UNIVERSITI PUTRA MALAYSIA

IMPROVEMENT OF HYDROPONIC MEDIUM TO PROMOTE ROOTING AND CONTROL OF ROOT GALL DEVELOPMENT ON BLACK PEPPER CUTTING

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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October 2021

Chair : Patricia King Jie Hung, PhD
Faculty : Agricultural Science and Forestry

Stem cutting is the common planting material for black pepper (*Piper nigrum* L.) farmers, but soil propagation renders both the planted material and new developing plant susceptible to soil-borne parasites such as root knot nematodes (RKN). Hydroponic cultivation technique thus offers a platform for manipulating root growth conditions, commencement of plant production under zero risk of soil parasites, hence a more extensive root system with improved strength to resist soil pathogens. The present research thus aimed to; (i) establish the most suitable nutrient composition and stem cuttings for promoting root-stock growth of black pepper (*P. nigrum* cv. 'Kuching') plants through hydroponic cultivation (ii) assess the effect of potassium silicate and salicylic acid on RKN infestation in black pepper plants (iii) evaluate the oxidative stress by assessing the production of superoxide anion (O_2•-) and hydrogen peroxide (H_2O_2) in black pepper plants after inoculation of the plant rhizosphere with RKN and identifying cellular sites of O_2•- plus H_2O_2 accumulation in leaves of inoculated black pepper plants. A total of 210 stem cuttings were hydroponically planted in uniformly-sized growing trays and arranged in a completely randomized design in the laboratory. Seven nutrient solutions (NS) were investigated, with each NS containing an equal number of stem cuttings with adventitious roots (R) and stem cuttings without any root (U) at the time of planting. Plant response data was collected for a total period of 4 weeks. Hoagland solution supplemented with 0.005 mM potassium silicate solution (T4) and Hoagland solution supplemented with 2 mM salicylic acid solution (T6) showed faster root initiation whereas T1 (Hoagland solution only) produced the highest increment in root length of plants. The least suitable nutrient composition was T5 [T4 + 6 mL of 1 M Ca $(NO_3)_2.4H_2O$ solution]. Roots for plants whose stem cuttings did not have any root at the time of planting (U) increased in root length faster than plants whose stem cuttings had some adventitious roots at the time of planting (R). Hydroponic plants in T1, T4 and T6 were transplanted into the soil medium and introduced to greenhouse conditions while maintaining the NS for each plant. After acclimatization to both soil and greenhouse conditions, plants were inoculated with RKN. The number of *P. nigrum* cv. 'Kuching' plants exhibiting foliar symptoms of RKN infection followed the order T1 > T6 > T4 > uninoculated plants. Aerial growth of RKN-inoculated plants was highest in plants irrigated with T4, followed by T6. Total number of root galls followed the order T1 > T6 > T4. Present results indicate that supplementation of the NS with potassium silicate (T4) provided the highest plant resistance against the influence of RKN. This finding was additionally reflected in the oxidative stress experiment which revealed least $O_2^{\bullet-}$ and H_2O_2 production in leaves of RKN-inoculated plants irrigated with T4 compared to plants irrigated with T1 and T6. Results of this study could be used by black pepper farmers for inducing RKN resistance of the plant through soil nutritional amendment with potassium silicate.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENAMBAHBAIKKAN MEDIUM HIDROPONIK BAGI MENGGALAKKAN PENGAKARAN DAN PENGAWALAN PERKEMBANGAN PURU AKAR PADA KERATAN BATANG LADA HITAM

