



**UNIVERSITI PUTRA MALAYSIA**

***EFFECTS OF MOLYBDENUM APPLICATION ON NUTRIENT UPTAKE  
AND YIELD OF RICE AND NITROGENASE ACTIVITY OF  
RHIZOBACTERIA***

**HAMED ZAKIKHANI**

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**By**

**HAMED ZAKIKHANI**

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

**June 2016**

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## DEDICATION

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our heart.

This is dedicated to:

My parents



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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**June 2016**

**Chairman : Professor Mohd Khanif Yusop, PhD**  
**Faculty : Agriculture**

In tropical soils, the deficiency of Mo may be seen in the rice plants. Hence, elucidating the optimum levels of Mo in soil and plants will be helpful for understanding the plant growth. The current study was conducted to assess Mo status in soils and plants, and to determine the influences of Mo on in vitro nitrogenase activities of some plant growth promoting rhizobacteria (PGPR). Five rice cultivars (MR219, HASHEMI, MR232, FAJRE and MR253) and 4 levels of Mo (0, 0.01, 0.1, and 1.0 mg L<sup>-1</sup>) were used for the first experiment. The highest Mo uptake in shoots (0.70 µg plant<sup>-1</sup>) and roots (0.66 µg plant<sup>-1</sup>) were seen in the MR232 at the highest level of Mo (1 mg L<sup>-1</sup>) in solution culture. Iron rates in shoot decreased with increasing Mo levels in solution culture and reached 39.93 µg plant<sup>-1</sup> at the highest level of Mo. Also, phosphorus uptake in all cultivars increased with enhancing Mo in medium culture and reached highest (0.6 %) in cultivar MR232. In other experiment, Mo combination (0, 0.01, 0.05, 0.1, 0.5, 1, 1.5, 2, 2.5 mg L<sup>-1</sup>) was applied to the growth medium contained four nitrogen fixation bacteria (UPMB10, UPMB12, Sb16 and R19). Nitrogenase activity enhanced with increasing Mo levels in growth medium of all bacteria strains except Sb16. The strongest correlation ( $r = 0.78^{**}$ ,  $p < 0.01$ ) was found between Mo and ethylene production in UPB10, and the weakest one was seen in R19 ( $r = 0.49^{*}$ ,  $p < 0.05$ ). The highest ethylene productions in UPMB10 (99.6 µmol mol<sup>-1</sup> hour<sup>-1</sup>), UPMB12 (87.2 µmol mol<sup>-1</sup> hour<sup>-1</sup>) and R19 (80.1 µmol mol<sup>-1</sup> hour<sup>-1</sup>) were seen in treatments contained 2.5 ppm Mo. We collected eleven soil series from two depths of Kedah and Kelantan paddy fields. Sequential extractions of soil Mo fractions indicated that Kranji series contained highest plant available amount of Mo (0.26 mg kg<sup>-1</sup>) in comparison with other seven series of Kedah soils (surface layer), and highest medium plant available Mo (0.24

mg kg<sup>-1</sup>) was seen in Rotan and Sedaka series of Kedah area (surface layer). In upper layers of Kedah soil series, we could not detect any acid soluble Mo (associated with calcium). In surface layers of Kelantan, highest amount of plant available Mo (0.23 mg kg<sup>-1</sup>) was found in in Lating series but medium plant available of Mo (0.15 mg kg<sup>-1</sup>) was obtained from Cempaka series. In lower depths of Kedah, Guar series contained highest plant available mount of Mo (0.28 mg kg<sup>-1</sup>) and medium plant available rate of Mo (0.35 mg kg<sup>-1</sup>) was seen in Rotan series. In lower layers of Kelantan, Lating series contained highest amount of plant available Mo (0.34 mg kg<sup>-1</sup>) and medium plant available of Mo rate (0.07 mg kg<sup>-1</sup>) was only found in Batu Hitam series. We found out that cation exchange capacity was positively correlated with total amount of Mo in upper layers of Kedah soil series ( $r= 0.61$ ;  $P \leq 0.05$ ), and no statistically correlation was found between Mo contents and soil properties in Kelantan soil series.

