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EFFECTS OF SELECTED HERBICIDES ON ACTIVITY OF Stenotrophomonas maltophilia AND GROWTH OF AEROBIC RICE, AND THEIR PERSISTENCE IN SOIL

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EFFECTS OF SELECTED HERBICIDES ON ACTIVITY OF *Stenotrophomonas maltophilia* AND GROWTH OF AEROBIC RICE, AND THEIR PERSISTENCE IN SOIL

By

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EFFECTS OF SELECTED HERBICIDES ON ACTIVITY OF *Stenotrophomonas maltophilia* AND GROWTH OF AEROBIC RICE, AND THEIR PERSISTENCE IN SOIL

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April 2015

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Aerobic rice cultivation has come up as a promising alternative for flood-irrigated rice system in terms of water use recently. Bio-fertilizer, consisting of beneficial N_2 fixing bacteria can promote crop production and nutrient uptake efficiency. Herbicides, the most succesfull chemical weed management approach in direct seeded rice may cause hazardous effects on plant and soil microbes when they come into contact. This study was conducted in laboratory and glasshouse conditions with the following objectives; (i) to determine the effects of three selected rice herbicides on growth and N_2 fixing activity of diazotrophic *Stenotrophomonas maltophilia* (Sb16) (ii) to determine the effects of Sb16 bacterial inoculation and application of three selected rice herbicides on growth of aerobic rice and the soil microbial and chemical properties (iii) to determine the effect of Sb16 on persistence of three selected rice herbicides in soil. The effects of paraquat, pretilachlor and 2, 4-D on growth and N_2 fixing activity of Sb16 and pH of Jensen N-free medium were determined *in-vitro* at every 24-h interval within 7 days of incubation period. The effects of Sb16 bacterial inoculation and application of paraquat, pretilachlor and 2, 4-D on growth of aerobic rice, soil microbial population and chemical properties and their persistence in sterilized and non-sterilized soil were determined under glasshouse condition. Results from *in-vitro* experiment showed the significant ($P \leq 0.05$) decrease of growth of Sb16 by 7.29 and 7.22 $\log_{10}$ cfu.mL^{-1} in samples amended with full and double doses of herbicides compared to control and half dose (7.37 $\log_{10}$ cfu.mL^{-1}). N_2 fixing activity of Sb16 significantly ($P \leq 0.05$) increased by 1.66 Nmol C_2H_4/mL/h with half dose of 2, 4-D compared to control (0.58 Nmol C_2H_4/mL/h). The growth parameters of aerobic rice, population of total bacteria and diazotrophs and soil chemical properties showed higher values in inoculated samples treated with herbicides compared to non-inoculated samples. The longest half lives of paraquat in sterilized and non-sterilized soil were

recorded by 866.38 and 198.3 days in non-inoculated samples treated with double dose. Half lives of 58.74 and 99.01 days were obtained in inoculated and non-inoculated sterilized soil samples, respectively treated with double dose of pretilachlor. Sb16 is recommended as a beneficial biofertilizer in aerobic rice cultivation. Sb16 can promote the aerobic rice production and nutrient uptake in soil applied with paraquat, pretilachlor and 2, 4-D at their recommended dose. Sb16 might be useful in decontamination of aerobic rice field soil applied with pretilachlor and 2, 4-D at their recommended dose under natural soil conditions.

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

KESAN-KESAN RACUN RUMPAI TERPILIH PADA AKTIVITI *Stenotrophomonas maltophilia* DAN PERTUMBUHAN PADI AEROB, DAN KETAHANAN DALAM TANAH

