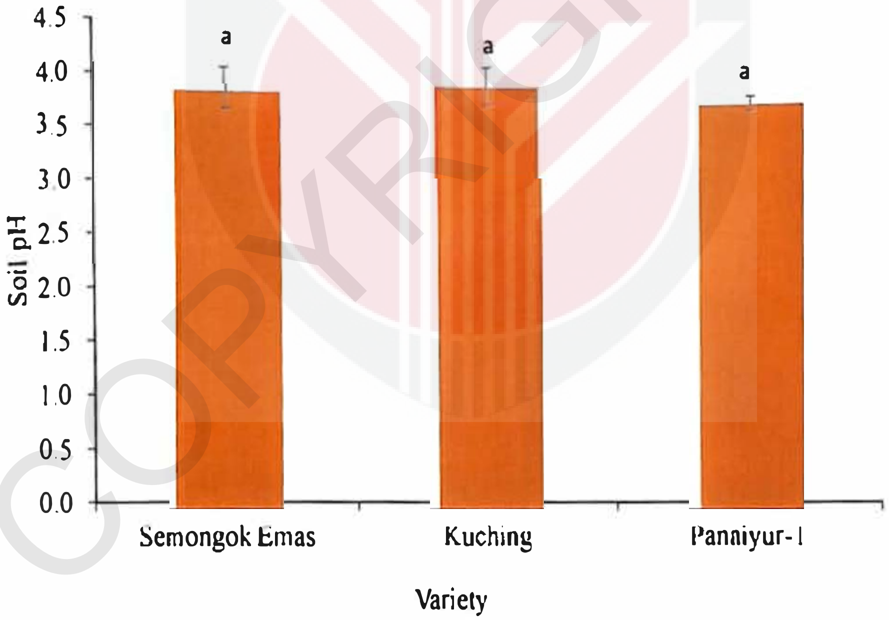

Figure 4.1 Soil pH of different variety of black peppers at Ladang Samarakan. Error bars indicate the standard deviation. "a" labelled in bars indicate not significant under Tukey's HSD test ($p > 0.05$)

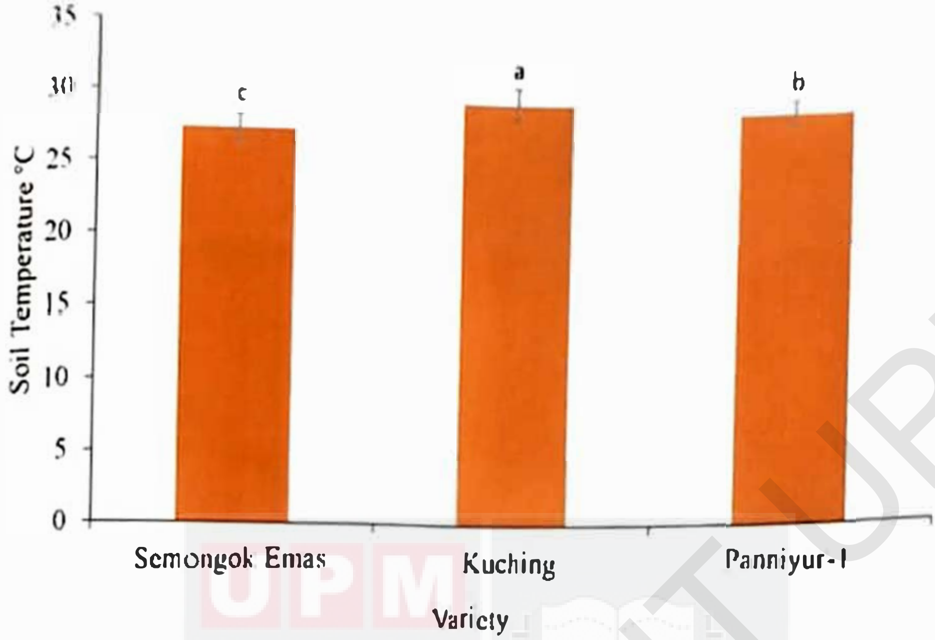

Figure 4.2 Soil temperature of different variety of black peppers at Ladang Samarakan. Error bars indicate the standard deviation. "a", "b" and "c" labelled in bars indicate significant differences under Tukey's HSD test ($p > 0.05$)