Oleh

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Keratan batang adalah bahan penanaman yang biasa digunakan oleh petani lada hitam (*Piper nigrum* L.). Namun demikian, penanaman dalam tanah akan menyebabkan keratan batang yang ditanam dan tumbuhan yang baru bercambah mudah diserang parasit bawaan tanah seperti nematoda simpul akar (RKN). Teknik pengkulturan hidroponik menawarkan platform untuk memanipulasi keadaan pertumbuhan akar dan memastikan titik permulaan pengeluaran tanaman tanpa risiko serangan parasit tanah, sekaligus memanjangkan sistem akar tumbuhan, di samping mempunyai rintangan yang tinggi terhadap patogen tanah. Oleh yang demikian, kajian ini dijalankan untuk (i) menyediakan komposisi nutrisi dan keratan batang yang paling sesuai untuk menggalakkan pertumbuhan akar pokok lada hitam (*P. nigrum* cv. 'Kuching') melalui teknik pengkulturan hidroponik, (ii) menilai kesan kalium silikat dan asid salisilik ke atas serangan RKN dalam tumbuhan lada hitam, (iii) menilai tekanan oksidatif dengan menilai pengeluaran anion superoksida (O_2•-) dan hidrogen peroksida (H_2O_2) dalam pokok lada hitam selepas inokulasi rizosfera tumbuhan dengan RKN dan mengenalpasti tapak selular O_2•- dan pengumpulan H_2O_2 dalam daun pokok lada hitam yang telah diinokulasi. Data tindak balas tanaman dikumpul selama 4 minggu. Keratan batang dalam dulang yang mengandungi larutan Hoagland + 0.005 mM larutan kalium silikat (T4) dan larutan Hoagland + 2 mM larutan asid salisilik (T6) menunjukkan permulaan pertumbuhan akar yang pantas manakala T1 (larutan Hoagland sahaja) menghasilkan pertambahan tertinggi bagi panjang akar tumbuhan. Komposisi nutrisi yang paling tidak sesuai adalah T5 [T4 + 6 mL larutan 1 M Ca $(NO_3)_2.4H_2O$]. Pertumbuhan akar (panjang) bagi keratan batang yang tidak mempunyai akar pada masa penanaman (U) adalah lebih pantas berbanding dengan keratan batang yang mempunyai akar adventif pada masa penanaman (R). Tumbuhan hidroponik dalam T1, T4 dan T6 dipindahkan ke dalam tanah dan diperkenalkan kepada keadaan rumah hijau disamping mengekalkan NS bagi setiap tumbuhan. Setelah menyesuaikan dengan keadaan tanah dan

rumah hijau, tumbuhan diinokulasi dengan RKN. Bilangan pokok *P. nigrum* cv. ‘Kuching’ yang menunjukkan simptom jangkitan RKN pada daun, mengikut urutan, ialah T1 > T6 > T4 > tumbuhan yang tidak diinokulasi. Pertumbuhan sistem pucuk bagi tumbuhan yang diinokulasi dengan RKN yang tertinggi dapat dilihat pada tumbuhan yang ditanam dan disiram dengan larutan T4, diikuti dengan larutan T6. Jumlah puru akar mengikut urutan adalah T1 > T6 > T4. Data menunjukkan NS yang mengandungi kalium silikat (T4) memberikan rintangan tertinggi terhadap RKN. Penemuan ini juga dapat dilihat daripada analisis tekanan oksidatif, di mana pengeluaran O_2•- and H_2O_2 dalam daun tumbuhan (yang diinokulasi dengan RKN) yang dibekalkan dengan larutan T4 adalah yang terrendah berbanding tumbuhan yang dibekalkan dengan larutan T1 dan T6. Keputusan dan penemuan yang diperoleh daripada kajian ini boleh digunakan oleh para petani lada hitam bagi meningkatkan kerintangan tanaman mereka terhadap RKN melalui pengubahsuaian komposisi nutrisi tanah dengan kalium silikat.