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Penanaman padi aerob telah menjadi alternatif yang baik terhadap sistem penanaman anaerob dalam kecekapan penggunaan air. Bio-fertilizer, mengandungi bakteria pengikat N_2 yang meningkatkan hasil tanaman dan kecekapan pengambilan nutrien. Racun rumpai, bahan kimia yang paling Berjaya dalam pengawalan rumpai dalam kaedah tabor terus padi boleh mendatangkan kesan yang berbahaya kepada tumbuhan dan mikrob tanah apabila berlaku sentuhan. Kajian ini teluh dilakukan di lab dan rumah kaca dengan objektif berikut: i) Untuk mengkaji kesan tiga jenis racun rumpai yang terpilih terhadap pertumbuhan dan aktiviti N_2 bakteria *Stenotrophomonas maltophilia* (Sb16) ii) Untuk mengkaji kesan inokulasi Sb16 dan aplikasi tiga jenis racun rumpai terhadap pertumbuhan padi aerob dan mikrob tanah dan sifat-sifat kimia iii) Untuk mengkaji kesan Sb16 terhadap ketahanan racun rumpai dalam tanah. Kesan paraquat, pretilachlor dan 2, 4-D terhadap pertumbuhan dan aktiviti pengikatan N_2 oleh Sb16 dan pH media Jensen bebas N ditentukan secara in-vitro pada setiap 24 jam selamasa dalam masa 7 hari inkubasi. Kesan inokulasi Sb16 dan aplikasi paraquat, pretilachlor serta 2, 4-D terhadap pertumbuhan padi aerob, populasi mikrob tanah dan sifat-sifat kimia dan ketahanan dalam tanah steril dan tak-steril ditentukan dalam keadaan rumah kaca. Keputusan kajian in-vitro mendapati kesan yang signifikan ($P \leq 0.05$) pengurangan pertumbuhan Sb16 sebanyak 7.29 dan 7.22 Log_{10} cfu.mL^{-1} dalam sampel yang mengandongi dos penuh dan dua kali gand dos penuh racun rumpai berbanding kawalan dan dos separon (7.37 Log_{10} cfu.mL^{-1}. Aktiviti pengikatan N_2 oleh Sb16 meningkat ($P \leq 0.05$) sebanyak 1.66 Nmol C_2H_4/mL/h dengan separuh dos 2, 4-D berbanding kawalan (0.58 Nmol C_2H_4/mL/h). Parameter pertumbuhan padi aerobic, populasi keseluruhan bacteria dan sifat-sifat kimia tanah menunjukkan peningkatan terhadap sampel yang diinokulasi oleh bacteria dan racun rumpai berbanding sampel yang tidak diinokulasi. Separuh hayat terpanjang paraquat dalam tanah steril dan

tidak disteril mencatatkan sebanyak 866.38 dan 198.3 hari. Sampel yang tidak di steril dan di rawat dengan dos dua kali ganda. Separuh hayat bagi sampel yang diinokulasi dan tidak diinokulasi tanah steril mencatatkan separuh hayat selama 58.74 dan 99.01 hari. Keduanya di rawat dengan dos dua kali ganda pretilachlor. Sb16 adalah disyorkan sebagai biobaja bermanfaat dalam penanaman padi aerobik. Sb16 boleh menggalakkan pengeluaran padi aerobik dan pengambilan nutrien dalam tanah digunakan dengan paraquat, pretilachlor dan 2, 4-D pada dos yang mereka cadangkan. Sb16 mungkin berguna dalam dekontaminasi daripada aerobik tanah sawah digunakan dengan pretilachlor dan 2, 4-D pada dos mereka disyorkan di bawah keadaan semula jadi tanah.

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I certify that a Thesis Examination Committee has met on 17 April 2015 to conduct the final examination of Armita Nahi on her thesis entitled "Effects of Selected Herbicides on Activity of *Stenotrophomonas maltophilia* and Growth of Aerobic Rice, and Their Persistence in Soil" in accordance with the Universities and University College Act 1971 and the Constitution of the University 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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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ARA	Acetylene Reduction Assay
BDL	Below Detectable Level
BNF	Biological Nitrogen Fixation
CEC	Cation Exchange Capacity
Cfu	Colony Forming Unit
CRD	Completely Randomized Design
DAA	Days After Application
DAT	Days After Transplanting
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonucleic acid
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
NA	Nutrient Agar
Nfb	Nitrogen Free Broth
OD	Optical Density
OM	Organic Matter
PDA	Potato Dextrose Agar
PGPB	Plant Growth Promoting Bacteria