For the last experiment, we used foliar and soil application methods in order to determine the optimum Mo rate at which highest rice grain is produced. We found out that grain yield of rice was correlated with total shoot dry weight and plant height with  $r$  value of 0.53\* and 0.74\* in treatments sprayed with Mo. The greatest grain yield (21 g plant<sup>-1</sup>) was seen in treatment of 5 mg Mo kg<sup>-1</sup> in soil, and treatment of 30  $\mu$ g Mo L<sup>-1</sup> supplied as foliar.

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

**KESAN MOLIBDENUM PERMOHONAN PENGAMBILAN NUTRIEN  
DAN HASIL NASI DAN NITROGENASE PEMEROLEHAN  
RHIZOBACTERIA**

By

**HAMED ZAKIKHANI**

Jun 2016

**Pengerusi : Profesor Mohd Khanif Yusop, PhD**  
**Fakulti : Pertanian**

Dalam tanah tropika, kekurangan Mo boleh dilihat dalam tanaman padi. Oleh itu, menjelaskan atas dasar tahap optimum Mo dalam tanah dan tumbuh-tumbuhan akan menjadi berguna untuk memahami pertumbuhan tumbuhan. Kajian ini telah dijalankan untuk menilai status Mo dalam tanah dan tumbuh-tumbuhan, dan untuk menentukan pengaruh Mo di dalam vitro aktiviti nitrogenase beberapa pertumbuhan tumbuhan menggalakkan rihizobacteria (PGPR). Five kultivar beras (MR219, HASHEMI, MR232, MR253 dan FAJRE) dan 4 tahap Mo (0, 0.01, 0.1, dan 1.0 mg L<sup>-1</sup>) telah digunakan untuk percubaan pertama. The Mo pengambilan tertinggi dalam pucuk (0.70 µg kilang<sup>-1</sup>) dan akar (0.66 µg tumbuhan<sup>-1</sup>) dilihat di MR232 di peringkat tertinggi Mo (1 mg L<sup>-1</sup>) dalam budaya penyelesaian. kadar zat besi dalam pucuk menurun dengan peningkatan tahap Mo dalam budaya penyelesaian dan mencapai 39.93 µg tumbuhan<sup>-1</sup> pada tahap tertinggi Mo. Juga, pengambilan fosforus di dalam semua kultivar meningkat dengan meningkatkan Mo dalam budaya sederhana dan mencapai tertinggi (0.6%) dalam kultivar MR232. Dalam eksperimen lain, gabungan Mo (0, 0.01, 0.05, 0.1, 0.5, 1, 1.5, 2, 2.5 mg L<sup>-1</sup>) telah digunakan untuk medium pertumbuhan yang terkandung empat bakteria nitrogen (UPMB10, UPMB12, Sb16 dan R19). Aktiviti nitrogenase dipertingkatkan dengan meningkatkan tahap Mo dalam medium pertumbuhan semua bakteria strain kecuali Sb16. Korelasi kuat ( $r = 0.78^{**}$ ,  $p < 0.01$ ) didapati antara Mo dan pengeluaran etilena dalam UPB10, dan yang paling lemah dilihat dalam R19 ( $r = 0.49^{*}$ ,  $p < 0.05$ ). Penghasilan etilena tertinggi di UPMB10 (99.6 µmol mol<sup>-1</sup> jam<sup>-1</sup>), UPMB12 (87.2 µmol mol<sup>-1</sup> jam<sup>-1</sup>) dan R19 (80.1 µmol mol<sup>-1</sup> jam<sup>-1</sup>) telah dilihat dalam rawatan terkandung 2.5 mg L<sup>-1</sup> Mo. Kami dikumpul sebelas siri tanah dari dua kedalaman sawah padi Kedah dan Kelantan. pengestrakan berurutan tanah Mo pecahan menunjukkan bahawa siri Kranji terkandung tertinggi tumbuhan jumlah yang ada bagi Mo (0.26 mg kg<sup>-1</sup>) berbanding dengan tujuh siri yang lain Kedah tanah (lapisan permukaan), dan paling tinggi kilang sederhana ada Mo (0.24 mg kg<sup>-1</sup>) dilihat dalam Rotan dan Sedaka siri kawasan Kedah (lapisan permukaan). Dalam lapisan atas siri tanah Kedah, kami tidak dapat mengesan apa-apa larut Mo asid (yang

berkaitan dengan kalsium). Dalam lapisan permukaan Kelantan, jumlah tertinggi tumbuhan ada Mo ( $0.23 \text{ mg kg}^{-1}$ ) didapati di dalam Lating siri tetapi tumbuhan medium yang ada bagi Mo ( $0.15 \text{ mg kg}^{-1}$ ) telah diperolehi dari siri Cempaka. Di kedalaman lebih rendah Kedah, siri Guar terkandung tumbuhan tinggi yang terdapat gunung Mo ( $0.28 \text{ mg kg}^{-1}$ ) dan sederhana kilang Kadar yang ada bagi Mo ( $0.35 \text{ mg kg}^{-1}$ ) telah dilihat dalam siri Rotan. Dalam lapisan bawah Kelantan, Lating siri terkandung jumlah tertinggi tumbuhan ada Mo ( $0.34 \text{ mg kg}^{-1}$ ) dan loji medium yang ada bagi Mo kadar ( $0.07 \text{ mg kg}^{-1}$ ) hanya dijumpai dalam siri Batu Hitam. Kami mendapati bahawa kapasiti pertukaran kation korelasi positif dengan Jumlah Mo di lapisan atas siri tanah Kedah ( $r = 0.61$ ;  $P \leq 0.05$ ), dan tidak ada korelasi statistik yang didapati antara kandungan Mo dan sifat-sifat tanah di Kelantan siri tanah.

Untuk percubaan terakhir, kami menggunakan kaedah permohonan foliar dan tanah untuk menentukan kadar Mo optimum di mana beras tertinggi dihasilkan. Kami mendapati bahawa hasil bijirin beras telah dikaitkan dengan jumlah berat menembak kering dan ketinggian tumbuhan dengan nilai  $r$  dari  $0.53^*$  dan  $0.74^*$  dalam rawatan disemur dengan Mo. The hasil bijirin besar ( $21 \text{ g kilang}^{-1}$ ) dilihat dalam rawatan  $5 \text{ mg Mo kg}^{-1}$  di dalam tanah, dan rawatan  $30 \mu\text{g Mo L}^{-1}$  dibekalkan sebagai foliar.



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

**Mohd Khanif Yusop**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Mohamed Hanafi Musa**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Radziah Othman**

Associated Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**

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Supervisory  
Committee \_\_\_\_\_

Signature: \_\_\_\_\_

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Member of  
Supervisory  
Committee \_\_\_\_\_

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## LIST OF ABBREVIATIONS

Mo	Molybdenum
MoO <sub>4</sub> <sup>2-</sup>	Molybdate
H <sub>2</sub> MoO <sub>4</sub>	Molybdic Acid
HMoO <sub>4</sub>	Hydroxy dioxymolybdenum
MoS <sub>2</sub>	Molibdenite
MoO <sub>2</sub>	Molybdenum dioxide
MoO <sub>3</sub>	Molydenum trioxide
CuMoO <sub>4</sub>	Copper Molybdenum tetraoxide
ZnMoO <sub>4</sub>	Zinc Molybdenum tetraoxide
PbH <sub>2</sub> MoO <sub>4</sub>	Lead Molybdate
CaH <sub>2</sub> MoO <sub>4</sub>	Calcium Molybdate
Fe(OH) <sub>3</sub>	Iron(III) oxide-hydroxide
Fe <sub>2</sub> O <sub>3</sub>	Iron(III) oxide
CaSO <sub>4</sub> .2H <sub>2</sub> O	Gypsum
PO <sub>4</sub> <sup>3-</sup>	Phosphate
NaHCO <sub>3</sub>	Sodium Hydrogen Carbonate
NaOH	Sodium Hydroxide
HCL	Hydrochloric Acid
HClO <sub>4</sub>	Perchloric Acid
DMRT	Duncan Multiple Range Test
MOP	Muriate of potash
K <sub>2</sub> O	Potassium oxide
HF	Hydrogen fluoride
HClO <sub>4</sub>	Perchloric acid
HNO <sub>3</sub>	Nitric acid
NH <sub>4</sub> OAC	Ammonium Acetate
ICP-EOS	Inductively coupled plasma optical emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ppm	Parts per million
µg g <sup>-1</sup>	micro gram per gram
mg g <sup>-1</sup>	milligram per gram
kg ha <sup>-1</sup>	Kilogram per hectare
µmol mol <sup>-1</sup> hour <sup>-1</sup>	Micromol per Mol per hour
H	hours
µM	Micro Molar

mM	Milli molar
$\mu\text{g Mo L}^{-1}$	Microgrsm Molybdenum per liter
g Plant <sup>-1</sup>	gram per plant
$\mu\text{g L}^{-1}$	microgram per kilogram
mg kg <sup>-1</sup>	megagram per kilogram
W:v	Weight per volume
RCBD	Randomized complete block design
FD	Factorial Design
PCA	Principal Component Analysis
CEC	Cation Exchange Capacity
ANOVA	Analysis of variance
OM	Organic matter
°C	Centigrade
S.O.V	Source of Variation

## CHAPTER 1

### INTRODUCTION

Molybdenum (Mo) is important in ecosystems as a micronutrient for plants, animals and some microorganisms. The average concentration of Mo in the earth's crust varies from 1.0 to 2.3 mg kg<sup>-1</sup> ranking it 53<sup>th</sup> in crustal abundance (Krauskopf and Bird, 1979). Molybdenum has five possible oxidation states (II, III, IV, V, and VI). In nature, the oxidation states of IV and VI predominate but the VI state is the most stable form. It also has industrial use and is an important fertilizer element in some farming systems. Gupta (1997) mentioned that Mo could be found in four main fractions in soils as follows; 1) water-soluble (plant available), 2) adsorbed and fixed on oxides and hydroxides such as iron and aluminium, 3) solid phases of Mo, and 4) associated Mo with organic compounds. Molybdenum occurs combined with other elements in nature so that most common mineral of Mo, which is molybdenite (MoS<sub>2</sub>), is found in granites, and the next most common mineral of Mo (PbMoO<sub>4</sub>) is found in oxidized zones of mineral deposits (Gupta, 1997; Adriano, 2001). The main Mo supply (nearly 95%) is mined from porphyry deposits associated with intrusive igneous rocks but marine black shales, lignite and lignitic sandstone, coal and phosphorite deposits are also recognized to contain large amounts of Mo (King *et al.*, 1973). Plant available and predominant aqueous species of Mo in natural systems is molybdate (MoO<sub>4</sub><sup>2-</sup>). In soil at pH above 4, molybdate is the dominant aqueous species but at the pH below 4, hydrogen molybdate (HMoO<sub>4</sub><sup>2-</sup>), Mo (OH)<sub>6</sub><sup>0</sup> and HMo<sub>2</sub>O<sub>7</sub><sup>-</sup> are the prevailing forms (Cruywagen and De Wet, 1988). Mo ions are components of several enzymes, including nitrogenase, nitrate reductase, xanthine oxidoreductase, aldehyde oxidase and sulfite oxidase (Hille *et al.*, 2011).

Nitrogenase enzyme is responsible for all biological nitrogen fixation activities. Since Mo is an essential element for nitrogen fixation, its absence can disrupt the biological fixation of nitrogen. Interaction between Mo and other nutrients is of great importance in plant nutrition and yield. In an experiment, application of Mo enhanced shoot phosphorus (P) uptake and root P concentration, and P increased shoot Mo concentration and uptake in rapeseed (Liu *et al.*, 2010). They also concluded that co-application of Mo and P was necessary to improve plant growth. In soil solution, higher concentrations of nitrate are presented to increase the Mo requirement by plants (Gupta and Lipsett, 1982) but higher concentrations of ammonium may reduce uptake of Mo. The nitrate positive influence on uptake of Mo can be due to pH enhancement and release of OH<sup>-</sup> ions. Also, greater uptake of ammonium may release more H<sup>+</sup> ions in the rhizosphere and reduce pH, consequently declining Mo uptake by the plants. In a pot experiment,



application of calcium (Ca) fertilizer increased the uptake of Mo in tobacco (Eivazi *et al.*, 1983). They also added that the effect of Ca fertilizer was greater when higher levels of sodium molybdate fertilizer were applied. Iron accumulation in plants seems to be inversely proportional with application of Mo. Hanger (1965) reported that Mo-induced apical chlorosis and stunting in red clover were eliminated once chelated iron was added to growth medium.

Availability of Mo to the plants are influenced by some factors, including soil pH, organic compounds, iron and aluminum oxides, soil texture and type and drainage. When pH increases in soils, availability of essential micronutrients declines but molybdenum's availability increases hence, in alkaline soils, availability of Mo increases and becomes more available to the plants. In acid soils, mobility of Mo reduces as its adsorption to sorbent sites increases (Reddy *et al.*, 1997). Molybdenum acts like an anion in soil and illustration of its adsorption mechanism(s) on organic matter is difficult. However, some workers reported that a close relationship between levels of adsorbed Mo and organic matter was seen (Karimian and Cox, 1978). Also, Kaiser *et al.* (2005) mentioned that poorly drained soils (peat marshes) tend to accumulate high concentrations of molybdate, but, by contrast, well-drained sandy soils leach significant levels of applied Mo. Penetration of molybdate into microspores and iron oxide interdomains can be resulted in immobilisation of molybdate. Lang and Kaupenjohann (2003) demonstrated that the rate of molybdate immobilisation by iron oxides surfaces is dependent on pore system geometry and crystallinity of iron oxides (Lang and Kaupenjohann, 2003). Also, there are some soil factors affecting the thresholds of Mo toxicity. McGrath *et al.* (2010) concluded that ammonium oxalate-extractable iron and organic carbon were the best predictors of toxicity threshold values for Mo.

Diazotrophic bacteria, such as *Bacillus* genera, belong to plant growth promoting rhizobacteria. These bacteria are able to convert dinitrogen into ammonia which is used by the plants. Molybdenum is known as an essential micronutrient for the biological nitrogen fixers and its deficiency limit their biological nitrogen-fixing activities (Campo *et al.*, 2000). In a field experiment, inoculation Mo-rich seeds produced crops with enhanced Mo and nitrogen rates in seeds and higher seed yield. They also mentioned that foliar applications of Mo increased concentrations of Mo in soybean seed by 3000% in comparison with regular grains produced without any supply of Mo (Campo *et al.*, 2009).

## 1.1 Justification

Molybdenum deficiency is common in acid soils and it has crucial roles in plants and microorganisms via molybdoenzymes. Research data on rice responses to Mo are not as extensive as those of other micronutrients like zinc, copper, boron, iron, and manganese. Symptoms of Mo deficiency are more common than toxicity in crops under certain soils. If high concentrations of Mo are present, depressing effect of Mo on physiological availability of copper will cause molybdenosis in ruminants (Majak *et al.*, 2006). Nevertheless toxicity of Mo is uncommon and is seen only when large concentrations of Mo are present. Antagonistic impacts of Mo on absorption of other nutrients by the plants can be considered as nutrient-suppression effect and also synergistic influences of Mo can produce a catalysing effect on uptake of nutrients by the plants in environments. Availability of Mo in acid soils is very low. Since Malaysian soils are predominantly acidic, evaluation of Mo in both soils and plants could be vitally important. In line with the national agriculture policy, Malaysia wishes to increase rice self-sufficiency (Liew *et al.*, 2010). There has been no report on Mo deficiency in crop plants productions in Malaysia, and this may account for the lack of specific information on soil and plant Mo status in data resources. Hence, understanding the Mo status in rice plants and paddy fields, and study of its influences on other nutrients uptake, are of importance. We hypothesized that molybdenum application increase the yield of rice. A particular focus was on quantifying the concentrations of Mo within the rice cultivars. We also hypothesized that Mo availability in irrigated paddy soils are dependent on chemical and physical characteristics of soils. Another hypothesis was that Mo application improves nitrogenase activity of some soil rhizobacteria.

## 1.2 Objectives

1. To evaluate Mo status of selected rice soils of Malaysia
2. To determine the effects of rice varieties on Mo nutrition of rice
3. To determine the effects of Mo on nitrogenase activities of selected PGPR
4. To determine the optimum Mo rate at which highest rice grain yield is produced



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