ABSTRACT

Bakanae disease is a major rice disease caused by Fusarium fujikuroi. Infected plants show slender and hyper elongated internodes due to the over-production of gibberellic acid. Application of Trichoderma spp. as biocontrol agent is gaining attention due to high capability in hyperparasitize the soil borne pathogen. The studies aimed to screen and evaluate the bio-efficacy of Trichoderma spp. with antagonistic activities against F. fujikuroi and plant growth-promoting properties. All the 65 Trichoderma isolates were isolated from healthy rice rhizosphere soil. Thirty eight out of 65 Trichoderma isolates exhibited more than 45 Percentage of Inhibition Radial Growth (PIRG) against F. fujikuroi in dual culture plate testing. All selected Trichoderma isolates were further in vitro screened for antagonistic testing: volatile compounds production and hydrogen cyanide production and plant growth-promotion properties: IAA production and phosphate solubilization. Twelve Trichoderma isolates were selected for further evaluation on antagonistic activity against F. fujikuroi, germination rate, plumule and radical lengths and vigor index. Finally, seven of the most potential Trichoderma isolates were selected for greenhouse evaluation. The bakanae disease incidence and disease severity in rice plant treated with respective selected Trichoderma isolates were significant reduced as compared with untreated plant. However, there was no significant increase in plant height between Trichoderma inoculated and uninoculated plants. Moreover, rice plant treated with Trichoderma T61 showed significantly increase in total plant dry biomass as compared to untreated plants. The selected Trichoderma isolates have potential to be developed as biological control agent against F. fujikuroi and also as an alternative for bakanae management.

Key words: Trichoderma spp., bakanae disease, Fusarium fijikuroi, biocontrol, plant growth promotion

INTRODUCTION

Rice (Oryza sativa L.) is a staple food crop in many Asian countries. Rice production is required to increase by 25%, in order to meet the estimated population of around 8 billion in year 2025. However, worldwide rice production is threatening by diseases, included bakanae disease caused by F. fujikuroi. The over production of fusaric and gibberellic acids by Fusarium fujikuroi are responsible for the symptoms of bakanae disease (Kandhari, 2010). Affected rice seedlings are thin, pale yellowish green, elongated abnormally and some will die before producing grains. Some other
symptoms are discoloration at the lower nodes and the production of adventitious roots in one or
more nodes above the water level. Infected plants in some cases are severally stunted instead of
elongated (Kandhari, 2010) and affected the productivity. In Malaysia, yield losses about 30-50%
have been reported in direct-seeded rice field and under heavy infestation; lodging of infected
plants may occur and caused total yield loss (Watanabe et al., 2000).

Recently, the application of biological control agent in plant disease management is gaining
huge momentum in crop production systems. Various studies have been conducted to focus on
application of plant growth-promoting microorganism to improve plant health and productivity in
various crops. *Trichoderma* spp. were reported have the ability to reduce several plant diseases by
inhibiting plant pathogens mainly found in the soil roots, through antagonistic and mycoparasitic
potential (Viterbo et al., 2010). For instance, studies conducted by Ha (2010) in Vietnam, indicated
that *Trichoderma* spp. had the ability to suppress growth of fungal pathogens and enhance plant
growth. However, no research has been conducted to evaluate the bio-efficacy of *Trichoderma* spp.
in plant growth promotion and bakanae disease management especially in rice variety MR 219. The
objectives of the study were to screen and to evaluate the bio-efficacy of *Trichoderma* spp. with
antagonistic activities against *F. fujikuroi* and plant growth-promoting properties in rice variety
MR 219. The application of *Trichoderma* spp. is an alternative in bakanae management toward
sustainability in rice production systems.

**MATERIALS AND METHODS**

**Isolation of *Trichoderma* spp.:** *Trichoderma* spp. were isolated from the rhizosphere soils of
healthy rice plants collected from Kedah, Kelantan and Terengganu, Malaysia. *Trichoderma*
Medium E agar (TME) was used to isolate the *Trichoderma* spp. with serial dilution technique. The
soil solution was shaking for 30 min. The serial dilution of 10^{-1 }-10^{-4} was prepared by sequentially
transferring 1 mL of the solution samples into respective test tubes containing 9 mL of sterile
distilled water. A 0.1 mL of each solution was then pipette onto TME agar and evenly spread using
sterilized bent glass rod. The Petri dishes were incubated at 28±2°C for seven days (Naidu et al.,
2010). After incubation, colony forming unit was enumerated as CFU mL^{-1}. All colonies were
further transferred to Potato Dextrose Agar (PDA) for maintaining. Distinct morphological
characteristics of *Trichoderma* spp., such as shape, size and texture were observed and identified
based on morphological and cultural characteristics (Soesanto et al., 2011).