PGPR	Plant Growth Promoting Rhizobacteia
RCBD	Randomized Complete Block Design
RNA	Ribonucleic acid
SAS	Statistical Analysis System
SD	Standard deviation
SPAD	Soil Plant Analysis Division: Chlorophyll meter
UV- VIS- NIR- Spectrophotometric	Ultraviolet- Visible-Near Infrared Reflection Spectrophotometric
WHC	Water Holding Capacity
2, 4-D	2, 4-dichlorophenoxy acetic acid

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is considered the chief food for majority of the population globally (Chauhan and Johnson., 2011). Water plays a significant role in rice production system. Therefore, water-efficient strategies are required to be applied. Aerobic rice system has emerged as the most promising water-wise approach in rice culture system where the crop is cultivated through direct seeding on non-puddled and non-swamped soils (Anwar *et al.*, 2010). Aerobic rice is more inclined toward sustainable agriculture due to lower water requirement during cultivation. In aerobic rice cultivation, lower yield is anticipated compared to flooded rice due to poor water access and seed germination, unfavorable crop stand, high weed competition and nutrients stress.

The use of beneficial microorganisms would further increase the efficiency of natural resources in rice cultivation. In recent decades, soil-plant-microbe interaction has turned out a significant issue in sustainable agriculture system. Many kinds of microorganism have been known to play significant role in plant growth and development, as they inhabit in soil, especially rhizosphere. Therefore, altering the rhizosphere microflora by seed, soil or root inoculation with specific organism is a possible alternative. The use of diazotrophs (N_2 fixing bacteria) as bio-fertilizer could reduce the need for inorganic fertilizer (Sofi and Wani., 2007). A diazotrophic strain Sb16 (previously isolated from rice rhizosphere) as an inoculant was introduced to soil and formed natural association with rice plant (Naher et al., 2009). Investigations have pointed out that indigenous microflora play a key role in the establishment of the introduced microbes.

On the other hand, aerobic rice, causes higher weed pressure (Balasubramanian and Hill., 2002) compared to flood irrigated rice. Hence, an effective weed management approach in aerobic rice cultivation is required toward sustainable weed management. Chemical control is considered the most efficient, cost-effective and practical weed management approach among various alternatives including physical, cultural, biological and chemical control (Hussain *et al*., 2008). The herbicide has been recommended as a viable alternative to hand weeding in direct seeded rice by many researchers working on weed management (Anwar *et al*., 2012). Herbicides might develop a wide range of toxic side effects to the environment despite of their benefits. Since they can cause a contamination in groundwater due to leaching or become immobile and persist on top soil, their fate in soil is gaining a great importance (Ayansina *et al*., 2003). Most herbicides applied will ultimately reach to soil where they come into contact with various microflora performing biochemical transformations related to the plant mineral nutrition. The soil microbes are the first to be influenced directly or indirectly by the herbicides. As they respond immediately to stressful conditions, they are considered as more suitable indicators than other organisms or chemical parameters (Filip., 2002).

Herbicides applied to soil may be removed by soil indigenous microorganisms as well as inoculated microbe, which would lead to an enhancement in microbial population and root and shoot biomass. Microbes could develop extremely well on herbicide compounds in the soil by utilizing them as nutrients and energy sources. Some strains of microbes are capable of utilizing the chemicals and change them to useful compounds, leading to multiplication of the microbes. This will result in an improvement of crop production through immunizing the plant from hazards of herbicides. High amounts of herbicides are applied to soil, as farmers have perceived their benefit. They may come in contact with non-target organisms and show toxicity to soil microflora and plants. The biofertlizer containing bacteria can protect the plant from herbicidal effect through either the improvement of growth of plant or degradation of herbicides in soil. There is no study on the effects of herbicides and N_2 fixing bacteria on aerobic rice. Moreover, there is very little information on effect of introduced bacteria on persistence of herbicides applied to the soil under natural conditions. Therefore this study was conducted with following objectives:

1. To determine the effects of three selected rice herbicides on growth and N_2 fixing activity of *Stenotrophomonas maltophilia* (Sb16)

2. To determine the effects of Sb16 and three selected rice herbicides on growth of aerobic rice, microbial population and chemical properties of soil

3. To determine the effect of Sb16 on persistence of three selected rice herbicides in soil cropping with aerobic rice

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