Malaysian Journal of Microbiology, Vol 11(1) 2015, pp. 20-26
http://dx.doi.org/10.21161/mjm.61714

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Susceptibility of Malaysian rice varieties to *Fusarium fujikuroi* and *in vitro* activity of *Trichoderma harzianum* as biocontrol agent

Wan Nur Ain Wan Abd Halim[1], Azmi Abd Razak[2], Jambari Ali[3] and Nur Ain Izzati Mohd Zainudin[4*]

[1, 3, 4]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Malaysian Agricultural Research and Development Institute, Beg Berkunci No. 203, Pejabat Pos Kepala Batas, 13200 Seberang Perai, Pulau Pinang, Malaysia.
Email:, ainizzati@upm.edu.my

Received 21 February 2014; Received in revised form 6 October 2014; Accepted 21 October 2014

ABSTRACT

Aims: Bakanae disease on rice has been widely distributed in all countries where rice is grown commercially, especially in Asian countries including Malaysia. As an alternative measure in controlling *Fusarium fujikuroi*, two approaches have to be adapted i.e. by using resistant varieties and biocontrol agents as reported in the present study.
Methodology and results: A total of 31 Malaysian rice varieties were used in screening and results showed that variety MR211 was the most susceptible and MR220 was slightly susceptible. Out of 60 isolates of *Trichoderma harzianum* isolated from soils in Malaysia and tested against the pathogen under *in vitro* condition, 13 isolates showed high percentage of inhibition (PIRG > 60%). All isolates of *T. harzianum* showed that the PIRGs were significantly different at $p \leq 0.05$ with those of control plates.
Conclusion, significance and impact of study: Biocontrol agent and resistant variety are better alternative for controlling plant diseases. We found a variety MR220 was slightly susceptible, but none of tested varieties is resistant towards pathogen of bakanae disease. *T. harzianum* has the ability to inhibit the growth of *F. fujikuroi* (T3068P) under *in vitro* condition. The findings of the Malaysian susceptible/resistant variety and potential *T. harzianum* isolate as a biocontrol agent of bakanae are important for future tests in the plant house and field trials.

*Keywords: S*creening, *Fusarium fujikuroi*, bakanae, *Trichoderma harzianum*, rice variety, biocontrol

INTRODUCTION

Bakanae of rice caused by *Fusarium fujikuroi* Nirenberg, was first described in Japan. The disease is also known as foot rot or elongation disease and is widely distributed in all rice-growing areas, especially in Asia. In Malaysia, it was seriously observed in 1985 during the second rice-planting season (Saad, 1986). If the disease becomes an outbreak due to lack of prevention through early detection, there will therefore be problems to worldwide food resources, especially for the majority of Asian.

Bakanae is a seedborne disease; thus, by sowing the seeds using direct casting in infested soil will be giving first infection onto the seedlings. The infected seedlings usually die at advanced stage of infection. Some infected seedlings could also be stunted and chlorotic. The classic and most conspicuous symptom of the bakanae disease is abnormal elongation; this symptom can be seen from a distance in fields and seedbeds. The affected plants may several inches taller than normal plants, thin, yellowish green, and may produce adventitious roots at the lower nodes of the culms (Nur Ain Izzati *et al.*, 2008a). The pathogen has ability to produce the growth hormone gibberellin, which is responsible for abnormal elongation (Nur Ain Izzati *et al.*, 2008b). The diseased plants bear few tillers and leaves dry-up quickly. The affected tillers usually die before reaching maturity stage (Karov *et al.*, 2009).

In Malaysia, there are lacks of information on Malaysian rice varieties that are resistant to bakanae disease. Therefore, this study was conducted to distinguish the resistance of rice varieties against the disease. The indiscriminate use of chemical fungicides to control the rice bakanae pathogen may lead to the appearance of new resistant strains and increased the toxicological lead to the environment (Hajieghrari *et al.*, 2008). Therefore, biocontrol agents are useful as an alternative method beside fungicide use in nurseries and rice field. Several organisms such as species of *Trichoderma*, *Bacillus* and *Streptomyces* have been demonstrated to be effective as biocontrol agents.