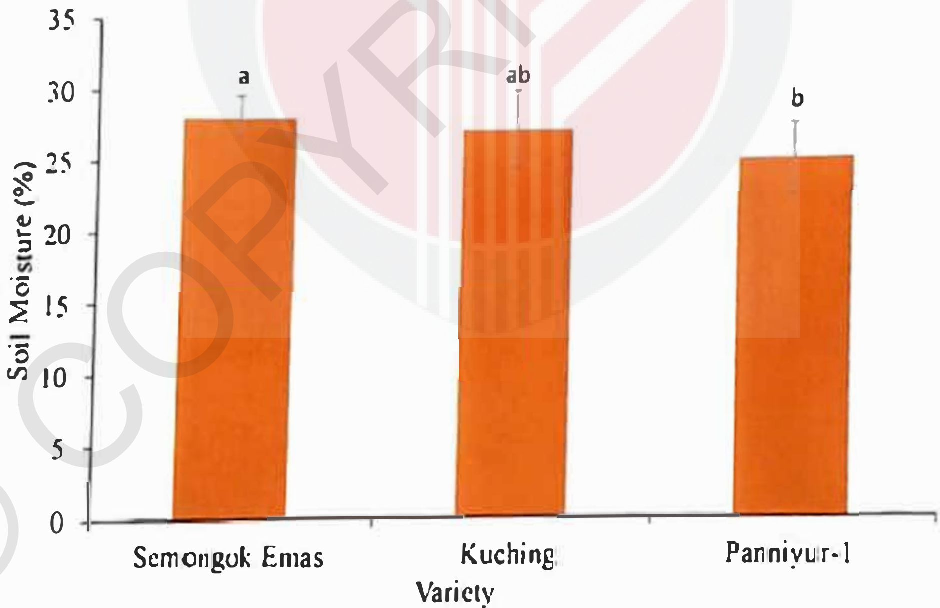

Figure 4.3 Soil moisture of different variety of black peppers at Ladang Samarakan. Error bars indicate the standard deviation. Different letters labelled in bars indicate significant differences under Tukey's HSD test ($p > 0.05$)

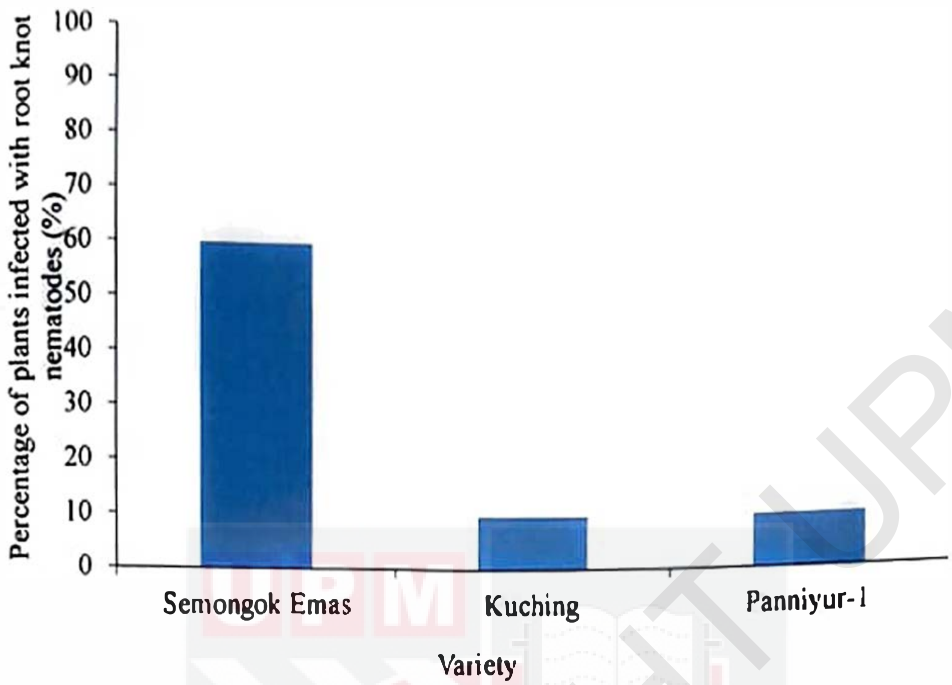

Figure 4.4 Percentage of plants infected with root knot nematodes (%) based on different varieties of black pepper plants at Ladang Samarakan

