



***EFFECTS OF NANO SILVER AS BACTERICIDE ON BENEFICIAL
BACTERIA, UPTAKE BY PLANT AND IMPACT ON PLANT GROWTH***

POOPAK SOTOODEHNIA

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By

POOPAK SOTOODEHNIA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Doctor of Philosophy**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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POOPAK SOTOODEHNIA

January 2019

Chairman : Norida Mazlan, PhD
Faculty : Agriculture

Nanotechnology refers to the use of nano –size particles (measuring < 100 nm) in various fields including agriculture. Nanoparticles can be in the form of nano pesticide, nano fertilizer, waxing and sensors that are currently being used in agriculture. However, there is little information on the effects of these particles on microorganisms in soil and its impact on the environment. Therefore, this study was undertaken to evaluate the effect of nano-silver as bactericides on selected plant growth-promoting bacteria, residue in soil and its uptake in plant and impact on plant growth. In the initial study, the minimum inhibitory concentration (MIC) of nano-silver on beneficial microbes from the soil and pathogen was determined. To evaluate the MIC of silver nanoparticles (AgNPs), the size and shape of nanoparticles were determined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). To evaluate the effect of nano-silver on beneficial microbes, a total of 13 beneficial microbes tested for this study by using microdilution broth method. In the second study the effect of nano-silver against *Ralstonia solanacearum* and its effect on seed germination was studied. Based on the MIC results 40 ppm of nano- silver was used while different treatments were set for this study. On the third study the leaching potential of nano- silver in different soils samples were investigated. For leaching study, 196 µl of nano- silver was added to the two soils samples and leachate were collected every day for 14 days. Three replications including control were arranged as a completely randomized design (CRD) experiment and later the silver were measured by inductively coupled plasma optical emission spectrometry (ICP–OES). The forth study was aimed to analyze the residue of nano- silver in plant and also effect of nano-silver on plant growth. In this study *Cucumis sativus*'s seed were soaked with different concentration of nano- silver (16, 24, 32 and 40 ppm) and control was not treated with nano-silver. This study was conducted in a glasshouse and the treatments were chosen based on the MIC results and the Munchong series

soil, sand and organic matter were used as mixture for this study. Samples were analyzed with ICP-OES to detect the residue of silver using destructive sampling. Three different time (3WAP, 6WAP and 8WAP) were used for sampling time. Results showed that nanoparticles were pure spherical entities with 16 ± 6 nm in size. Nano- silver inhibited the activity of bacteria and pathogen on the range of 12 – 40 ppm. Results showed that silver nano particles increased the seed germination and protect the seed from pathogen. The physico- chemical data indicated that Munchong series soil sample was acidic (pH~ 5) and the amount of clay was approximately 70 %. However, the reference soil sample (with ratio of 2:2:1 Munchong series soil, sand and organic matter) was slightly acidic (pH ~ 6.6) and the amount of sand was 60 %. Results showed that the cation exchange capacity (CEC), electrical conductivity (EC), micro and macro elements were higher in the reference soil sample which could be due to the organic matter (cow dung) that was used. Results also showed that no silver was detected in leachate of all treatments that might be due to the amount of clay, organic matter in samples. However, another possibility could be due to less amount of silver that was applied to the soil. For the uptake study, results showed that residue of silver was detected in root, stem, flower and fruit during different sampling times. Results also indicated that by increasing the concentration of nano-silver the dry weight of plant was also increased. However, nano- silver did not affect the length of stem, root length. Silver nanoparticles is shown to affect root diameter (RD) and root volume (RV) and leaf area index (LAI) which depends on the time of exposure. Thus at the third sampling, silver increased the LAI, RD and RV.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KESAN NANO PERAK SEBAGAI PEMBUNUH BAKTERIA ATAS
BAKTERIA BERFAEDEH, PENYERAPAN OLEH TUMBUHAN DAN IMPAK
KEPADA PERTUMBUHAN FLORA**