Several isolates of *Trichoderma* have been developed as biocontrol agents against fungal plant pathogens (Howell, 2003; Harman, 2006; De Souza *et al.*, 2008; Suhaida and Nur Ain Izzati, 2013). In addition, Howell (2003) reported that *Trichoderma* were successfully

*Corresponding author

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

applied as biocontrol agents against several plant diseases in commercial agriculture. De Souza *et al.* (2008) stated that *Trichoderma* species is a mycoparasite that has proven effective as a biocontrol agent against a range of important plant pathogens.

Trichoderma species is a filamentous fungus that is highly interactive in root, soil and foliar environments. It can produce a wide range of antibiotic substances (Sivasithamparam and Ghisalberti, 1998) and they parasitize other fungi (De Souza *et al.*, 2008). *Trichoderma* could easily detect the other fungi and grow rapidly towards them. *Trichoderma* species have the ability to compete with soil microorganisms for space and nutrients consumption, in particular. *Trichoderma* species have also the ability to inhabit soilborne pathogens and grows in association with plant roots (Harman, 2000). These mycoparasites secreted degrading enzymes on the cell walls of fungal pathogens and subsequently function as elicitors of plant protection mechanisms (Kubicek *et al.*, 2001; Woo *et al.*, 2002). In this study, the biological potential of some *Trichoderma* isolates were evaluated against *F. fujikuroi* under *in vitro* conditions.

MATERIALS AND METHODS

Pathogen and rice varieties

In previous study, the pathogenicity test was conducted by using a rice variety MR 211 (Nur Ain Izzati *et al.*, 2008a). The result showed, among 34 isolates of five species of *Fusarium*, the isolate of *F. fujikuroi* T3068P was highly virulent to rice and caused bakanae disease (Nur Ain Izzati *et al.*, 2008a). Thirty-one varieties of rice obtained from Malaysian Agricultural Research and Development Institute (MARDI), Seberang Perai, Pulau Pinang were used to screen the resistant variety against *F. fujikuroi*.

Conidial suspension and artificial inoculums

Isolate T3068P was cultured on potato dextrose agar (PDA) and incubated for 7 days. The plates were flooded with sterile water and the conidial suspensions were pooled before adjusted to 1×10^6 conidial/mL. The seeds were soaked in 50 mL spore suspension for 12 h but the control (non-inoculated) seeds were soaked in 50 mL sterile water. Inoculated and control seeds were sown on rice field soils in plastic trays (38×28×10 cm). Fifteen seeds were planted in each tray of triplicates and arranged in complete randomized design (CRD) in the plant house at University Agricultural Park, Universiti Putra Malaysia. The seedlings were irrigated daily with the tap water and the fertilizer 15N:15P:15K was given every 10 days.

Development of symptoms and Disease Severity Index (DSI)

The height of the seedlings and the external disease symptoms were observed continuously based on the disease scale of 0 to 4 as shown in Table 1. Disease Severity Index (DSI) was calculated as follows:

$$DSI = \frac{\sum \text{numbers of plants in the specific scale} \times \text{disease scale}}{\text{Total number of plants observed}}$$

$$DSI = \frac{\sum (n\times0) + (n\times1) + (n\times2) + (n\times3) + (n\times4)}{\text{Total number of plants observed}}$$

DSI was calculated and all the data analyzed by using SPSS programme version 17.0. The resulting DSIs were classified as follows with slight modifications for bakanae disease of rice (IRRI, 2002).

DSI	Susceptibility reaction
0 - 0.29	Resistant
0.30 - 1.99	Slightly susceptible
2.00 - 2.99	Moderately susceptible
3.00 - 4.00	Susceptible

Table 1: Disease scale and disease symptoms for seedling scoring (adapted from Nur Ain Izzati *et al.*, 2008a).

Disease scale	Disease symptoms
0	healthy and uninfected plants (no external symptoms)
1	normal growth but leaves beginning to show yellowish-green content
2	abnormal growth (shorter or taller than normal), thin and yellowish green leaves
3	abnormal growth (shorter or taller than normal), chlorotic, thin and brownish leaves
4	seedlings with fungal mass on the surface of infected plants or died

Isolation of *Trichoderma* species

Trichoderma isolates used in whole trials were obtained from soil samples in Selangor and Terengganu, Malaysia. Isolation of fungal was done using the soil dilution-plating technique following Nur Ain Izzati and Faridah (2008). One mL from 10^{-3}, 10^{-4}, 10^{-5} soil dilutions were spread on Rose Bengal Agar (RBA) and incubated for 7 days at room temperature (28±1 °C). The colonies of *Trichoderma* species were subcultured and single-spored on PDA to obtain the pure isolate. All isolates were identified based on morphological characteristics according to Harman and Kubicek (1998) and Rahman *et al.* (2011).