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I certify that a Thesis Examination Committee has met on 29 October 2021 to conduct the final examination of Babirye Khadijah on her thesis entitled "Improvement of Hydroponic Medium to Promote Rooting and Control of Root Gall Development on Black Pepper Cutting" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Date: 10 February 2022

Declaration by the graduate student

I hereby confirm that:

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Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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Signature: ______________________
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RKN	Root Knot Nematodes
J2	Second Stage Juvenile
J4	Fourth Stage Juvenile
UPMKB	Universiti Putra Malaysia Kampus Bintulu
ROS	Reactive oxygen species
dai	days after inoculation
NBT	Nitro Blue Tetrazolium
DAB	3,3' diaminobenzidine
SA	Salicylic acid
SAR	Systemic Acquired Resistance

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Black pepper (*Piper nigrum* L.) is a perennial vine belonging to family *Piperaceae* (Izzah & Wan Asrina, 2019), whose berries are highly valued as a spice and medicine (Meghwal & Goswami, 2013; Ahmad *et al.,* 2012; Zhigang *et al.,* 2010). The plant requires optimum supply of both macro and micronutrients for productive yield (Izzah & Wan Asrina, 2019; Kevin *et al*., 2018; Ann, 2012). According to Izzah and Wan Asrina (2019) plus Dinesh *et al.* (2012), a clear understanding of black pepper's nutrient dynamics coupled with timely provision of essential nutrients are key components in achieving sustainable black pepper yields. Black pepper is an economically important crop in Malaysia (Aarthi & Kumar, 2019; Izzah & Wan Asrina, 2019; Lau *et al.,* 2012) and an important cash crop of Sarawak (Izzah & Wan Asrina, 2019; Pau, 2012). The cultivars commonly grown include 'Semenggok Emas', 'Semenggok Aman', 'Semenggok Perak' and 'Kuching' (Izzah & Wan Asrina, 2019; Lau et al., 2012; Sim & Rosmah, 2011). 'Kuching' is the traditional cultivar, with high yields (Izzah & Wan Asrina, 2019) but is the most highly prone to major root parasites and diseases e.g. root knot nematodes (Izzah & Wan Asrina, 2019; Megir & Paulus, 2011).

Root-knot nematodes (RKN) still remain one of the leading causes of economic damage in agricultural production all over the world (Naz *et al.,* 2020; Ye *et al.,* 2015; Claudia *et al*., 2013). They are obligate sedentary endoparasites (Ye *et al*., 2019, 2015; Akyanzi & Felek, 2013; Trinh, 2010) with over 80 species known (El-Nuby *et al.,* 2019; Mattei *et al.,* 2016; Ann, 2012). Yield losses in field crops due to RKN are estimated at > US $ 100 billion per year globally (Naz *et al.,* 2020; Sikora *et al.,* 2005). *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria* and *Meloidogyne hapla are* the commonly widespread *Meloidogyne* species (El-Nuby *et al.,* 2019; Akyanzi & Felek, 2013; Naz *et al.,* 2012) as they have a wide host range (Akyanzi & Felek, 2013; Hunt & Handoo, 2009; Adam *et al.*, 2007). In Malaysia, these species are ubiquitous, associated with several economically important agricultural crops and are among the major causes of reduced yields and quality in black pepper (Ravindra *et al.,* 2014; Pau, 2012). The resulting yield losses depend on the quantity of nematodes infesting the roots. Moreover, only 10 juveniles are enough to cause 16% reduction in black pepper plant growth (Koshy *et al.,* 1979).

Timely detection plus identification of *Meloidogyne* spp. is key in the design and implementation of the most appropriate RKN management strategy in farmlands (Ye *et al.,* 2019, 2015; Cunha *et al.,* 2018; Baidoo *et al.,* 2016) because it provides reliable information on which RKN species is dominant in the field (Cunha *et al.,* 2018; Naz *et al.,* 2012). Moreover, more than one RKN species can sometimes be found in the same plant root (Devran & Sogut, 2009). Traditionally, RKN species are identified morphologically by examining the different life cycle stages (Ye *et al.,* 2019, 2015; Cunha *et al.,* 2018; Hunt & Handoo, 2009). According to Hunt and Handoo (2009), valuable clues onto which *Meloidogyne* spp. is infesting a particular farm can additionally be obtained by examining characteristics of the root galls viz; shape, distribution on the root system, and size. Therefore, a combination of more than one identification approach provides reliable diagnosis of RKN distribution in the farm for timely and appropriate intervention measures.

