UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF DIFFERENT LEVELS OF IRON AND MOLYBDENUM ON NITROGEN FIXATION AND PHOSPHATE SOLUBILIZATION IN AEROBIC RICE

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By

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June 2019

Chair : Professor Radziah binti Othman, PhD
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Growth and yield of aerobic rice (*Oryza sativa* L.) is affected by macro and micro-elements in soil. Biological nitrogen fixation (BNF) and phosphate solubilization by microorganisms are alternatives to reduce the use of high chemical fertilizers in rice cultivation. Iron (Fe) and molybdenum (Mo) are part of nitrogenase enzyme and abundance or shortage of these elements may affect N_2-fixation as well as phosphate solubilization. The following studies aimed to determine i) the effect of different concentrations of Fe and Mo on growth of N_2-fixing bacteria (NFB) and P-solubilizing bacteria (PSB), ii) the effect of inoculation with NFB and PSB on growth of rice applied with different concentrations of Fe and Mo and iii) the effect of microbial inoculation with combined Fe and Mo on growth and nutrient uptake of aerobic rice. Studies were conducted in the laboratory and glasshouse conditions. Four concentrations of Fe (0, 2, 10, 50 ppm) and Mo (0, 0.05, 5, 10 ppm) were evaluated on growth of two *Bacillus* sp. strains in modified nitrogen free broth and nutrient broth. Bacterial enumeration was done at the end of incubation. In the following study, the same concentrations of Fe and Mo were applied to aerobic rice seedlings in sand culture. Seven days old seedling was inoculated with NFB and PSB each as single and combined inoculum and grown for 40 days. Modified Yoshida nutrient solution was applied to each pot twice per week. The best concentrations of iron and molybdenum from previous experiment were selected and evaluated on aerobic rice plant as a combined micronutrient and inoculated with a single and a combined NFB and PSB for 60 days. Inoculum and nutrient solution applied was similar to previous experiment. Results showed that different concentrations of Fe and Mo significantly affected NFB and PSB and P-solubilizing activity. Application of different concentrations of Fe and Mo and inoculated with NFB and PSB as single or combination significantly affected plant biomass, plant height, leaf chlorophyll content, leaf area index, root development, N and P content, and nutrient uptake of aerobic rice. Combination of Fe and Mo inoculated with single and combined NFB and PSB significantly affected plant biomass, plant height, leaf chlorophyll content, leaf area index, root development, N and P content, nutrient uptake and total bacterial population in rhizosphere and endophyte. In conclusion, application 50 ppm of Fe and 10 ppm of Mo along with combined NFB and PSB enhanced plant growth and uptakes of aerobic rice.

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

PENGARUH KEPEKATAN FERUM DAN MOLIBDENUM YANG BERBEZA PADA BAKTERIA PENGIKAT NITROGEN DAN PELARUT FOSFORUS TERHADAP PADI AEROB

Oleh

SITI NUR' AIN BTE SAMBUDIN

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Pertumbuhan dan hasil tanam padi aerob (*Oryza sativa* L.) dipengaruhi oleh unsur makro dan mikro dalam tanah. Penetapan nitrogen secara biologi (BNF) dan solubilisasi fosfat oleh mikroorganisma adalah alternatif untuk mengurangkan penggunaan baja kimia yang tinggi dalam penanaman padi. Ferum (Fe) dan molybdenum (Mo) adalah sebahagian daripada enzim nitrogenase dan lebihan atau kekurangan unsur-unsur ini boleh mempengaruhi penetapan N_2 serta larutan fosfat. Kajian berikut bertujuan untuk menentukan i) kesan kepekatan yang berbeza Fe dan Mo terhadap pertumbuhan bakteria N_2-penetapan (NFB) dan bakteria pelarut fosfat (PSB), ii) kesan inokulasi NFB dan PSB secara individu atau gabungan pada pertumbuhan padi aerob dengan kepekatan Fe dan Mo yang berbeza dan iii) kesan inokulasi mikrob dengan gabungan Fe dan Mo mengenai pertumbuhan dan pengambilan nutrien beras aerobik. Kajian dijalankan di makmal dan rumah kaca. Empat kepekatan Fe (0, 2, 10, 50 ppm) dan Mo (0, 0,05, 5, 10 ppm) dinilai pada populasi dua *Bacillus* sp. strain dalam larutan bebas nitrogen yang diubahsuai dan larutan nutrien. Pengirasan jumlah populasi bakteria telah dilakukan pada akhir inkubasi. Dalam kajian berikut, kepekatan Fe dan Mo yang sama digunakan untuk benih padi aerob dalam medium pasir. Anak benih berusia tujuh hari telah disuntik dengan NFB dan PSB masing-masing secara tunggal atau gabungan dan ditanam selama 40 hari. Larutan nutrien Yoshida yang telah diubah suai diberikan pada setiap tanaman dua kali seminggu. Kepekatan Fe dan Mo yang terbaik dari eksperimen terdahulu telah dipilih sebagai mikronutrien gabungan dan diinokulasi dengan NFB dan PSB secara tunggal atau gabungandan