Oleh

POOPAK SOTOODEHNIA

Januari 2019

Pengerusi : Norida Mazlan, PhD
Fakulti : Pertanian

Nanoteknologi merujuk kepada penggunaan partikel yang bersaiz nano (kiraan $< 100\text{nm}$) dalam pelbagai industri termasuk industri pertanian. Partikel-partikel nano terdapat dalam bentuk pembunuh serangga nano, baja nano, penggilap dan penderia yang sekarang digunakan dalam arena pertanian. Namun begitu, terdapat kekurangan maklumat tentang kesan nanopartikel kepada mikroorganisma dalam tanah dan akibatnya kepada alam semula jadi. Oleh itu, kajian ini diambil untuk menilai kesan nano-perak sebagai pembunuh bakteria terhadap beberapa bakteria yang menggalakkan pertumbuhan flora. Dalam kajian permulaan, konsentrasi penghalang minimum (MIC) bagi nano-perak terhadap mikrob berfaedah daripada tanah dan patogen telah ditetapkan. Bagi menilai MIC nanopartikel perak (AgNPs), bentuk dan saiz nanopartikel telah ditentukan melalui mikroskop transmisi elektron (TEM) dan mikroskop elektron pengimbas (SEM). Bagi menilai kesan nanopartikel perak atas mikrob berfaedah, sebanyak 13 mikrob berfaedah telah dikaji untuk kajian ini dengan menggunakan kaedah mikropencairan kaldu. Dalam kajian kedua, kesan nano-perak terhadap *Ralstonia solanacearum* dan kesannya terhadap percambahan biji benih telah dikaji. Berdasarkan atas keputusan MIC, 40 ppm nano-perak telah digunakan sementara rawatan yang berlainan ditetapkan bagi kajian ini. Dalam kajian ketiga, potensi melarut resap nano-perak dalam pelbagai sampel tanah telah disiasat. Bagi kajian melarut resap, 196 μl nano-perak telah ditambahkan kepada dua sampel tanah, dan larut resapan telah dikumpulkan setiap hari sepanjang 14 hari. Tiga replika termasuk satu replika kawalan telah digunakan, disusun sebagai eksperimen "completely randomized design" (CRD) dan selepas itu, perak telah diukur dengan kaedah "inductively coupled plasma optical emission spectrometry" (ICP-OES). Kajian keempat bertujuan untuk menganalisis residu nano-perak dalam tumbuhan dan juga kesan nano-perak atas ketumbuhan flora. Dalam kajian ini, benih

Cucumis sativus's telah direndam dengan konsentrasi nano-perak yang berbeza (16, 24, 32, dan 40 ppm) dan biji benih kawalan tidak dirawat dengan nano-perak. Kajian ini dijalankan dalam sebuah rumah kaca dan rawatan-rawatan telah dipilih berdasarkan atas keputusan MIC, serta tanah, pasir dan bahan organik siri Munchong digunakan sebagai campuran untuk kajian ini. Sampel telah dianalisis dengan ICP-OES untuk mengesan residu perak menggunakan pensampelan merosakkan (destructive sampling). Tiga waktu berbeza (3WAP, 6WAP dan 8WAP) telah digunakan sebagai waktu kajian. Keputusan menunjukkan bahawa nanopartikel perak adalah entiti sfera tulen bersaiz 16 ± 6 nm. Nano-perak menghalang aktiviti bakteria dan patogen dalam julat 12-40 ppm. Keputusan menunjukkan bahawa nanopartikel perak telah menambahkan percambahan benih dan melindungi benih daripada patogen. Data fisio-kimia menunjukkan bahawa siri sampel tanah Munchong adalah berasid (pH ~ 5) dan jumlah tanah liat adalah lebih kurang 70%. Namun begitu, sampel tanah rujukan (dengan ratio 2:2:1 siri tanah, pasir dan bahan organik Munchong) adalah sedikit berasid (pH ~ 6.6) dan jumlah pasir adalah 60%. Keputusan menunjukkan bahawa kapasiti pertukaran kation (CEC), konduksian elektrik (EC), serta elemen mikro dan makro adalah lebih tinggi dalam sampel tanah rujukan. Ini mungkin disebabkan oleh bahan organik (tinja lembu) yang digunakan. Keputusan juga menunjukkan bahawa tiada perak yang dikesan dalam larut resapan daripada semua rawatan, ini mungkin disebabkan oleh jumlah tanah liat atau bahan organik di dalam sampel. Walau bagaimanapun, kemungkinan lain boleh disebabkan oleh kekurangan jumlah perak yang digunakan dalam tanah. Bagi kajian penyerapan, keputusan menunjukkan bahawa residu perak telah dikesan di akar, batang, bunga dan buah tumbuhan semasa waktu pensampelan berbeza. Keputusan juga menunjukkan bahawa apabila konsentrasi nano-perak dinaikkan, keberatan kering tumbuhan juga menaik. Namun begitu, nano-perak tidak dapat mempengaruhi kepanjangan batang dan akar. Nanopartikel perak telah ditunjukkan untuk memberi kesan kepada diameter akar (RD), isi padu akar (RV) dan indeks luas daun (LAI) yang bergantung kepada masa pendedahan. Oleh itu, perak menaikkan LAI, RD dan RV.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Norida binti Mazlan, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Halimi Mohd Saud, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Samsuri Abd, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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Name and Matric No: Poopak Sotoodehnia GS34779