**Antagonistic activities:** The pure cultures of *Fusarium fujikuroi* was obtained from Plant
Protection Department of University Putra Malaysia (UPM), Serdang, Selangor. The antagonistic
activities of *Trichoderma* spp. against *F. fujikuroi* were evaluated using dual culture plate testing,
volatile compounds production and Hydrogen cyanide (HCN) production.

**Dual culture plate testings:** Five mm diameter of mycelial disc from the margin of the seven
days-old culture of *F. fujikuroi* was transferred onto PDA plates and allowed to grow for two days
before introducing the respective *Trichoderma* isolates. The *F. fujikuroi* disc was placed
approximately 4 cm apart from the respective *Trichoderma* spp. The experimental unit was
arranged in completely randomized design with five replications. In control, only *F. fujikuroi* was
placed on the PDA plate. Inoculated plates were incubated at 28±2°C for seven days. The inhibition
zone was measured when the mycelium of *F. fujikuroi* in control plates had reached the edge of the
plates. The suppression effect of all *Trichoderma* spp. isolates were evaluated by using Percentage Inhibition in Radial Growth (PIRG) of *F. fujikuroi* based on the following formula (Gaigole et al., 2011).

PIRG value:

\[
\text{PIRG} = \frac{R1 - R2}{R1} \times 100\%
\]

R1 = Radial growth of *F. fujikuroi* in the absence of the antagonist (control)
R2 = Radial growth of *F. fujikuroi* in the presence of the antagonist (treatment)

**Volatile compounds production:** Five millimeter mycelial disc of *F. fujikuroi* and *Trichoderma* spp. obtained from the margin of the seven days-old culture of *F. fujikuroi* and *Trichoderma* spp. were transferred onto PDA plate. Both discs of *F. fujikuroi* and the *Trichoderma* spp. were placed on the opposite (placed inversely) at equal distance from the centre of plates. A PDA plate without *Trichoderma* spp. isolate paired with *F. fujikuroi* served as control. The pairs of each plate (without the lids) were sealed together with parafilm and incubated at 28±2°C for seven days. The radius of *F. fujikuroi* disc was recorded and the percentage inhibition of radial growth was determined after seven days of incubation by using the same formula as described in dual culture plate testing.

The most potential *Trichoderma* spp. was evaluated based on *in vitro* antagonistic and volatile substances (Dubey et al., 2007). Five replications were conducted for each treatment.

**Hydrogen cyanide (HCN) production:** For HCN production, *Trichoderma* spp. was grown on Tryptic Soy Agar (TSA) supplemented with 4.4 g L\(^{-1}\) of glycine for two days. White filter paper discs were cut in the same size and soaked in picric acid solution (0.5% picric acid in 2% (w/v) sodium carbonate in 1 L of water). The sheets of filter papers were placed on the upper lid of each plate. The plates were sealed with Parafilm and incubated for seven days at 28±2°C. After incubation, HCN production was observed by the colour changes of the filter paper from yellow to light brown or reddish brown which indicated the production of HCN (Meera and Balabaskar, 2012). The coloured filter paper was then eluted by placing the filter paper in a clean test tube containing 10 mL distilled water and the optimum density (absorbance) was measured at 625 nm by using spectrophotometer (Manwar et al., 2011). Five replications were maintained for each isolate.

