UNIVERSITI PUTRA MALAYSIA

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EVALUATION OF CHITOSAN FOR BLAST DISEASE SUPPRESSION IN MR 219 RICE VARIETY

BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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ENDORSEMENT

This project report entitled "Evaluation of chitosan for blast disease suppression in MR 219 rice variety" is prepared by Farhana Binti Burhanudin and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

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LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
cm	Centimeter
mm	Millimeter
kg	Kilogram
g	Gram
mg	Milligram
ml	Milliliter
µl	Microliter
mt	Metric ton
ha	Hectare
DNA	Deoxyribo nucleic acid
mRNA	Messenger Ribo nucleic acid

ABSTRACT

Rice (*Oryza sativa* L.) from family Poaceae is the most important grain crop in Malaysia. Blast disease caused by the fungus *Pyricularia oryzae* is an important disease that affects rice production. Chitosan is a nontoxic biopolymer derived by deacetylation of chitin that shows antifungal effect against several pathogens hence, was successful in controlling diseases of several plants. However, chitosan is not known to suppress blast disease on MR 219 rice variety. Thus, this study was conducted, 1) to determine the effect of chitosan on mycelial growth of *P. oryzae in vitro* and 2) to assess the effect of different chitosan concentrations on disease development. Poison Agar Assay was used to test four chitosan concentrations (1, 2, 3 and 4%) and control *in vitro*. The radial growth was measured in the 14 days after inoculation resulted a highly significant difference among percent inhibition between all treatments. *P. oryzae* growth was totally inhibited at 4% concentration, however, the inhibiting effect was relatively low at 2 and 1% concentrations. The result showed a highly significant difference between all treatments where chitosan at 4% concentration caused a 19.40% disease incidence (DI) while 10.95% disease severity (DS) on rice plants compared to 84.08% DI and 71.15% DS in the control plants. Present results inferred that chitosan could act as an alternative natural compound in controlling blast disease on MR 219 rice variety.

ABSTRAK

Padi (*Oryza sativa* L.) dari keluarga Poaceae adalah tanaman bijirin yang paling penting di Malaysia. Penyakit karah disebabkan oleh kulat *Pyricularia oryzae* adalah penyakit penting yang menjejaskan pengeluaran padi. Kitosan adalah biopolimer bukan toksik yang diperolehi oleh proses diasetil kitin yang menunjukkan kesan antikulat terhadap beberapa patogen oleh sebab itu ia telah berjaya mengawal penyakit pada beberapa tanaman. Namun begitu, tidak diketahui sama ada kitosan boleh menyekat penyakit karah pada varieti padi MR 219. Oleh itu, kajian ini dijalankan, 1) untuk menentukan kesan kitosan terhadap pertumbuhan miselium *P. oryzae in vitro* dan 2) untuk menilai kesan kepekatan kitosan yang berbeza terhadap perkembangan penyakit. Ujian Agar Racun telah digunakan untuk menguji empat kepekatan kitosan (1, 2, 3 dan 4%) dan 0% sebagai kawalan secara *in vitro*. Pertumbuhan radius miselium diukur dalam masa 14 hari selepas inokulasi dan keputusan menunjukkan perbezaan yang amat ketara bagi peratus perencatan antara semua rawatan. Pertumbuhan *P. oryzae* terencat sepenuhnya pada kepekatan 4%, walaubagaimanapun, kesan merencat adalah lebih rendah pada kepekatan 2 dan 1%. Keputusan menunjukkan perbezaan yang amat ketara antara semua rawatan yang mana kitosan pada kepekatan 4% menyebabkan 19.40% kejadian penyakit manakala 10.95% keterukan penyakit pada tanaman padi MR 219 berbanding dengan 84.08% kejadian penyakit and 71.15% keterukan penyakit pada tanaman kawalan. Keputusan kajian meyimpulkan bahawa kitosan boleh bertindak sebagai alternatif sebatian semula jadi untuk mengawal penyakit karah pada varieti padi MR 219.

CHAPTER 1

INTRODUCTION

Oryza sativa L. from family Poaceae is the most important grain crop in Malaysia. Rice becomes carbohydrate and protein source for people, especially in developing countries (FOA, 2004). According to the statistics published by the Sub-Sector Food Crop, Malaysia's rice cultivation area in 2013 is 688,207 hectares, while the rice yield is 3,817 kg/ha (DOA, 2013). Fasahat *et al.* (2012) claimed that more than 90% of the rice cultivation area in Malaysia was grown with MR 219 due to their high yield potential.

Malaysian *Indica* variety, MR 219 resulted from a cross between MR 137 and MR 151 released by Malaysian Agricultural Research and Development Institute (MARDI), in January 2001 (Alias, 2002). MR 219 was moderately resistance against blast disease when it was first developed. However, new blast pathotypes have been developed after a long period cultivation, seeds regeneration as well as environmental factors that lead to the blast disease susceptibility (Miah *et al.*, 2014).

Blast disease caused by fungus *Pyricularia oryzae* is one of the important disease that affecting rice production in Malaysia. The existence of this serious disease is threatening the rice industry, because of its large distribution and high incidence under favorable conditions (Ou, 1985). Koutroubas *et al.* (2009) claimed this disease

caused about 10-20% yield reduction in susceptible varieties, but the loss may be up to 80% in severe situations.

Therefore, various commercial fungicides which provide superior disease suppression and greater user convenience have been used to control rice blast disease since the early part of the century (Froyd and Froeliger, 1994). Despite of that, Kuyek *et al.* (2000) stated most fungicides offerings the best disease management is expensive for the resource poor farmers of Asia. Moreover, the proportion of the fungicide that is not absorbed by the plants will be moved and transferred to the environment through wind, water and soil (Ahmad *et al.*, 2014).

According to US EPA (2012) Fuji-one contains Isoprothiolane 40% EC is classified as "moderately toxic" in terms of fish toxicity while Score contains Difenoconazole 25% EC is categorized as "moderately toxic" in terms of algae eco-toxicity (Ahmad *et al.*, 2014). The latest fungicide, Filia contains Tricyclazole and Propiconazole launched by Syngenta Malaysia in Pendang, Kedah for the control of neck blast. Snelder *et al.* (2008) claimed the increment of fungicide use will raise the risks to human health and the environment in the near future.

The usage of natural compound is encouraged for blast disease management to meet economic viability and safety requirement for environment. Chitosan is nontoxic polymer and partially deacetylated polymer of N-acetyl glucosamine (GlcNAc) has been widely proposed for its potential in various areas including food (Shahidi and Synowiecki, 1991), agriculture (Yamada *et al.*, 1993), cosmetics (Majeti and Kumar

2000) and pharmaceutical (Kato *et al*., 2003). Bio-waste of aquatic organisms was used for chitosan production commercially and terrestrial crustaceans, terrestrial organisms while mushrooms introduced as alternative sources (Nwe *et al.*, 2004).

Li *et al.* (2009) reported that chitosan successfully inhibited *Fusarium sulphureum* growth and controlled potato tuber dry rot disease. In addition, chitosan showed a strong antifungal effect against *Rhizoctonia solani* as well as reduced the sheath blight severity of rice seedlings irrespective to the mode of application in a greenhouse (He *et al.*, 2012). On the other hand, Algam and Elwagia (2015) stated that early blight disease was well controlled by using chitosan and increased growth parameter of tomato.

The opportunity for using organic compounds to control blast disease should be developed as an alternative approach because the chemical application for rice blast control give rise to environmental concerns after a long practice which also lead to the health issue. Up to now, much information about the antifungal activity of chitosan on *P. oryzae* have been generated from previous studies (Rodrı´guez *et al.*, 2007; Suprapta *et al.*, 2014), however, chitosan is not known whether it can suppress blast disease on rice, particularly MR 219 rice variety.

Thus, the objectives of this study were, 1) to determine the effect of chitosan on mycelial growth of *P. oryzae in vitro* and 2) to assess the effect of different chitosan concentrations on disease development.

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