Challenging *T. harzianum* isolates against *F. fujikuroi* isolate T3068P

Six mm diameter of colonies of *T. harzianum* isolates and T3068P were placed 5 cm apart on PDA. Plates were incubated at room temperature for 5 days. The control plates inoculated with T3068P without *T. harzianum* isolate. Radial growth of both fungal was measured using the following formula:

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Percentage of inhibition growth rate (PIRG) = (R1-R2)/R1
R1= Diameter (cm) of colony growth of *F. fujikuroi* in control
R2= Diameter (cm) of *F. fujikuroi* in antagonist-tested plate

PIRG was calculated and all the data analyzed by using SPSS programme version 17.0. The descriptive assessment for the antagonistic activity was converted as follows (Soytong, 1998):

+ + + + = very high antagonistic activity (>75 PIRG)
+ + + = high antagonistic activity (61-75 PIRG)
+ + = moderate antagonistic activity (51-60 PIRG)
+ = low antagonistic activity (<50 PIRG)
- = no antagonistic activity

RESULTS AND DISCUSSION

Development of symptoms and disease severity index (DSI)

Based on screening study, no Malaysian varieties tested were resistant to bakanae disease, but the most slightly susceptible variety was MR220. Seedlings inoculated with conidial suspensions of isolate T3068P showed the typical symptoms of bakanae disease with abnormal growth, thin, yellowish green and produced adventitious roots at the lower nodes of the culms (Figure 1A-D). The DSI for all varieties increased after day 10 to 30 (Table 2). The DSI was significantly ($p \leq 0.05$) different between days and varieties. The highest DSI was recorded from MR211 seedlings at day 40 which is 3.20 followed by MR27 (2.93) and MR123 (2.80). The lowest DSI was MR220, which is 0.68 and followed by MR219 (0.85) and MR185 (0.90). All the DSI for each variety were significantly different at $p \leq 0.05$ from the control seedlings (Figure 2). The most critical period for the infection occurred in the first 3 days during germination of seeds. This is because of secretion of amino acids and sugars that act as rich energy substrates for the effectively growing pathogen. Hence, the infected plants were taller than control after 5 to 40 days and DSI was significantly ($p \leq 0.05$) different between days.

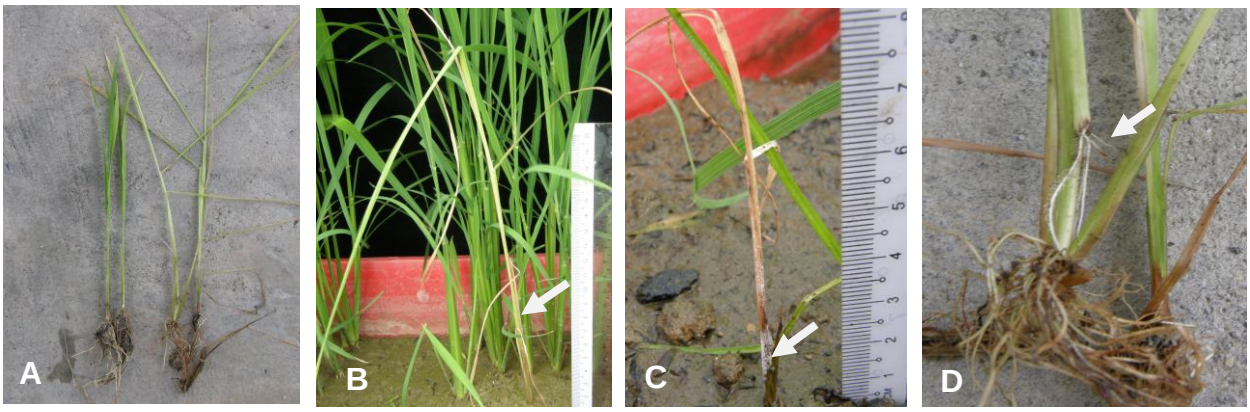

Figure 1: Typical symptoms of bakanae disease. A, normal and healthy plants (n) and infected seedlings showing abnormal elongation (i); B, infected seedling, showing abnormal elongation, thin and yellowish leaves (arrow); C, pinkish fungal mass above water level on dried-up seedling (arrow); D, infected tiller produced wiry (stiff) adventitious roots (arrow).