**Indole acetic acid (IAA) production:** Five discs of each *Trichoderma* spp. were transferred into respective universal bottles containing 10 mL of Potato Dextrose Broth (PDB) and incubated on the incubator shaker for 24 h. After 24 h of incubation, 1 mL of fungal inoculum was transferred into 250 mL conical flask containing 100 mL of sterile PDB with 5 mL of 0.2% (w/v) L-tryptophan and incubated at 28±2°C for 72 h. Conical flask without *Trichoderma* spp. served as controls or blanks. A 1.5 mL of aliquot was sampled and centrifuged at 3,000 rpm for 30 min, 1 mL of the supernatant was then added with two drops of orthophosphoric acid and 4 mL of salkowskis reagent (50 mL, 35% perchloric acid; 1 mL 0.5 M ferric chloride, FeCl\(_3\)). To determine the amount of IAA produced from the isolates, the colour density (absorbance) was measured at 535 nm using spectrophotometer (Noori and Saud, 2012). The IAA produced was compared to the standard graft and expressed as μg mL\(^{-1}\).
Phosphate solubilizing activity: All *Trichoderma* spp. isolates were screened for inorganic phosphate solubilization. The respective fungal isolates were cultured in PDB for seven days, 100 μL of the mycelium broth were spotted onto National Botanical Research Institute’s Phosphate (NBRIP) medium containing inorganic phosphate, respectively by using a micro pipette. The inoculated plates were incubated at 28±2°C for seven days. After incubation, the colonies with clear halo zones (solubilizing zone) around colony indicated positive solubilization of mineral phosphate. Phosphate solubilization activities were screened by measuring the clearing zone surrounding the fungal colony as phosphate solubilization index (Noori and Saud, 2012).

$$\text{Phosphate solubilization index} = \frac{A}{B} \times 100\%$$

A = Total diameter (*Trichoderma* colony+halo zone)
B = Diameter of *Trichoderma* colony

Selection of potential *Trichoderma* spp.: The selection of potential *Trichoderma* spp. was conducted based on mean separation by LSD using Statistical Product and Service Solutions (SPSS) programme version 20.0. The potential antagonistic and plant growth-promotion *Trichoderma* spp. were further confirmed by seed germination testing and rice seedling vigor index before evaluated under greenhouse conditions. The antagonistic activity of the selected *Trichoderma* isolates was conducted as the method described previously.

Rice seed germination testing: Rice seeds variety MR219 were surface sterilized for 5 min with 1% sodium hypochlorite solution followed by three times of rising with sterile distilled water. *Trichoderma* spp. inoculums (1×10⁸ CFU mL⁻¹) were prepared by using haemocytometer. Sterilized rice seeds were immersed in respective *Trichoderma* suspension for 1 h and 45 min. Rice seeds immersed in distilled water served as control. Ten *Trichoderma* inoculated rice seeds were placed in a petri dish layered with moisten filter paper and incubate at 28±2°C under dark condition. Germinated seeds were recorded at the 3rd day after incubation. Germination rate was calculated as the formula below:

$$\text{Seed germination rate} = \frac{\text{No. of germinated seed}}{\text{Total No. of seeds}} \times 100\%$$

The experiment was conducted twice with five replicates per treatments. The germination rates were analyzed by Friedman test, Least Significant Different (LSD) (Bandyopadhyay et al., 2003).

Rice seedlings vigor index: The rice seedlings from the germination testing were further assessed at the 5th day after incubation for plumule and radical lengths. The germination rate, plumule and radical lengths were used to calculate for vigor index using the following formula (Farooq et al., 2005):

$$\text{Vigor index} (%) = (\text{Plumule length+Radical length}) \times \text{Germination rate}$$

Second selection of the potential *Trichoderma* spp.: The selection of potential *Trichoderma* spp. was conducted as the method mentioned above. The seven most potential *Trichoderma* isolates (T40, T43, T45, T46, T50, T59 and T61) were selected for bio-efficacy evaluation under greenhouse conditions.
Bio-efficacy evaluation of the selected *Trichoderma* spp.: The bio-efficacy of the selected *Trichoderma* isolates was conducted under greenhouse conditions using rice variety MR 219. The sterilized rice seeds were soaked in the suspension of *F. fujikuroi* (1×10⁸ CFU mL⁻¹) for 24 h before sowing. Rice seeds soaked in distilled water served as control. Ten *F. fujikuroi* treated rice seeds were sown in poly bags containing 4 kg of top soil. The pots were maintained under greenhouse conditions. The treatment of selected *Trichoderma* isolates was presented in the Table 1.