Plant roots produce root exudates and volatile compounds which contain among other components chemoattractants plus chemorepellants (Dam & Bouwmeester, 2016; Wicher, 2012; Devine & Jones, 2002) that are distributed into the surrounding soil through water and rhizospheric pores (Liu & Park, 2018). Root knot nematodes present in the soil not only detect chemoattractants but are (RKN) also preferentially attracted to those roots whose fraction/ratio of chemoattractants production is greater than the chemorepellants (Liu & Park, 2018). For them to penetrate into the host/roots, RKN secrete enzymes responsible for degrading the cell wall and breakdown of cell wall components, causing damage of the root cell membrane and leakage of root cellular contents (reviewed by Haegeman *et al.,* 2012, 2011). Additionally, RKN target plant hormones related to defense and manipulate plant defense responses through secretion and translocation of effectors into the host, to disrupt the host cell function (Fernandez *et al.,* 2015; Truong *et al*., 2015; Cotton *et al.,* 2014). Nematode effectors are secreted directly into plant roots by use of RKN stylets (Fernandez *et al.,* 2015; Mantelin *et al*., 2015; Truong *et al.*, 2015) by the second-stage juveniles or secreted into the apoplast (Truong *et al.,* 2015; Vieira *et al.,* 2011). Root knot nematodes incite formation of permanent food sinks (giant cells) into the plant roots through manipulation of hormonal signaling pathways (Haegeman *et al*., 2012). Survival of the nematode is guaranteed as long as the induced feeding structure is not damaged by plant defense responses (Haegeman *et al.,* 2012). To further suppress plant defense responses and finally establish themselves, some RKN coat their surfaces with antioxidant proteins to counteract the effects of reactive oxygen species produced as a plant defense response (Haegeman *et al*., 2012; Robertson *et al.,* 2000).

Advances that have been employed to control RKN include crop rotation and provision of a fallow season, fumigation, application of wide spectrum chemical nematicides as seed coatings or drenched in the soil, use of bioagents like antagonistic plants/extracts, bacteria, and fungi to produce compounds which halt RKN egg hatching or deter development of other life stages, and many more (Naz *et al*., 2020; Hu *et al.,* 2013; Mitkowski & Abawi, 2003). However, each of these advances has associated challenges that limit its sustainability; the broad host spectrum of *Meloidogyne* spp., in addition to the requirement for increased expertise and integrated management practices limit the use of crop rotation (Mitkowski & Abawi, 2003). Fumigants and nematicides although effective, pose a threat to both humans and the ecosystem due to their gradual environmental toxicity (Naz *et al.,* 2020; El-sherif *et al*., 2016; Silva *et al.,* 2015). This potential environmental damage in addition to the increased global demand for organic food has resulted into both the total ban on and restricted use of chemical fumigants and nematicides (Zhan *et al.,* 2018; Silva *et al.,* 2015; Ardakani, 2013). In addition, fumigants are expensive thus out of reach to low-income farmers (Mukhtar *et al.,* 2013; Silva *et al.,* 2010; Ghini & Kimati, 2000). Furthermore, resistance- breaking populations of *Meloidogyne,* due to prolonged use of fumigants and nematicides are challenging the use of these control measures (Naz *et al.,* 2020; Ghini & Kimati, 2000). The use of genetic control and bioagents is most times limited to scarcity of high-resistant material, local climatic condition, or target species, and has thus led to limited success and variability of results (Franzener *et al.,* 2007).