4.2 Potential of black pepper rootstock cultivation in hydroponics

Based on Table 4.2, after 12 weeks, fully aerated black pepper shown the highest percentage of survival, rooting and sprouting. Fully aerated black pepper hydroponics shown 100% survival compared to half aerated black pepper hydroponics and non-aerated black pepper hydroponics which had shown 0% survival after the period of 12 weeks. Fully aerated black pepper hydroponics shown 100% rooting compared to the 0% of half aerated black pepper hydroponics and non-aerated black pepper hydroponics after the period of 12 weeks. Fully aerated black pepper hydroponics shown 75% of sprouting as compared to the 0% sprouting of non-aerated and half aerated black pepper hydroponics after the period of 12 weeks.

Table 4.2 Parameters of plants under different hydroponics treatment.

Parameter	Treatment		
	Non Aeration (Control)	Half Aeration	Full Aeration
Survival (%)	0	0	100
Rooting (%)	0	0	100
Sprouting (%)	0	0	75

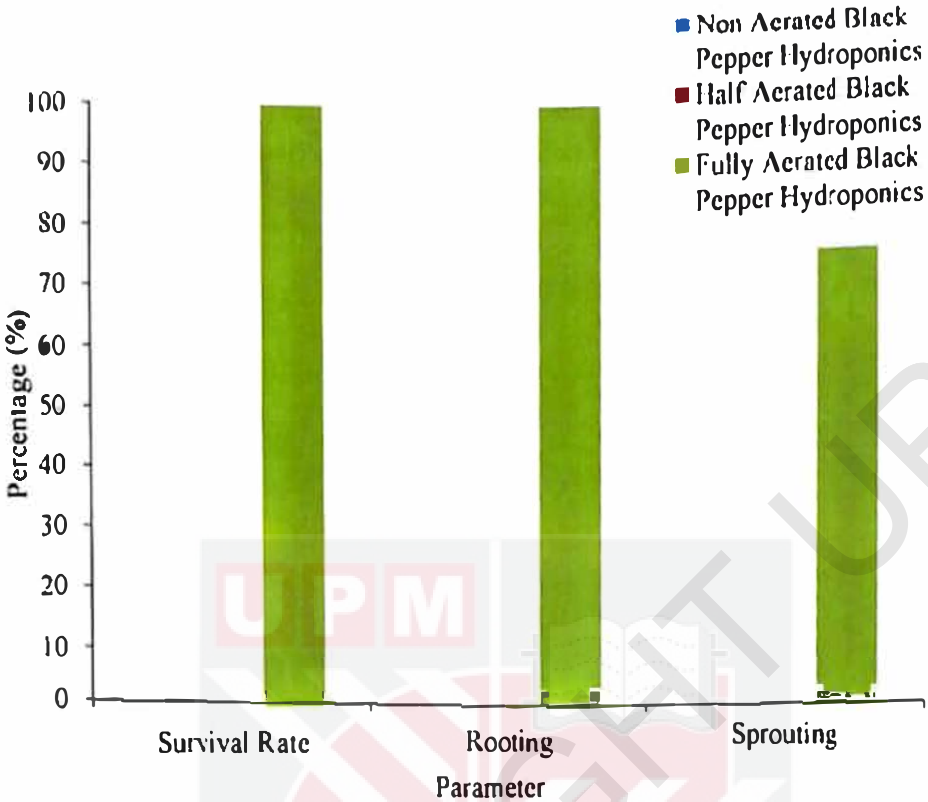

Figure 4.5 Percentage of survival, rooting and sprouting of different treatment of black peppers in hydroponics after 12 weeks

4.3 Characterization of Black Peppers Growth in Fully Aerated Hydroponics

As recorded in Table 4.3, the growth of black peppers in fully aerated hydroponics was recorded over 12 weeks. The increase in stem length for the black peppers in fully aerated hydroponics was 6.0 ± 4.60 cm. The increase in the longest root for the black peppers in fully aerated hydroponics was 24.28 ± 3.10 cm. The total number of leaves of the black pepper plants in fully aerated hydroponics after 12 weeks was 2.8 ± 1.2.