At 7 days after sowing, the rice seedlings were inoculated with respective *Trichoderma* inoculums (1×10⁸ CFU mL⁻¹) through soil drenching at 10 mL per pot. The experimental units were arranged in a Randomized Complete Block Design (RCBD) with five replications per treatment. The day and night temperatures in greenhouse at University Malaysia Terengganu ranged 30.3-35.1 and 23.3-30.6°C, respectively. Rice seedlings were fertilized every two weeks with 3 g/poly bag of N:P:K = 21:21:21 and irrigated daily with tap water (Nur Ain Izzati et al., 2008).

The bakanae disease severity, incidence and growth parameters of rice seedlings in the glasshouse were evaluated at vegetative stage: 35 days after sowing (MacLean et al., 2002). The disease incidence was calculated by using the formula as described by Teng and James (2001):

\[
\text{Disease incidence (\%) = \left( \frac{\text{Total No. of infected plants per pot}}{\text{Total No. of plants per pot}} \right) \times 100}\%
\]

The disease symptoms were evaluated based on to the disease scales from 0-4 (Table 2). The Disease Severity Index (DSI) was calculated following the calculation described by Ooi (2002) using the following equation:

\[
\text{DSI} = \sum \frac{\text{No. of plants in specific scale} \times \text{disease scale}}{\text{Total No. of plants}}
\]

\[
= \sum \frac{(n \times 0) + (n \times 1) + (n \times 2) + (n \times 3) + (n \times 4)}{\text{Total No. of plants(n)}}
\]

**Data analysis:** All treatments were arranged in Randomized Complete Block Design (RCBD), consisting five replications for each treatment. Post Hoc test- Least Significant Different (LSD) was
applied when one way ANOVA revealed significant differences (p<0.05). All statistical analyses were performed with Statistical Product and Service Solutions (SPSS) programme version 20.0.

RESULTS

A total of 65 *Trichoderma* spp. were obtained from the healthy rice rhizosphere soil obtained from Kedah, Kelantan and Terengganu, Malaysia. All isolates were confirmed as *Trichoderma* spp. based on morphological and cultural characteristics. Dual culture plate testing was used as a benchmark to evaluate the antagonistic capability of *Trichoderma* isolates against *F. fujikuroi*. Thirty eight out of 65 *Trichoderma* isolates exhibited excellent antagonistic activity against *F. fujikuroi* with PIRG value more than 45% (Table 3). *Trichoderma* isolate T40 was identified as the strongest antagonist against *F. fujikuroi* with significantly high of PIRG value (77.33%). Five *Trichoderma* isolates (T8, T9, T37, T40 and T41) exhibited PIRG value for more than 55% (Table 3 and Fig. 1).

For volatile compounds production testing, all *Trichoderma* isolates tested shown inhibition effect against *F. fujikuroi*. Isolate T59 exhibited the highest inhibition effect against *F. fujikuroi*.

Table 3: Antagonistic activities and plant growth-promotion properties of *Trichoderma* isolates