The continuous pursuit for effective, sustainable, and environmentally-benign approaches to control RKN infestation in black pepper (*P. nigrum*) fields has prompted researchers to graft susceptible varieties onto nematode-resistant rootstocks as an alternative method to developing RKN-resistant *P. nigrum* rootstock (Aarthi & Kumar, 2019). Several countries such as India and Malaysia have grafted the shoots of *P. nigrum* on top of *P. colubrinum* Link as an ecologically-sound strategy to reduce RKN infestation in the former plant (Lau *et al.,* 2012; Sim & Rosmah, 2011). *P*. *colubrinum* is a wild relative of *P*. *nigrum* having resistance genes to nematodes (Tameling *et al.*, 2002; Ravindran & Remashree, 1998). However, attempts to reduce and/or resist RKN infestation in *P. nigrum* through use of grafted RKN-resistant rootstocks have achieved short-lived success and are therefore still futile (Lau *et al.,* 2012; Eng, 2001). According to Vanaja *et al*. (2007) plus Ravindran and Remashree (1998), efforts to graft *P*. *nigrum* shoots onto *P*. *colubrinum* rootstocks yielded high initial success but delayed graft incompatibility (Sim & Rosmah, 2011; De Waard & Zeven, 1969) led to poor plant survival in later years (Lau *et al.,* 2012). From our knowledge, no grafted RKN-resistant *P. nigrum* rootstocks have stood the test of time (exceeding at least four years) in Malaysia till date because the available germplasm resources are not long-term. Therefore, grafting technique of *P. nigrum* for root knot nematode resistance is not sustainable yet.

Black pepper (*P. nigrum*) being both a nutritionally demanding and weather-sensitive crop (Izzah & Wan Asrina, 2019; Dinesh *et al.,* 2012), hydroponic farming thus provides a good research platform for producing plants from a growth environment that is not hindered by adverse effects of sudden weather, and soil nutritional uncertainties that would compromise proper growth. Hydroponic farming involves cultivation of crops in a soilless medium (Wada, 2019; De Souza *et al.,* 2018; Eduardo *et al*., 2015), in which the plant's growth conditions are manipulated timely to suit the experimental design (Sambo *et al.,* 2019). It is a positive response towards a friendlier agriculture in comparison with soil-based farming (Tajudeen & Taiwo, 2018; Benke & Tomkins, 2017; Maucieri *et al.,* 2017) and has gained much attention worldwide, notably in vegetable production (Sambo *et al.,* 2019; De Souza *et al.,* 2018; Spehia *et al.,* 2018). This continuous positive trend is attributed to the comparative advantages that hydroponic cultivation offers over traditional field production notably timely supply of the desired nutrient composition and concentration to the plant, allows for a more detailed analysis of the whole root system in addition to easier monitoring of root growth and morphology without interference compared to field-grown plants, facilitates easier screening of nutrient deficiencies and toxicities in the growing medium and root system, kick-start of the crop production cycle in absence of soil-borne pests and diseases, flexibility and/or ability to manipulate environmental growth conditions such as light intensity, pH, temperature, and alike (Islam *et al.,* 2018; Pignata *et al.,* 2017; Rodriguez-Ortega *et al.,* 2017). This combination translates into faster growth rate of the plant. Hydroponic cultivation therefore presents a farming alternative for producing healthier root stock of *P. nigrum* plants.

Soil-nutritional amendment is commonly practiced as a yardstick for disease and pest-control (Fan *et al.,* 2018; Hassan & Byoung, 2018; Jayawardana *et al.,* 2015). Although silicon (Si) is a non-essential nutrient, improved beneficial effects to plants in response to Si fertilization is well-documented including disease /pathogen suppression and its regulatory roles in the uptake of plant nutrients (Laane, 2018; Deshmukh *et al.,* 2017; Liang *et al.,* 2007). Amendment of the soil with Si has successfully suppressed RKN in different crops; it has decreased the RKN reproduction rate, root galling, population density and overall RKN development in cucumber, rice, sugarcane, coffee, among others (Zhan *et al.,* 2018; El-sherif *et al.,* 2016; Mattei *et al.,* 2016). Additionally, data concerning the carcinogenicity, mutagenicity, or developmental toxicity of Si application in hydroponically grown crops is not available yet (El-sherif *et al*., 2016). In this move, the potential of improving black pepper (*P. nigrum*) root growth through supplementing nutrient solutions with Si and reduction of RKN infestation in black pepper plants through Si nourishment, have not yet received much attention.