Table 4.3 Growth parameters of fully aerated black peppers hydroponics after 12 weeks.

Growth Parameter	Fully Aerated Black Peppers Hydroponics
Increase in stem length (cm)	6.0 ± 4.60
Increase in longest roots (cm)	24.28 ± 3.10
Total number of leaves	2.8 ± 1.2

Values are means of 4 replicates ± SD.

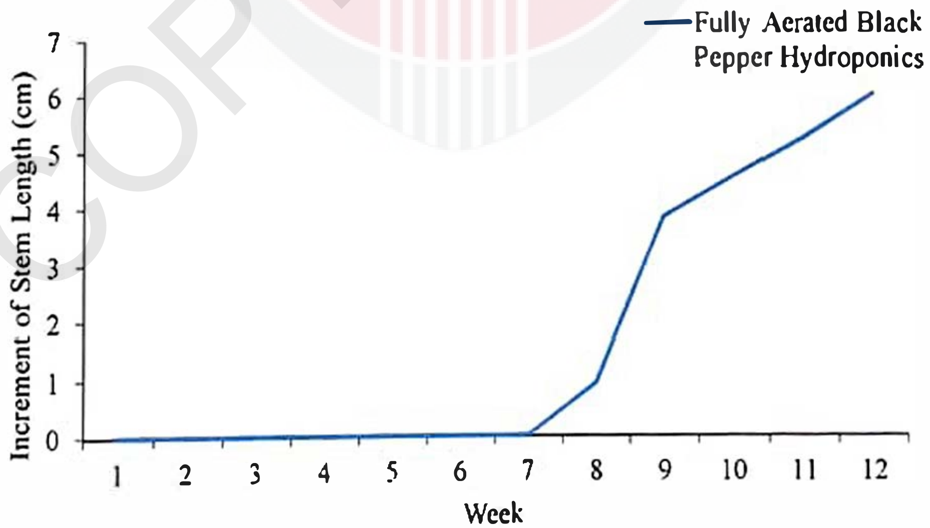

Figure 4.6 Growth curve of stem length in fully aerated black pepper hydroponics in 12 weeks

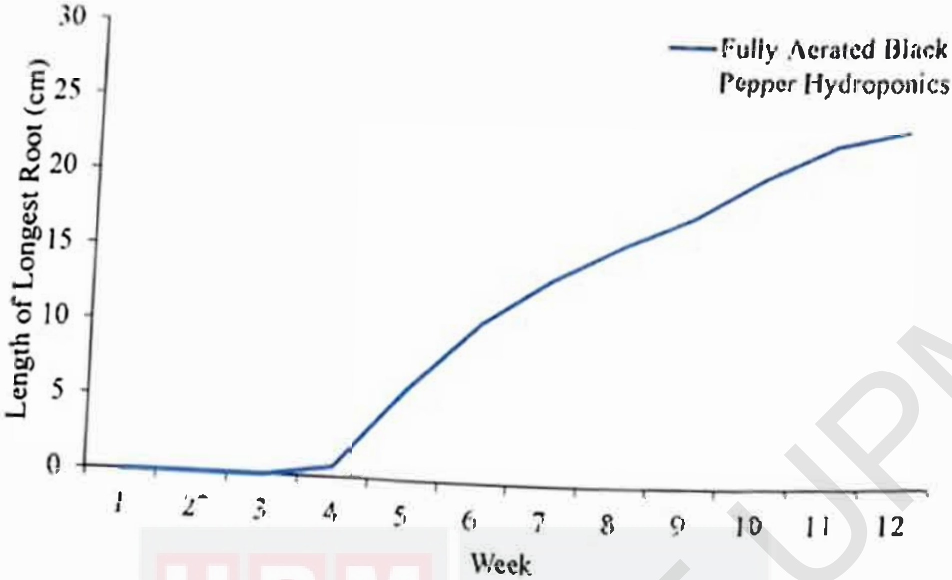

Figure 4.7 Growth curve of longest root in fully aerated black pepper hydroponics in 12 weeks