| No. of isolates | Trichoderma code | Dual culture plate testing (PIRG %) | Volatile compound production testing (PIRG %) | HCN production (nm) | IAA production (μg mL⁻¹) | Phosphate solubilization activity testing (index) |
|-----------------|------------------|-----------------------------------|---------------------------------------------|---------------------|--------------------------|-----------------------------------------------|
| 1               | T2               | 50.67 ±d                        | 30.55 ±bcd                                    | 0.01 ±a             | 18.31 ±g                | 0                                             |
| 2               | T3               | 52.00 ±d                        | 22.22 ±bcd                                    | 0.04 ±ghi           | 0'                       | 0                                             |
| 3               | T4               | 48.00 ±d                        | 15.28 ±hi                                     | 0.04 ±ghi           | 18.19 ±h                | 0                                             |
| 4               | T5               | 53.33 ±d                        | 22.22 ±bcd                                    | 0.05 ±jk            | 0'                       | 0                                             |
| 5               | T6               | 48.00 ±d                        | 26.38 ±bcd                                    | 0.04 ±ghi           | 0'                       | 0                                             |
| 6               | T7               | 52.00 ±d                        | 29.16 ±bcd                                    | 0.01 ±jk            | 14.94 ±f                | 0                                             |
| 7               | T8               | 56 ±d                          | 22.22 ±bcd                                    | 0.01 ±jk            | 15.44 ±g                | 0                                             |
| 8               | T9               | 58.67 ±d                       | 19.45 ±bcd                                    | 0.02 ±jk            | 0'                       | 0                                             |
| 9               | T12              | 50.67 ±d                       | 16.66 ±ghi                                    | 0'                 | 30'                      | 0                                             |
| 10              | T13              | 48 ±d                          | 19.44 ±bcd                                    | 0.04 ±ghi           | 0'                       | 0                                             |
| 11              | T16              | 50.67 ±d                       | 13.89 ±g                                      | 0.03 ±ghi           | 0'                       | 0                                             |
| 12              | T19              | 53.33 ±d                       | 27.78 ±bcd                                    | 0.03 ±ghi           | 6.88 ±a                 | 0                                             |
| 13              | T28              | 52 ±d                          | 25 ±bcd                                       | 0'                 | 0'                       | 0                                             |
| 14              | T29              | 48 ±d                          | 22.22 ±bcd                                    | 0.03 ±ghi           | 27'                      | 0                                             |
| 15              | T37              | 57.33 ±d                       | 33.33 ±bcd                                    | 0.04 ±ghi           | 0'                       | 0                                             |
| 16              | T38              | 45.33 ±d                       | 26.38 ±bcd                                    | 0.03 ±bcd           | 3.75'                    | 0                                             |
| 17              | T39              | 54.67 ±d                       | 30.55 ±bcd                                    | 0.05 ±ghi           | 0'                       | 0                                             |
| 18              | T40              | 77.33 ±a                       | 26.38 ±bcd                                    | 0.02 ±ghi           | 0'                       | 0                                             |
| 19              | T41              | 60.00 ±b                       | 27.77 ±bcd                                    | 0.03 ±ghi           | 35.63 ±c                | 0                                             |
| 20              | T42              | 46.67 ±d                       | 18.05 ±ghi                                    | 0.02 ±ghi           | 0'                       | 0                                             |
| 21              | T43              | 52 ±d                          | 23.61 ±bcd                                    | 0.05 ±fg            | 49.38 ±d                | 0                                             |
| 22              | T44              | 52 ±d                          | 25 ±bcd                                       | 0.07 ±f             | 6.25 ±e                 | 0                                             |
| 23              | T45              | 45.33 ±c                       | 16.67 ±ghi                                    | 0.08 ±e             | 7.5 ±i                  | 0                                             |
| 24              | T46              | 45.33 ±c                       | 31.94 ±bcd                                    | 0.12 ±a             | 0'                       | 0                                             |
| 25              | T47              | 45.33 ±c                       | 20.83 ±bcd                                    | 0.07 ±bcd           | 10.63 ±b                | 0                                             |
| 26              | T48              | 50.67 ±d                       | 30.55 ±bcd                                    | 0.08 ±bcd           | 0'                       | 0                                             |
| 27              | T49              | 48 ±d                          | 26.38 ±bcd                                    | 0.12 ±e             | 20.63 ±f                | 0                                             |
| 28              | T50              | 46.67 ±d                       | 29.16 ±bcd                                    | 0.09 ±p             | 0'                       | 0                                             |
| 29              | T51              | 52 ±d                          | 29.16 ±bcd                                    | 0.04 ±ghi           | 3.13 ±c                 | 0                                             |
| 30              | T52              | 45.33 ±c                       | 29.16 ±bcd                                    | 0.09 ±p             | 2.5 ±k                  | 0                                             |
| 31              | T54              | 45.33 ±c                       | 31.94 ±bcd                                    | 0.07 ±g             | 0'                       | 0                                             |
| 32              | T56              | 49.33 ±d                       | 23.61 ±bcd                                    | 0.05 ±fg            | 0'                       | 0                                             |
| 33              | T59              | 48 ±d                          | 44.44 ±ab                                     | 0.03 ±ghi           | 0'                       | 0                                             |
| 34              | T61              | 48 ±d                          | 38.89 ±ah                                     | 0.03 ±ghi           | 93.75 ±c                | 0                                             |
| 35              | T62              | 45.33 ±c                       | 36.11 ±abcd                                    | 0.05 ±fg            | 0'                       | 0                                             |
| 36              | T63              | 45.33 ±c                       | 25 ±bcd                                       | 0.03 ±ghi           | 0'                       | 0                                             |
| 37              | T64              | 45.33 ±c                       | 20.83 ±ghi                                    | 0.04 ±ghi           | 0'                       | 0                                             |
| 38              | T65              | 45.33 ±c                       | 37.50 ±abcd                                    | 0.05 ±ef            | 0'                       | 0                                             |