Relatedly, application of plant hormone regulators such as salicylic acid (SA) has been extensively used in improving nutrient uptake, stimulating root initiation, lateral and adventitious root formation, seed germination, vegetative growth, among other beneficial roles during plant growth (Pasternak *et al.,* 2019; Wani *et al*., 2016; Khan *et al*., 2015). Salicylic acid has also been reported for reducing egg mass numbers and reproduction rates of RKN in plants. Thus, there is hope of promoting black pepper (*P. nigrum* cv. 'Kuching') faster and healthier root systems that are less prone to RKN through nourishing the plants with SA.

Over time, plants have evolved defense responses/mechanisms which detect molecular patterns associated with pathogen, microbe or plant damage, using specialized receptors (Cabot *et al.,* 2019; Santamaria *et al.,* 2018; Duran-Flores & Heil, 2016). Production of hydrogen peroxide, superoxide anions, among other reactive oxygen species (ROS) is one of the defense responses exhibited by plants (Cabot *et al.,* 2019; Ali *et al.,* 2018; Bittner *et al.,* 2017). Presence and/or infestation of plant roots by RKN subjects plants to oxidative stress, which manifests as increased production of ROS. Although information regarding use of this approach to detect RKN infestation in black pepper (*P. nigrum*) fields is still scanty, its application and/or adoption could ultimately reveal which nutrient composition protects RKN-inoculated black pepper plants from deleterious oxidative impact.

The aim of the present study was to establish the most suitable nutrient composition and stem cuttings for promoting root-stock growth of black pepper (*P. nigrum* cv. 'Kuching') seedlings through hydroponic cultivation plus assessing the effect of potassium silicate and salicylic acid on RKN infestation in black pepper plants. This study was also aimed at evaluating the oxidative stress by assessing the production of superoxide anion ($O_2•-$) and hydrogen peroxide (H_2O_2) in black pepper plants after inoculation of the plant rhizosphere with RKN and identifying cellular sites of $O_2•-$ plus H_2O_2 accumulation in leaves of inoculated black pepper plants. To our knowledge, this was the first study documenting the growth of black pepper (*P. nigrum* cv. 'kuching') in a hydroponic system, acclimatizing hydroponic plants to the soil medium and greenhouse conditions, reducing RKN infestation in black pepper plants by use of potassium silicate, simulating the production of $O_2•-$ plus H_2O_2 through RKN inoculation, and detecting cellular sites of $O_2•-$ plus H_2O_2 in black pepper leaves. Positive results of this study may be adopted by black pepper farmers for RKN management such as induced resistance through soil nutritional amendment.

1.2 Problem statement, objectives, and hypotheses

Currently, there is a dearth of information on the effect of soil nutrient modification on root parasites and pathogens that infest black pepper (Nerrisa *et al.,* 2018). Therefore, the impact of *P. nigrum* nourishment with potassium silicate and salicylic acid on root growth and RKN infestation is worth exploring. In addition to commencement of plant production under zero risk of soil parasites plus easier screening of nutrient deficiencies in the growing medium, hydroponic cultivation offers a farming alternative that facilitates continuous, timely and detailed monitoring of overall root development while growth proceeds. The present study therefore aimed at;

a. Establishing the most suitable nutrient composition and stem cuttings for promoting root-stock growth of black pepper (*P. nigrum* cv. 'Kuching') plants through hydroponic cultivation
b. Assessing the effect of potassium silicate and salicylic acid on RKN infestation in black pepper plants
c. Evaluating the oxidative stress by assessing the production of superoxide anion ($O_2•-$) and hydrogen peroxide (H_2O_2) in black pepper plants after inoculation of the plant rhizosphere with RKN and identifying cellular sites of $O_2•-$ plus H_2O_2 accumulation in leaves of inoculated black pepper plants.

This study hypothesized that supplementation of nutrient solutions with potassium silicate and salicylic acid would both promote root growth of black pepper plants and reduce root galling.

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