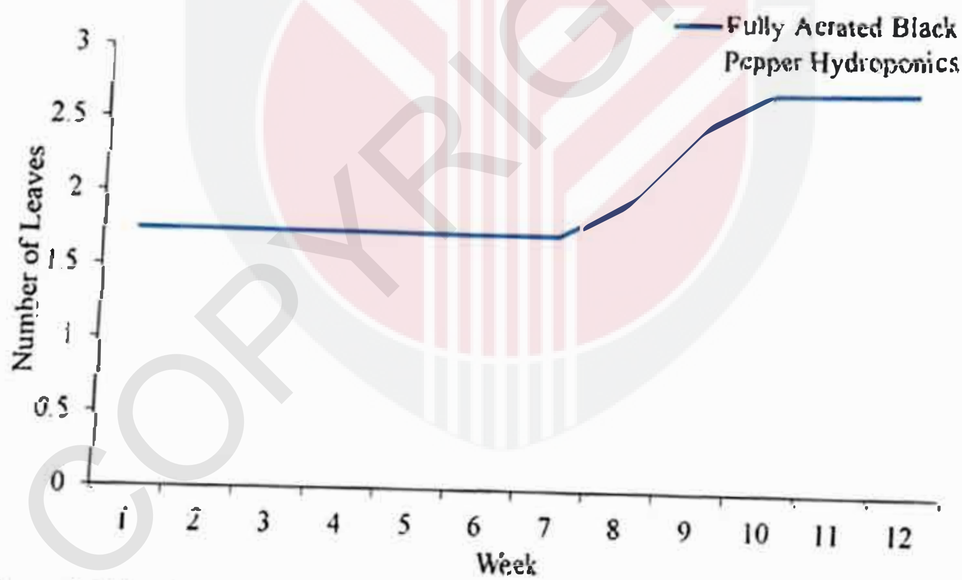

Figure 4.8 Number of leaves in fully aerated black pepper hydroponics in 12 weeks

CHAPTER 5

DISCUSSION

5.1 Relationship between prevalence of root knot nematode and the soil properties

There was a negative correlation between soil temperature and the percentage of plants infected with root knot nematodes at Ladang Samarakan at $p < 0.05$ which is 95% degree of confidence (Appendices C.1). This may be due to the higher temperature areas in the soil which could cause nematodes to migrate to lower or cooler soil areas, in this case, the Semongok Emas area. It is known that high temperature in the soil can reduce the number of nematodes for example through a process known as soil solarisation (Yucel *et al.* 2017).

There was no correlation between soil moisture, soil pH and the percentage of plants infected with root knot nematodes at Ladang Samarakan at $p < 0.05$ (Appendices C.1).

As the soil texture and soil colour was uniform at Ladang Samarakan, this meant that there was no significant effect on the prevalence of root knot nematode of black pepper plants.

According to Ravindran (2000), the infection of root knot nematode in black pepper plants can be identified by the yellowing of the leaves. However, at Ladang Samarakan, there were no distinct observable differences between infected and non-infected black pepper plants in term of canopies and the yellowing of the leaves. This may be due to the poor management of the farm itself as it can be seen that that the farm had low pH value at mean averages of 3.89, 3.92 and 3.81. It has been stated that optimum soil pH value for black pepper farms should be at 4.80–6.25 (Hamza *et al.* 2007). With lower pH than the optimum value at Ladang Samarakan, it could be expected that the canopies of the black pepper plant to not achieve optimum size and that yellowing and wilting of leaves between health and infected black pepper plants cannot be distinctly differentiated using only observations (Appendices A, Plate A.3 and Plate A.4).