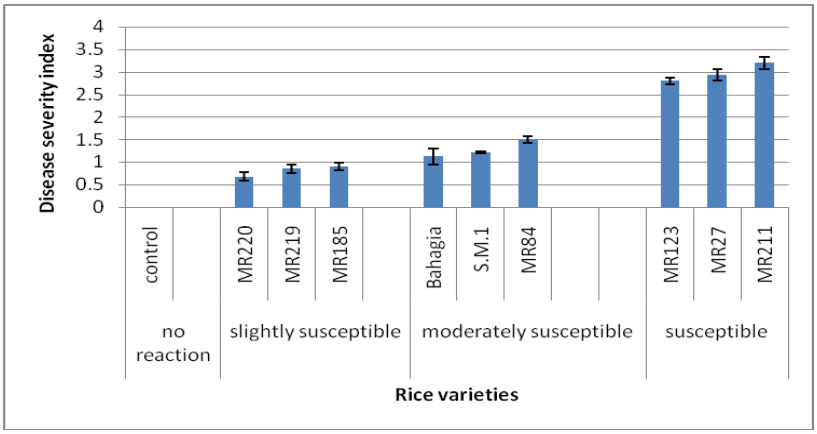

Figure 2: Comparison DSI value of inoculated and control rice varieties at day 40 after inoculation.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: Disease Severity Index (DSI) of inoculated and control rice seedlings at different days after inoculation (sowing).

No	Variety	[+] Disease Severity Index (DSI) at different days					[++] Susceptibility reaction
		5d	10d	20d	30d	40d	
1	CONTROL	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	No- reaction
2	MR220	0^{a}	0.26^{b}	0.46^{b}	0.46^{b}	0.68^{b}	Slightly susceptible
3	MR219	0^{a}	0.38^{b}	0.48^{b}	0.85^{cd}	0.85^{bc}	Slightly susceptible
4	MR185	0^{a}	0.38^{b}	0.50^{bc}	0.68^{bc}	0.90^{bcd}	Slightly susceptible
5	Ria	0^{a}	0.43^{bc}	0.75^{cd}	1.05^{def}	1.05^{cde}	Slightly susceptible
6	MR1	0^{a}	0.42^{bc}	0.68^{bcd}	0.91^{cde}	1.07^{cde}	Slightly susceptible
7	MR52	0^{a}	0.43^{bc}	0.78^{d}	0.93^{cde}	1.10^{de}	Slightly susceptible
8	Bahagia	0.39^{bcd}	0.59^{cde}	0.86^{de}	1.13^{ef}	1.13^{de}	Slightly susceptible
9	MR232	0.20^{ab}	0.36^{b}	0.51^{bc}	0.70^{c}	1.13^{de}	Slightly susceptible
10	MR10	0.23^{bc}	0.58^{cd}	0.83^{de}	1.10^{ef}	1.20^{ef}	Slightly susceptible
11	S.M.I	0.55^{defg}	0.78^{fgh}	0.89^{def}	1.22^{fg}	1.22^{ef}	Slightly susceptible
12	Mahsuri	0.63^{efgh}	0.94^{h}	1.40^{hi}	1.40^{ghi}	1.40^{fg}	Slightly susceptible
13	Jaya	0^{a}	0.59^{cde}	0.72^{bcd}	1.30^{fgh}	1.40^{fg}	Slightly susceptible
14	MRQ50	0.35^{bcd}	0.90^{gh}	1.05^{efg}	1.20^{fg}	1.41^{ef}	Slightly susceptible
15	S.M.II	0.47^{de}	0.63^{def}	0.83^{de}	1.30^{fgh}	1.48^{gh}	Slightly susceptible
16	MR7	0.63^{efg}	0.78^{efgh}	1.33^{gh}	1.50^{hij}	1.50^{ghi}	Slightly susceptible
17	MR84	0.53^{defg}	0.83^{gh}	1.40^{hi}	1.50^{hij}	1.50^{ghi}	Slightly susceptible
18	MR167	0.50^{def}	0.70^{defg}	1.13^{fgh}	1.13^{ef}	1.55^{ghi}	Slightly susceptible
19	Malinja	0.52^{defg}	0.90^{gh}	1.39^{hi}	1.39^{ghi}	1.68^{hij}	Slightly susceptible
20	MR106	0.50^{def}	0.80^{fgh}	1.33^{gh}	1.58^{ijk}	1.73^{hij}	Slightly susceptible
21	MR103	0.40^{bcde}	0.85^{gh}	1.13^{fgh}	1.43^{ghij}	1.75^{ij}	Slightly susceptible
22	Murni	0.73^{gh}	0.90^{gh}	1.32^{gh}	1.50^{hij}	1.82^{jk}	Slightly susceptible
23	MR71	0.53^{defg}	0.81^{fgh}	1.16^{gh}	1.46^{ghij}	1.87^{jk}	Slightly susceptible
24	MR81	0.73^{fgh}	0.83^{gh}	1.40^{hi}	1.78^{k}	2.00^{kl}	Moderately susceptible
25	MRQ74	0.43^{cde}	0.88^{gh}	1.28^{gh}	1.68^{ijk}	2.03^{kl}	Moderately susceptible
26	MR73	0.53^{defgh}	0.75^{defgh}	1.28^{gh}	1.68^{ijk}	2.13^{l}	Moderately susceptible
27	MR127	0.73^{fgh}	1.40^{ij}	1.65^{ij}	2.23^{l}	2.61^{m}	Moderately susceptible
28	MR159	0.45^{de}	0.85^{gh}	1.65^{ij}	2.25^{l}	2.68^{m}	Moderately susceptible
29	MR77	0.45^{de}	0.88^{gh}	1.65^{ij}	2.33^{l}	2.73^{mn}	Moderately susceptible
30	MR123	0.48^{de}	1.63^{k}	2.20^{k}	2.20^{l}	2.80^{mn}	Moderately susceptible
31	MR27	0.53^{defg}	0.93^{h}	1.83^{j}	2.80^{m}	2.93^{n}	Moderately susceptible
32	MR211	0.90^{h}	1.18^{i}	2.25^{k}	2.98^{m}	3.20^{o}	Susceptible