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Signature: _____

Name of Chairman
of Supervisory
Committee:

Associate Professor Dr. Norida binti Mazlan

Signature: _____

Name of Member
of Supervisory
Committee:

Associate Professor Dr. Halimi Mohd Saud

Signature: _____

Name of Member
of Supervisory
Committee:

Dr. Samsuri Abd

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LIST OF ABBREVIATIONS

CB	Carbon Base
CeO ₂	Cerium Oxide
CNT	Carbon Nano Tube
CRD	Completely Randomized Design
DOM	Dissolved Organic Matter
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
ICTA	International Centre of Technology Assessment
MIC	Minimum Inhibitory Concentration
NFB	Nitrogen Fixation Bacteria
NM	Nano Material
NP	Nanoparticles
TiO ₂	Titanium Dioxide

CHAPTER 1

INTRODUCTION

1.1 Introduction

Nanotechnology refers to the use of nano –size particles (measuring < 100 nm) in various fields including agriculture. Nanotechnology is used in the entire food production chain, during cultivation (e.g. pesticides, fertilizer), industrial processing, and packaging of food. Moreover, nanotechnology is utilized to enhance the nutritional aspects of food through nano-scale additives and nutrients as well as nano-sized delivery systems for bioactive compounds (Senjen, 2007; Abbasian et al, 2012). Nanotechnology in agriculture could in theory enhance the yield of crop and protect plants from diseases. Researchers have reported the effect of nanoparticles on rice, corn and tomatoes whereby their application increased yield of crop. Nanoparticles in the form of pesticides are being used from cultivation up till storage. Nanopesticides reduce the application rate and losses through the small size of the particles and in turn this reduces environmental contamination (Müller et al, 2007; Moorman et al, 2001).

Pesticides containing nanoparticles might contaminate the soil and groundwater due to its longer persistence and increased toxicity. These particles could be taken up by plants when it is applied in the form of pesticides or fertilizer and easily enter into the food chain. The NPs uptake by plants is dependent upon several factors, such as the type and age of the plant, the duration of exposure, the size, type, chemical composition, solubility and the surface structure of the nanoparticles. Some plants will be able to take up and accumulate the NPs. Therefore, the risk of using NPs in agriculture, especially in the form of pesticides and fertilizers, is fast becoming the topic of interest in the context of preserving the quality of both the food chain and the environment. Among all nano particles, silver nano particles are used as bactericides and herbicides due to its antibacterial properties (Navarro et al, 2008).

The most obvious problem from the use of silver nano particles is its impact on the environment, especially to ecological organisms (Drake and Hazelwood, 2005). Once released into the environment, NPs will be able to move from one environmental compartment to another, i.e. from water to sediment, soil to groundwater, from water to microorganisms, fishes, insects, or mammals. One of the major challenges of nanotechnology is the development of a protocol to detect and investigate the behavior of NPs in the environment and how they impact biological systems. It is unclear how much nano silver have been released into the environment, and it is imperative that the environmental risks

of these particles are properly determined for the protection of the people and the environment. The lack of data and knowledge pertaining to the risk of nano silver to humans and the environment remains an important issue in agriculture (Wickson et al, 2011).

Nanoparticles are more effective vis-à-vis its cleansing effect compared to its larger counterparts (Lkhagvajav et al, 2011). The usage of nano silver results in faster growth and increased length of plant roots (Nia, 2009; Abbasian et. al, 2012). The application of nano- silver on olive trees prior to its cultivation decreased the incidence of root diseases, while its application on strawberry plants increased their resistance to diseases and increased the size of the fruits and leaves (Pulit et al, 2011). Nowadays, nano-Ag is used in pesticides despite the risk that it may contaminate the soil and groundwater. Plants are capable of absorbing these particles and accumulating them in plant tissue. The risk of plants as a source of nourishment increases with the uptake of NPs which might be passed on to the food chain, and subsequently consumed by humans. Therefore, it is important that the environmental effect of these NPs be determined.

1.2 Objectives

The objectives of this study are:

1. To characterize nano silver bactericide and determine the minimum inhibitory concentration of nano-silver on beneficial microbes as well as its effect on seed germination in Japanese Cucumber (*Cucumis sativus*) against *Ralstonia solanacearum*,
2. To determine the leaching potential of nano-silver in two different soils samples,
3. To determine the uptake and distribution of nano-silver in cucumber and its effect on plant growth.

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