ditanam selama 60 hari. Cara inokulasi bakteria dan larutan nutrien adalah serupa dengan eksperimen terdahulu. Hasil kajian menunjukkan bahawa kepekatan Fe dan Mo mempengaruhi populasi NFB, PSB dan aktiviti pelarut fosfat. Penggunaan kepekatan Fe dan Mo yang berlainan dan inokulasi NFB dan PSB secara tunggal atau gabungan juga mempengaruhi biomas tumbuhan, ketinggian tumbuhan, kandungan klorofil dalam daun, indeks luas permukaan daun, perkembangan akar, kandungan N dan P, dan pengambilan nutrien padi aerob. Gabungan Fe dan Mo yang digabungkan dengan NFB dan PSB secara tunggal dan gabungan juga mempengaruhi biomas tumbuhan, ketinggian tumbuhan, kandungan klorofil dalam daun, indeks luas permukaan daun, perkembangan akar, kandungan N dan P, pengambilan nutrien dan jumlah populasi bakteria dalam rhizosphere dan endophyte. Sebagai kesimpulan, penggunaan 50 ppm Fe dan 10 ppm Mo bersama dengan gabungan.

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

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LIST OF ABBREVIATIONS

cfu	Colony forming unit
PGPR	Plant growth promoting rhizobacteria
NFB	Nitrogen fixing bacteria
PSB	Phosphate solubilizing bacteria
NA	Nutrient agar
NBRIP	National Botanical Research Institute's phosphate growth medium
SPAD	Soil Plant Analysis Division: Chlorophyll Content
DAT	Day after transplanting
SAS	Statistical analysis software
ANOVA	Analysis of Variance
N	Nitrogen
P	Phosphorus
Fe	Iron
Mo	Molybdenum

CHAPTER 1

INTRODUCTION

Aerobic rice cultivation is a new technology in rice culture where it is cultivated in well drained, non-puddled and non-saturated soil. Other than water supply, plant needs adequate nutrient for its growth and high yields. Micronutrient is important for plant growth and development as similar as importance of macronutrients and imbalanced in any of these micro-elements such as iron (Fe) and molybdenum (Mo) may retard its growth and development (Das, 2014). According to Fan et al. (2012) the shift from anaerobic to aerobic cultivation caused changes in gas content in the soil where it will lead to changes in nutrient dynamics and decrease bioavailability of micronutrients especially iron (Fe). Mongon et al. (2017) stated that the growth and development of rice is dependable on availability of essential micronutrient such as Fe. Iron reduction is a serious problem in aerobic cultivation because it is important in respiration and photosynthesis and the insufficient leads to interveinal chlorosis and reduced yield (Sarma et al., 2018). According to Bala and Hossain (2008) the main reason of low yield in rice cultivation is due to imbalance fertilizer or lack of soil micronutrients, especially Mo. Molybdenum plays an important role in nitrogen metabolism, protein synthesis and the development of the reproductive parts of rice plant (Das, 2014). This micronutrient is essential for most organisms including plants (Graham and Stangoulis, 2015) and bacteria (Williams and Frausto da, 2002). The Mo and Fe are essential elements of nitrogenase enzyme which is responsible for biological nitrogen fixation (Khan et al., 2014). The nitrogenase enzyme consists of two proteins: Fe protein (component containing iron and protein) and Mo-Fe protein (component containing molybdenum, iron and protein) (Hageman and Burris, 1978). The bacteria responsible for nitrogen fixation are known as diazotrophs and they encode the nitrogenase, enzyme complex that catalyses the conversion of N_2 gas to ammonia (Santi et al., 2013).

Beneficial soil microorganisms enhanced plant nutrient requirement through a wide range of biological processes including the transformation of unavailable nutrients into available forms for plant uptake (Babalola and Glick, 2012). The nitrogen fixing bacteria (NFB) helps in the conversion of dinitrogen gas into ammonium and nitrate (Naher et al., 2013) and phosphate solubilizing bacteria (PSB) solubilize the insoluble phosphate into available form for plant uptake (Bhattacharyya and Jha, 2012). The use of beneficial microorganisms such as NFB and PSB in agriculture can help to reduce the requirement of N and P fertilizers and reduce environmental problems caused by leaching and precipitation of chemical fertilizer in the soil (Naher et al., 2013; Othman and Panhwar, 2014).

Currently, there are insufficient information available on the role of Fe and Mo in the N_2-fixation and phosphate solubilization activities by N_2-fixing and phosphate solubilizing bacteria. Hence, this study was conducted to determine:

1. The effect of different concentrations of Fe and Mo on growth of N_2-fixing and phosphate solubilizing bacteria.
2. The effect of inoculation with N_2-fixing and phosphate solubilizing bacteria on growth of rice applied with different concentrations of Fe and Mo.
3. The effect of microbial inoculation with combined Fe and Mo on growth and nutrient uptake in aerobic rice.

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LIST OF PUBLICATIONS

Journal papers:

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Conference/Congress:

Siti Nur Ain Sambudin, Radziah Othman and Mohd Khanif Yusop (2013). Effects of different levels of iron (Fe) and molybdenum (Mo) on growth of rice inoculated with nitrogen fixing bacteria. In: *International Conference on Crop Improvement, 25- 26 November 2013.* Equatorial Hotel, Bangi, Selangor, Malaysia. (Poster Presentation)

Siti Nur Ain Sambudin, Radziah Othman and Mohd Khanif Yusop (2015). Effect of iron and molybdenum on growth of rice inoculated with phosphate solubilizing bacteria. In: *Soil Science Conference of Malaysia*. The Everly Hotel, Putrajaya, Malaysia. (Best Poster Award)

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