The root knot formation on the feeder roots of the black pepper plants at Ladang Samarakan was collected and taken back to the laboratory for further inspection using a scalpel and light microscope. The images of the nematodes were taken using Leica Imaging Software (Appendices A, Plate A.2 and Plate A.6).

5.2 Potential of Black Pepper Rootstock Cultivation in Hydroponics

It was determined that aeration is an important aspect of hydroponics especially in the case of propagation of rootstock of black pepper plants. It showed higher percentage of survival, rooting and sprouting in the fully aerated black pepper hydroponics. According to Nakayama (1950), high aeration in hydroponics will result in better growth in terms of roots for the tree planted in hydroponics. Better aeration will also lead to better growth of black pepper plants in the soil especially for the propagation of black pepper plants (Nybe *et al.* 2016). Since better aeration leads to the better growth of black pepper plants in hydroponics, it also leads to faster sprouting of new leaves of the black pepper plants.

The 75% sprouting of black pepper plants in fully aerated hydroponics was due to one of the black pepper cuttings could not reach the water level in the first week resulting in slightly wilting of the cutting. After noticing and lowering the cutting nearer to the water level of the nutrient solution of the hydroponics, the cutting recovered from wilting. However, the delay from accessing the nutrient solution and the slight wilting resulted in slower growth rate compared to the other cuttings in the fully aerated hydroponics (Appendices B, Table B.1 and B.3).

Single node stem cutting dipped with IBA solution in the standard cocoa propagator with coir dust as the medium resulted in 75% rooting in 21 days (Sadanandan 2000) compared to the 100% rooting of black pepper plants in hydroponics. It can be seen that propagation of black pepper plants in hydroponics yielded better results in terms of rooting of the cutting.

Propagation methods using tissue culture for black pepper plants require longer time and even new buds take three months to form in the culture (Jong 2011). Therefore propagation method using hydroponics can be much faster and requires less preparation time. Propagation of black peppers using tissue culture also have high chances of contamination and lower survival percentages if the aseptic protocol is not followed completely (Philip *et al.* 1992). As for hydroponics, black peppers require less control on the environment in processing and maintaining the black pepper cuttings.

5.3 Characterization of Black Peppers Growth in Fully Aerated Hydroponics

Black pepper cuttings were able to grow new roots, increased in stem length and sprouted new leaves within 12 weeks of the study period (Appendices B, Table B.1, Table B.2 and Table B.3). This growth along with the high percentage of survivability and rooting shown that fully aerated hydroponics can yield a lot of roots for rootstock cultivation or researches on roots. According to (Shavrukov *et al.* 2012) hydroponics is suitable to be used as research on the factors affecting the plant and its growth factors, environment and more. It is especially true in regards to the rooting system of a plant in hydroponics. Therefore, diseases that affect the rooting system of black pepper plant can be done through the use of hydroponics grown black pepper plants.

For researching of root diseases especially root knot nematodes on black peppers, hydroponics is one of the suitable condition due to the capability of inspecting the roots directly without hurting the roots. Pulling up the black pepper cuttings in hydroponics does not hurt the roots of the black pepper and cause any major growth inhibition due to damages to the rooting system. There are many more research that are conducted on the roots of plants grown in hydroponics such as to investigate the root exudation of spruce seedlings in hydroponics (Heim *et al.* 2001) and the investigate of quantitative trait loci of maize grown in hydroponics (Tuberosa *et al.* 2002) shown positive results in regards to the plants grown in hydroponics. This proves the feasibility of doing researches on plant grown in hydroponics.

As for research on nematodes for example root knot nematodes, other researchers also have done researches on root knot nematodes under hydroponics condition using tomato plants (Lambert *et al.* 1992). Therefore, it is safe to say the same experiment can be done on black pepper hydroponics. However, further research is required for conducting the black pepper hydroponics experiment of rearing root knot nematodes such as *Meloidogyne incognita* or *Radopholus similis* that are major causes of root diseases for black pepper plants (Ravindran 2000).