Means within column with same letters are not significantly different by Least Significant Different (LSD) test using SPSS at p<0.05. Each value represents the mean of five replications of two independent experiments.
Fig. 1(a-b): *Fusarium fujikuroi* colony in control plate (a): Suppression effect of *Trichoderma* spp. against *F. fujikuroi* in dual culture plate testing, (b) Inhibition effect against *F. fujikuroi* after seven days of incubation at 28±2°C, (c): Colony of *Fusarium fujikuroi* and (d): Colony of *Trichoderma* isolate

Fig. 2(a-b): (a) *Fusarium fujikuroi* colony in control plate and (b) Suppression effect on *F. fujikuroi* colony through the production of volatile compounds by *Trichoderma* isolate after seven days of incubation at 28±2°C with PIRG value 44.44% followed by isolate T61 (38.89%), T65 (37.50%), T62 (36.11%) and T37 (33.33%) (Table 3 and Fig. 2). Only 35 *Trichoderma* isolates shown positive HCN production with the formation of orange colour on the picric acid treated filter paper. The density of the colour indicated the presence of cyanide activity. *Trichoderma* isolates: T46 and T49 have shown the significantly high colour density (Table 3).

The plant growth-promotion activities of *Trichoderma* isolates were assessed based on Indole Acetic Acid (IAA) production and capability in phosphate solubilization. Seventeen out of 38 *Trichoderma* isolates were able to produce IAA ranged 2.5-93.75 μg mL⁻¹ (Table 3). *Trichoderma* T61 produced significantly high IAA, 93.75 μg mL⁻¹. However, none of the *Trichoderma* isolates screened capable in phosphate solubilizing on NBRIP agar (Table 3).

From the *in vitro* screenings conducted, twelve most potential *Trichoderma* isolates: T9, T40, T41, T43, T45, T46, T49, T50, T52, T59, T61 and T65 were selected based on the
Table 4: Antagonistic and plant growth-promotion testing of the selected potential *Trichoderma* isolates

| Dual culture plate testing (PIRG %) | Germination rate (%) | Plumule length (cm) | Radical length (cm) | Vigor index (%) |
|------------------------------------|----------------------|---------------------|---------------------|-----------------|
| Control                            | 96.67a               | 2.38ab              | 4.58ab              | 67.88ab         |
| T9                                 | 81.98a               | 100.00a             | 2.24ab              | 4.84ab          |
| T40                                | 84.99ab              | 100.00a             | 2.73a               | 5.41a           |
| T41                                | 81.98b               | 100.00a             | 2.35ab              | 4.13b           |
| T43                                | 84.99ab              | 100.00a             | 2.92a               | 5.05b           |
| T45                                | 89.49b               | 100.00a             | 2.30ab              | 4.44ab          |
| T46                                | 87.99a               | 96.67a              | 2.03ab              | 3.77ab          |
| T49                                | 84.99ab              | 100.00a             | 2.31ab              | 4.21ab          |
| T50                                | 87.99a               | 100.00a             | 1.65a               | 2.48a           |
| T52                                | 85.99b               | 100.00a             | 1.83a               | 2.98a           |
| T59                                | 91.98b               | 100.00a             | 2.34ab              | 5.15a           |
| T61                                | 86.99b               | 100.00a             | 1.93b               | 3.07b           |
| T65                                | 77.98b               | 96.67a              | 2.35ab              | 3.74ab          |

Means within column with same letters are not significantly different by Least Significant Different (LSD) test using SPSS at p ≤ 0.05. Each value represents the mean of five replications of two independent experiments.