[+] DSI in each column with different letters is significantly different at $p \leq 0.05$
[++] 0-0.29, Resistant; 0.30-1.99, Slight susceptible; 2.00-2.99, Moderate susceptible; 3.00-4.00, Susceptible

In Malaysia, there are lacks of information on screening varieties of rice that resistance to bakanae disease, this study was therefore conducted. However, screening varieties of blast have been practiced for many decades. For example in 1900 to 1910, the Japanese varieties Kameji and Aikoku rice were considered highly resistant to blast, while, Shinriki variety was very susceptible to blast. In this study, the slightly susceptible rice variety against bakanae disease was MR220 and the most susceptible variety was MR211. MR220 is reported that resistant to blast, bacteria leaf blight and tungro (MARDI, 2006).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Many factors would influence the disease development of bakanae. Other than variety of rice as a host, the pathogen and environmental conditions such as temperature, wind, moisture, sunlight, nutrition, and soil quality have major impact on development and severity of the disease (Doohan, 2005). In order for disease to occur, a pathogen must be virulent toward and compatible with specific host. The aggressiveness of a pathogen also influences disease severity (Doohan, 2005). *Fusarium fujikuroi* isolate T3068P has been proven as highly virulent and caused bakanae disease (Nur Ain Izzati *et al.*, 2008a). Fungal genes encode proteins that make the fungal specific and virulent towards particular host, and similarly for the host to have genes that are susceptible or resistant to pathogen. Incompatible interactions result in no disease development and the plant will not be infected (Table 3). Only avirulent-resistant (AR) interaction is resistant to the pathogen, which will induce the disease development in all cases (Doohan, 2005).

Table 3: The gene of fungal and plant interaction (adapted from Doohan 2005).