CHAPTER 6

CONCLUSION

There was a relationship between soil temperature and the prevalence of nematodes in black pepper plants at Ladang Samarakan. There was potential in using fully aerated hydroponics as rootstock cultivation of black pepper plants. The black pepper plants could grow in fully aerated hydroponics condition. The findings of these study will contribute to the further research of using hydroponics for black pepper plants in order to facilitate the study of root knot nematodes on the roots of black pepper plants. Therefore, this will bring us closer to the effective management of root knot nematodes in black pepper farms.

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APPENDICES A

Plate A 1 Hydroponics set up

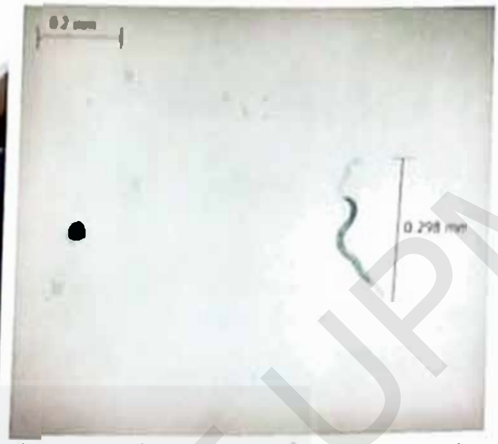

Plate A 2 Image of microscope under light microscope (100x magnification)

Plate A 3 Healthy black pepper plant

Plate A 4 Infected black pepper plant with root knot nematodes

Plate A 5 Root knot formation in black pepper plants

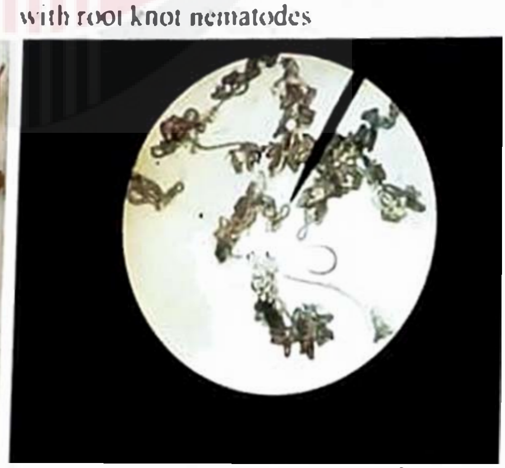

Plate A 6 Root knot nematodes observed under light microscope (100x magnification)

Plate A.7 Canopies of Panniyur-1 and Kuching

Plate A.8 Canopies of Semongok Emas

APPENDICES B

Table B.1 Increment in stem length (cm) of black pepper cuttings in fully aerated hydroponics over 12 weeks

Plants	Increment in Stem Length (cm)											
	Week											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	5.2	6.8	8.5	10.5
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	2.7	3.5	4.3	5.0
D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	7.5	7.9	8.1	8.5

Table B.2 Length of longest root (cm) of black pepper cuttings in fully aerated hydroponics over 12 weeks

Plants	Length of Longest Root (cm)											
	Week											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0	0.0	0.0	0.0	2.3	7.7	10.1	12.6	14.5	17.2	19.1	20.0
B	0.0	0.0	0.0	0.0	5.6	9.5	13.8	15.3	18.9	21.2	24.1	25.1
C	0.0	0.0	0.0	1.9	6.4	11.1	14.4	16.7	17.7	20.2	23.7	24.6
D	0.0	0.0	0.0	0.8	10.1	14.3	17.0	20.5	22.1	25.7	26.2	27.4

Table B.3 Number of leaves of black pepper cuttings in fully aerated hydroponics over 12 weeks.

Plants	Number of leaves											
	Week											
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	2	2	2	2	2	2	2	2	3	4	4	4
C	2	2	2	2	2	2	2	2	3	3	3	3
D	2	2	2	2	2	2	2	3	3	3	3	3

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Prevalence of Root Knot Nematodes at Ladang Samarakan and Potential of Black Pepper Cultivation in Hydroponics for Rootstock" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.

TER HONG YIP
Date: