



UNIVERSITI PUTRA MALAYSIA

**MOLYBDENUM AND PHOSPHOROUS ROLES IN BIOLOGICAL
NITROGEN FIXATION USING *RHIZOBIUM* AND *AZOSPIRILLUM* IN
SOYBEAN (*GLYCINE MAX L.*)**

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FIXATION USING *RHIZOBIUM* AND *AZOSPIRILLUM* IN SOYBEAN
(*GLYCINE MAX L.*)**

By

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**Thesis Submitted to the School of Graduate Studies, University Putra
Malaysia in Fulfillment of Requirements of the Degree of
Doctor of Philosophy**

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DEDICATION



I dedicate this thesis to my father, my mother, my wife, and my children Hussein, Haya for their support during my study in Malaysia.



Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy.

Molybdenum and Phosphorous Roles in Biological Nitrogen Fixation Using *Rhizobium* and *Azospirillum* in Soybean (*Glycine Max L.*)

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December 2011

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Bacterial inoculation for legumes is one of the most important ways in the agricultural sector in improving yield quality and quantity of harvest. The role of *Rhizobium* is very important to produce better nodulation, nitrogen fixation, growth and higher yield. *Azospirillum* inoculated as a plant growth promoter gives positive effects in improving root development and the subsequent increase in the rate of water and mineral uptake. Molybdenum in plants is required for efficiency of the nitrogenase enzyme that is required by the nitrogen fixing bacteria whilst phosphorus is the second most limiting nutrient, after nitrogen, required for plant growth. A study was carried out to determine the role and interaction of inoculants, phosphorus and molybdenum in soybean under a drip irrigation system. A series of experiments were conducted in laboratory and glass house conditions with the following objectives;

- 1- To study the effect of molybdenum on the growth and survival of *Bradyrhizobium* and *Azospirillum* under laboratory conditions.

2- To study the effect of phosphorus and bacterial inoculation on the growth and yield of soybean under saline water irrigation.

3- To determine the effect of molybdenum, *Bradyrhizobium* and *Azospirillum* on the growth and yield of soybean under drip irrigation system.

4- To study the effect of molybdenum and co- inoculation on the growth and yield of soybean under drip irrigation system. Results from the experiments indicated that co-inoculation with *Rhizobium* and *Azospirillum* together with molybdenum stimulated total dry matter accumulation and root growth, stimulated uptake and higher concentration of N, P and K compared with inoculation by *Rhizobium* or *Azospirillum* alone. In the first experiment two *Bradyrhizobium* strains UPMR020 and UPMR047 and Iraqi isolate BK1 isolated from the roots of cowpea were used to select the best strain for the glass house conditions. Results showed that UPMR020 gave the best number of nodules, weight of nodules and plant dry weight compared to UPMR047 and isolate BK1, with a significant increase at ($P < 0.01$). In the second experiment the UPMR020 and UPMR047 were examined to reveal the ability of strains to survive in soil during six periods of incubation. The UPMR020 with 1 kg of Mo/ha showed a higher number of bacteria during five weeks of incubation compared with other treatments. Two *Azospirillum* species, *A.brasilense* isolated from roots of corn and *A.irakense* isolated from root of rice in Iraq were used. *Azospirillum.brasilense* with 1 kg of Mo/ha gave a significant 3rd numbers of bacteria compared with *A.irakense*. In fourth experiment different levels of phosphorus (0, 40, 80 and 120 kg of P/ha) with strain UPMR020 was studied together with irrigation using saline water (3.5 dS/m). The results showed an increase in all plant parameters under bacterial inoculation by

UPMR020 and 120 kg of P/ha under in spite of irrigation by saline water. From the above we can conclude that UPMR020 and UPMR047 was the best and most effective inoculants; both strains were used in subsequent experiments and it was found out that UPMR020 was better than UPMR047 in growth and survival in soil under laboratory conditions, and highest number of bacteria was under combination of 1 kg of Mo/ha and UPMR020. *A.brasilense* was the best and very effective inoculant compared with *A.irakense* in which *A.brasilense* gave highest number of bacteria under all treatments except interaction with 10 kg of Mo/ha. The phosphorus level at 120 kg of P/ha was the best treatment in increasing all plant parameters during the experiment especially when UPMR020 was added, and 3.5 dS/m saline water was suitable for soybean plants as alternative way of irrigation. One kg of Mo/ha with bacterial inoculation (*Rhizobium* or *Azospirillum*) gave highest plant yield and other plant parameters compared with other treatments, while 10 kg of Mo/ha decreased plant parameters with or without bacterial inoculation. Co- inoculation by *Rhizobium* and *Azospirillum* with 1 kg of Mo/ha gave highest plant growth parameters compared with other treatments followed by 1.5 kg of Mo/ha. Drip irrigation system proved its efficiency as a very important way in irrigated soybean plants and give better results. Generally co- inoculation by both bacterial strains effectively increased all plant parameters followed by *Rhizobium* inoculation alone then *Azospirillum* alone.

Abstrak tesis dibentangkan kepada Senat Universiti Putra Malaysia untuk memenuhi syarat Ijazah Doctor Falsafah.

Peranan Molibdenum dan Fosforus Dalam Pengikatan Nitrogen Menggunakan *Rhizobium* dan *Azospirillum* Bagi Kacang Soya (*Glycine Max L.*)

Oleh

BASSAM KANAAN ABDUL JABBAR

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Inokulasi bakteria tanaman kacang adalah salah satu kaedah terpenting dalam sektor pertanian bertujuan meningkatkan kualiti hasil tuaian. Peranan *Rhizobium* amat penting untuk meningkatkan pembintilan, pengikatan nitrogen, pertumbuhan dan hasil yang lebih tinggi. *Azospirillum* yang diokulasi sebagai bakteria penggalak tumbesaran tumbuhan memberi kesan positif terhadap perkembangan akar dan seterusnya meningkatkan kadar pengambilan air dan mineral. Molibdenum diperlukan oleh bakteria pengikat N untuk keberkesanan fungsi enzim nitrogenase manakala fosforus adalah unsur yang kedua terpenting selepas nitrogen untuk tumbesaran tumbuhan. Satu kajian telah dilaksanakan untuk menentukan peranan dan interaksi inokulan, fosforus dan molibdenum dalam kacang soya di bawah sistem pengairan titis. Satu siri kajian telah dilaksanakan dalam makmal dan persekitaran rumah kaca dengan objektif yang berikut:

1. Untuk mengkaji kesan molibdenum ke atas pembiakan dan kemandirian *Bradyrhizobium* dan *Azospirillum* dalam makmal
2. Untuk mengkaji kesan fosforus dan penginokulatan bakteria terhadap tumbesaran dan hasil kacang soya menggunakan pengairan larutan garam
3. Untuk menentukan kesan molibdenum, *Bradyrhizobium* dan *Azospirillum* ke atas tumbesaran dan hasil kacang soya menggunakan pengairan titis
4. Untuk mengkaji kesan molibdenum dan penginokulatan bersama ke atas tumbesaran dan hasil kacang soya menggunakan pengairan titis.

Keputusan kajian menunjukkan penginokulatan bersama *Rhizobium* dan *Azospirillum* bersama molibdenum merangsang penambahan berat kering dan pertumbuhan akar, pengambilan dan peningkatan kepekatan N, P dan K berbanding dengan penginokulatan *Rhizobium* atau *Azospirillum* sahaja. Dalam kajian pertama, dua strain *Bradyrhizobium* UPMR020 dan UPMR047 dan isolat Iraq (BK1) yang diasing dari akar kacang duduk (cowpea) diuji untuk memilih strain yang terbaik untuk kajian rumah kaca. Keputusan menunjukkan UPMR020 memberi hasil bintil, berat bintil dan berat kering pokok yang tertinggi berbanding dengan UPMR047 dan isolat BK1 pada aras yang signifikan ($P < 0.01$). Dalam kajian kedua, keupayaan UPMR020 dan UPMR047 bertahan hidup selama enam pusingan inkubasi telah dilakukan. UPMR020 dengan 1 kg Mo/ha menunjukkan bilangan bakteria yang lebih tinggi selepas inkubasi selama lima minggu berbanding rawatan yang lain. Dua spesis *Azospirillum* iaitu *A. brasilense* dari akar jagung dan *A. irakense* dari akar pokok padi di Iraq telah digunakan untuk kajian seterusnya. *A. brasilense* dan 1 kg Mo/ha memberi kesan signifikan ($P < 0.01$) bilangan bakteria berbanding *A. irakense*. Dalam kajian keempat, beberapa kadar fosforus (0, 40,

80 dan 120 kg P/ha) bersama strain UPMR020 telah dikaji menggunakan air larutan garam (3.5 dS/m). Keputusan menunjukkan peningkatan pada semua parameter untuk rawatan UPMR020 dan 120 kg P/ha walaupun diberi air garam. Berdasarkan keputusan di atas, UPMR020 dan UPMR047 dirumuskan sebagai inokulan yang paling berkesan dan terbaik. Kedua-dua strain telah digunakan untuk kajian selanjutnya dan didapati bahawa UPM020 adalah lebih baik daripada UPMR047 berdasarkan pembiakan dan kemandirian dalam tanah di persekitaran makmal. Jumlah terbanyak bakteria adalah dari gabungan 1 kg Mo/ha dan UPMR020. *A. brasilense* adalah yang terbaik dan paling efektif berbanding *A. irakense* di mana *A. brasilense* memberikan jumlah bakteria tertinggi dalam semua rawatan kecuali dengan 10 kg Mo/ha. Paras fosforus pada 120 kg P/ha adalah yang terbaik meningkatkan parameter semasa kajian terutamanya apabila ditambah UPMR020 dan kepekatan air garam 3.5 dS/m adalah sesuai sebagai alternatif pengairan. Satu kg Mo dengan penginokulan bakteria (*Azospirillum* atau *Rhizobium*) memberi hasil pokok tertinggi berbanding rawatan lain, manakala 10 kg Mo/ha mengurangkan parameter pokok dengan dan tanpa penginokulan bakteria. Penginokulan bersama *Rhizobium* dan *Azospirillum* dengan 1 kg Mo/ha memberikan parameter tertinggi berbanding rawatan lain disusuli oleh 1.5 kg Mo/ha. Pengairan titis membuktikan keberkesanannya sebagai satu kaedah yang penting dalam pengairan tanaman kacang soya.

Secara am, penginokulan bersama oleh kedua-dua bakteria meningkatkan dengan berkesan kesemua parameter tumbesaran diikuti oleh penginokulan *Rhizobium* sahaja dan *Azospirillum* sahaja.



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I certify that a Thesis Examination Committee has met on 22 December 2012 to conduct the final examination of Bassam Kanaan Abduljabbar on his thesis entitled “Molybdenum and Phosphorous Roles in Biological Nitrogen Fixation Using *Rhizobium* and *Azospirillum* in Soybean (*Glycine max* L.)” in accordance with the Universities and University College 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 Doctor of Philosophy.

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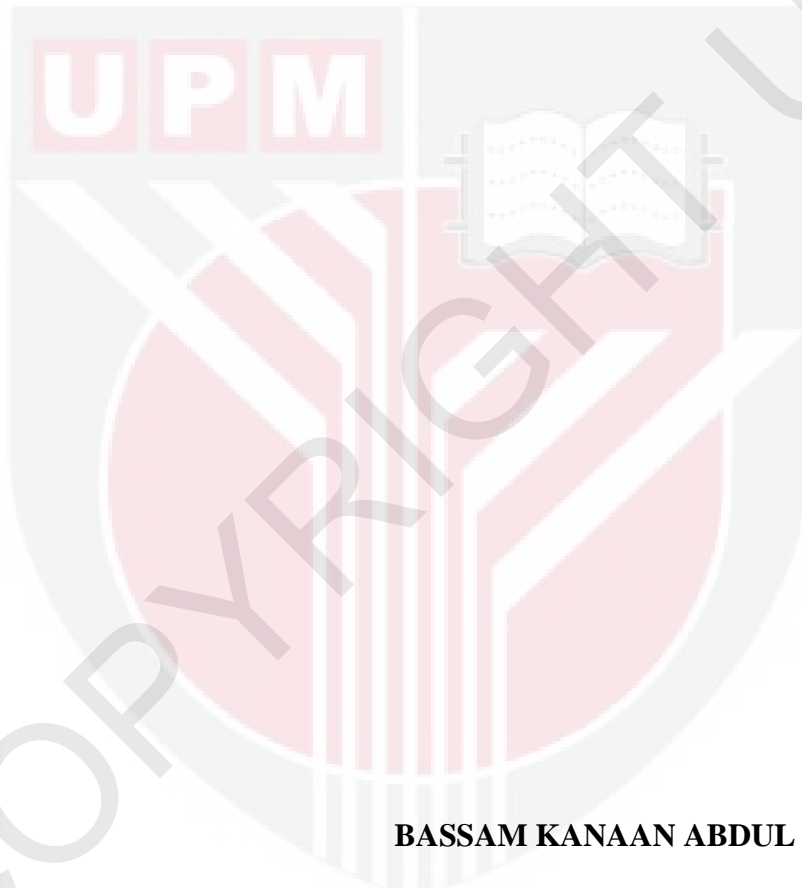
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DECLARATION

I declare that the thesis is my original work except for quotation and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted for any other degree at University Putra Malaysia or at any other institution.



BASSAM KANAAN ABDUL JABBAR

Date: 22 December 2011

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