Host Pathogen	Resistant (R)	Susceptible (r)
Avirulent (A)	AR(incompatible)	Ar (compatible)
Virulent (a)	aR (compatible)	ar (compatible)

Screening of biocontrol agent under dual culture

The antagonistic activity of 60 isolates of *T. harzianum* that were obtained from soil in various locations was recorded after day 5 of incubation (Table 4). *T. harzianum* isolated from Semenyih (isolate T31) demonstrated the highest response with PIRG of 65.51% (Figure 3) followed by isolates T07, T08, T11, T16, T24, T28, T36, T38, T40, T47, T48 and T55 in range 65.30-62.14%. Antagonistic activities of all *T. harzianum* isolates (95.51-42.52%) were significantly different with control plates (0%).

Table 4: The percentage of inhibition growth rate (PIRG) and antagonistic activity of *T. harzianum* isolates.

Crop; location (city, state)	Isolates	Mean value of PIRG[A]	Antagonistic activity[B]
Corn; Serdang, Selangor	T07, T12, T14, T15	46.96[a] - 48.39[a]	+
	T01, T02, T03, T04, T05, T09, T10, T13	50.14[a] - 59.01[a]	++
	T07, T08, T11	62.93[a] - 65.30[a]	+++
Rice; Kuala Selangor, Selangor	T20, T21, T22, T27, T29, T30	45.84[a] - 48.99[a]	+
	T17, T18, T19, T23, T25, T26	50.74[a] - 58.50[a]	++
	T16, T24, T28	62.49[a] - 64.29[a]	+++
Oil Palm; Semenyih, Selangor	T33, T34, T35, T37, T42, T44, T45	45.99[a] - 48.63[a]	+
	T32, T39, T41, T43	51.65[a] - 55.26[a]	++
	T31, T36, T38, T40	62.14[a] - 65.51[a]	+++
Kenaf; Kuala Terengganu, Terengganu	T50, T57, T59, T60	42.52[a] - 47.82[a]	+
	T46, T49, T51, T52, T53, T54, T56, T58	50.29[a] - 56.89[a]	++
	T47, T48, T55	63.83[a] - 64.45[a]	+++
Control	–	0[b]	–

[A]PIRG, percent inhibition of radial growth.
Means with the same letter in the same column are not significantly different at 5% level Tukey.
[B]Antagonistic activity: ++++, very high antagonistic activity (>75 PIRG); +++, high antagonistic activity (61-75 PIRG); ++, moderate antagonistic activity (51-60 PIRG); +, low antagonistic activity (<50 PIRG); –, no antagonistic activity.

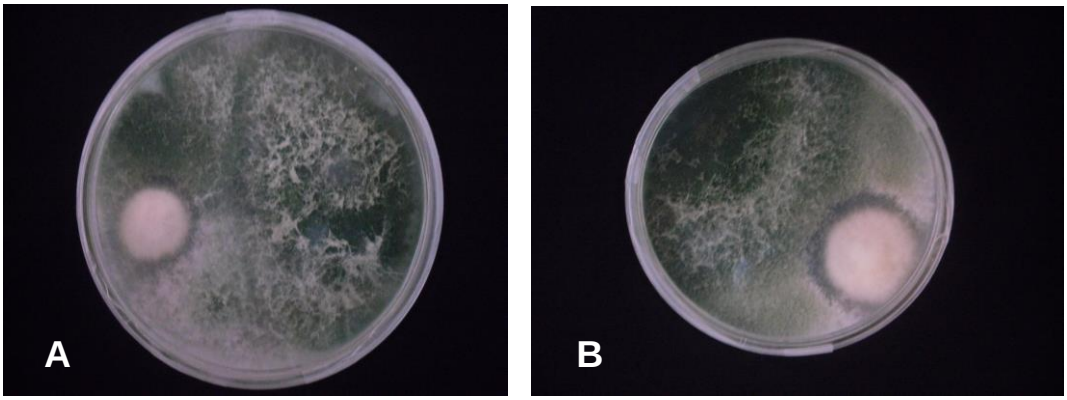

Figure 3: Percentage of inhibition growth rate (PIRG). A, T31 showed the highest antagonistic activity with the highest PIRG 65.51%; B, T50 the lowest antagonistic activity with PIRG 42.52%.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

T. harzianum was previously reported as an influential biocontrol agent against seedborne pathogens. Studies of the efficacy of *Trichoderma* spp. as a fungal biocontrol agent (Harman, 2000) are well known. However, no any report was documented on *T. harzianum* as biocontrol agent of bakanae disease. *Trichoderma* spp. have been intensively studied as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly common soil borne pathogens (Howell, 2003; Nur Ain Izzati and Faridah, 2008). Different isolates of *Trichoderma* have different optimum temperature for growth and have different strategies to inhibit the growth of pathogen (Kredics *et al.*, 2003; Hajieghrari *et a.*, 2008). In the present study under *in vitro* condition, all isolates of *T. harzianum* from soil have the ability to inhibit the growth of *F. fujikuroi* (T3068P), the pathogen of bakanae disease on PDA plates with PIRG between 42.52% and 65.51%. Somehow the isolates are only considered as promising antagonists when the PIRG exceeding 60% (Noveriza and Quimio, 2004). In this study, out of 60 isolates of *T. harzianum*, 13 isolates had shown promising antagonist (Table 4). Noveriza and Quimio (2004) reported that, *T. harzianum* as effective antagonists can grow very fast rate outpacing the growth of the pathogen (*F. fujikuroi*) and covered the entire medium surface after 5 days of incubation.

Trichoderma as biocontrol mechanisms can inhibit the fungal pathogens by mycoparasitism (Howell, 2003) and antibiosis (Sivasithamparam and Ghisalberti, 1998). The processes of mycoparasitism are recognition of the host, attack and subsequent penetration and killing. During this process, *Trichoderma* secretes a source of cell wall degrading enzymes that hydrolyze the cell wall of the host fungus and then releasing oligomers from the pathogen cell wall (Kubicek *et al.*, 2001; Howell, 2003; Woo *et al.*, 2006). It is believed that *Trichoderma* can detects the presence of another fungus by secretes of hydrolytic enzymes at a constitutive level and sense the molecules released from the host by enzymatic degradation (Harman *et al.*, 2004; Woo and Lorito, 2007).

The interaction between *Trichoderma* and fungal pathogen showed the inhibition of growth of the pathogen by competition for carbon, nitrogen and other growth factors, together with competition for space (Noveriza and Quimio, 2004). The presence of different carbon sources, such as mono- or polysaccharides, colloidal chitin, or fungal tissues, can encourage the secretion of source of cell wall degrading enzymes (Mach *et al.*, 1999). Hyakumachi (2000) reported that *Trichoderma* isolates can give a stable and obvious suppressive effect against different soilborne pathogen compared to *Penicillium* spp., *Mucor* sp., and *Fusarium equiseti* isolates.

CONCLUSION

It is therefore we can conclude that two alternative measurements can be applied for controlling *F. fujikuroi*, pathogen of bakanae disease, which is by using resistant variety and biocontrol agent. Among 31 Malaysian rice varieties, MR220 was found slightly susceptible variety against the disease; however, none of the tested varieties is resistant. Out of 60 isolates of *T. harzianum* tested, 13 isolates showed high percentage of inhibition, which that can be used as biocontrol agent for future trials.

ACKNOWLEDGEMENT

The authors would like to thank the valuable technical assistances of staff from University Agricultural Park, UPM. This work was partially supported by grant RUGs-UPM, project no: 05-03-08-0480RU (to Nur Ain Izzati M.Z.).

REFERENCES

De Souza, J. T., Bailey, B. A., Pomella, A. W. V., Erbe, E. F., Murphy, C. A., Bae, H. and Hebbar, P. K. (2008). Colonization of cacao seedlings by *Trichoderma stromaticum*, a mycoparasite of the witches' broom pathogen, and its influence on plant growth and resistance. *Biological Control* **46, 36-45.**

Doohan, F. (2005). Fungal pathogen of plants. *In:* Fungi: Biology and Application. Kavanagh, K. (eds.). John Wiley & Sons Ltd, England. **pp. 219-228.**

Hajieghrari, B., Torabi, G. M., Mohammadi, M. R. and Davari, M. (2008). Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *African Journal of Biotechnology* **7, 967-972.**

Harman, G. E. (2000). Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T22. *Plant Disease* **84, 377-393.**

Harman, G. E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* **96, 190-194.**

Harman, G. E. and Kubicek, C. P. (1998). *Trichoderma* and *Gliocladium*. Taylor & Francis, London, **pp. 278.**

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. *Natural Review Microbiology* **2, 43-56.**

Howell, C. R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Disease* **87, 4-10.**

Hyakumachi, M. (2000). Studies on biological control of soilborne plants pathogens. *Journal of General Plant Pathology* **66, 272-274.**

IRRI, (2002). Standard evaluation system (SES) for rice. International Rice Research Institute. **pp. 56.**

Karov, I. K., Mitrev, S. K. and Kostadinovska, E. D. (2009). *Gibberella fujikuroi* (Sawada) Wollenweber, the new parasitical fungus on rice in the republic of Macedonia. *Proceeding of Natural Science, Matica Srpska Novi Sad* **116, 175-182.**

Kredics, L., Antal, Z., Manczinger, L., Szekeres, A., Kevei, F. and Nagy, E. (2003). Influence of environmental parameters on *Trichoderma* strains with

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

biocontrol potential. *Food Technology and Biotechnology* **41, 37-42.**

Kubicek, C. P., Mach, R. L., Peterbauer, C. K. and Lorito, M. (2001). *Trichoderma* from genes to biocontrol. *Journal of Plant Pathology* **83, 11-23.**

Mach, L., Peterbauer, C. K., Payer, K., Jaksits, S., Woo, S. L., Zeilinger, S., Kullnig, C. M., Lorito, M. and Kubicek, C. P. (1999). Expression of two major chitinase genes of *Trichoderma atroviride (T. harzianum* P1) is triggered by different regulatory signals. *Applied Environmental Microbiology* **65, 1858-1863.**

MARDI. Malaysian Agricultural Research and Development Institute, (2006). Released Malaysian rice varieties. Sources: Released Brochure of Malaysian Rice Varieties.

Noveriza, R. and Quimio, T. H. (2004). Soil mycoflora of black pepper rhizosphere in the Philippines and their *in vitro* antagonism against *Phytophthora capsici* L. *Indon. Journal of Agriculture Science* **5, 1-10.**

Nur Ain Izzati, M. Z. and Faridah, A. (2008). Disease suppression of Ganoderma-infected oil palm seedling treated with *Trichoderma harzianum. Plant Protection Science*, **44, 101-107.**

Nur Ain Izzati, M. Z., Azmi, A. R. and Salleh, B. (2008a). Bakanae disease of rice in Malaysia and Indonesia: Etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. *Journal of Plant Protection Research* **48, 477-483.**

Nur Ain Izzati, M. Z., Azmi, A. R. and Salleh, B. (2008b). Secondary metabolite profiles and mating population of *Fusarium* species in section Liseola associated with bakanae disease of rice. *Malaysian Journal of Microbiology* **4, 6-13.**

Rahman, A., Begum, M. F., Bari, M. A., Ilias, G. N. M. and Alam, M. F. (2011). Isolation and identification of *Trichoderma* species from different habitats and their use for bioconversion of solid waste. *Turkish Journal of Biology* **35, 183-194.**

Saad, A. (1986). Penyakit bakanae di Malaysia. *Berita Penyelidikan MARDI* **8, 15.**

Sivasithamparam, K. and Ghisalberti, E. L. (1998). Secondary metabolism in *Trichoderma* and *Gliocladium*. *In:* Trichoderma and Gliocladium. Harman, G. E. and Kubicek, C. P. (eds.). Taylor and Francis Ltd., London. **pp.139-191.**

Soytong, K. (1998). Identification of species of *Chaetomium* in the Philippines. 3rd edn. Munoz, NuevaEcija, Philippines. **pp. 28-29.**

Suhaida, S. and Nur Ain Izzati, M. Z. (2013). The efficacy of *Trichoderma harzianum* T73s as a biocontrol agent of Fusarium Ear Rot disease of maize. *International Journal of Agriculture and Biology* **15, 1175-1180.**

Woo, S. L. and Lorito, M. (2007). Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. *In:* Novel Biotechnologies for Biocontrol Agent Enhancement and Management. Vurro, M. and Gressel, J. (eds.). IOS, Springer Press, Amsterdam, the Netherlands. **pp. 107-130.**

Woo, S. L., Scala, F., Ruocco, M. and Lorito, M. (2006). The molecular biology of the interactions between *Trichoderma* spp., phytopathogenic fungi, and plants. *Phytopathology* **96, 181-185.**

Woo, S., Fogliano, V., Scala, F. and Lorito, M. S. (2002). Synergism between fungal enzymes and bacterial antibiotics may enhance biocontrol. *Antonie van Leeuwenhoek* **81, 353-356.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538