Table 5: Bio-efficacy of the selected *Trichoderma* isolates under greenhouse conditions

| Treatments | Inoculated isolates | Disease incidence (%) | Disease Severity Index (DSI) | Plant height (cm) | Dry biomass (g) |
|------------|---------------------|------------------------|-------------------------------|------------------|-----------------|
| Control    | -                   | 0.00b                  | 18.24ab                      | 0.53ab           |
| Control    | *F. fujikuroi*      | 90.00b                 | 2.53ab                       | 18.69bc          | 0.47b           |
| Treatment 1 | *F. fujikuroi*+T40 | 39.63b                 | 22.14ab                      | 0.56ab           |
| Treatment 2 | *F. fujikuroi*+T43 | 22.50b                 | 21.04  ab                    | 0.48b            |
| Treatment 3 | *F. fujikuroi*+T45 | 21.94b                 | 21.51bc                      | 0.51b            |
| Treatment 4 | *F. fujikuroi*+T46 | 25.00b                 | 20.08bc                      | 0.52b            |
| Treatment 5 | *F. fujikuroi*+T50 | 24.76b                 | 21.33bc                      | 0.54b            |
| Treatment 6 | *F. fujikuroi*+T59 | 20.74b                 | 19.31bc                      | 0.58ab           |
| Treatment 7 | *F. fujikuroi*+T61 | 30.00b                 | 20.20bc                      | 0.67b            |

Means within column with same letters are not significantly different by Least Significant Different (LSD) test using SPSS at p ≤ 0.05. Each value represents the mean of five replications.

mean separation conducted. The selected isolates were further tested for germination rate, vigor seedling index, plumule and radical lengths.

The germination rate on rice seeds inoculated with *Trichoderma* isolates and control were no significant different. However, *Trichoderma* isolates: T9, T40, T41, T43, T45, T50, T52, T59 and T61 improved germination rate to 100% as compared to control, T46 and T65 at 96.67%, respectively (Table 4). For plumule and radical lengths, no significant increments were observed in *Trichoderma* inoculated seedlings compared to control. However, the rice seeds inoculated with T50, T52 and T61 stunted the growth of rice seedling with vigor index of 41.27, 48.13 and 50.00%, respectively (Table 4). Rice seeds inoculated with T43 shown highest plumule length (2.92 cm) and which contributed to the highest vigor index (79.70%) (Table 4).

Seven most potential *Trichoderma* isolates were selected for greenhouse evaluation. *Trichoderma* T45, T46, T50, T59 and T61 were selected based on prominent antagonist activity against *F. fujikuroi* and T40, T43 and T59 on growth performances (Table 4). Rice plant inoculated with respective *Trichoderma* isolates shown significantly low bakanae disease incidence and disease severity index (Table 5). Plant heights in all *F. fujikuroi* inoculated seedlings were not significantly different. Generally, rice plant inoculated with both *F. fujikuroi* and *Trichoderma* isolates were slightly higher than those in control (Fig. 2). In addition, plant dry biomass for *Trichoderma* T61 inoculated seedlings found significantly higher than rice seedlings inoculated with only *F. fujikuroi*, *F. fujikuroi* with T43, T45 and T46, respectively (Table 5).

DISCUSSION

All 65 isolates obtained from rhizosphere soil were confirmed as *Trichoderma* spp.: *T. harzianum* and *T. virens*. The *Trichoderma* spp. were reported abundant in the healthy rice
rhizosphere soil (Harman et al., 2004). The variable in suppression capability of Trichoderma isolates obtained against soil-borne fungal plant pathogen (F. fujikuroi) was also reported by Console et al. (2012). In dual culture plate testing, the mycelia of Trichoderma spp. extended outgrowths of F. fujikuroi representing a chemo-attractive mechanism of interaction between two isolates (Sharma, 2011). The biocontrol potential of Trichoderma spp. against phytopathogenic Fusarium was highly suggested by Calistru et al. (1997). This is associated with the strong saprotrophic and mycoparasitic behavior of Trichoderma spp. in colonizing the root epidermis and even a few cell layers below the epidermis (Harman et al., 2004). For instance, Trichoderma spp. were commonly use for effectively control of fungal pathogen in various fruit crops (Svetlana et al., 2010).

Besides, several mechanisms were also reported responsible for the suppression of pathogenic fungal including competition, antibiotic and metabolite production (Compant et al., 2005) and this was in line with our findings where the growth inhibition of F. fujikuroi was associated with the production of the volatile compounds and HCN. However, the PIRG values from the volatile compounds production were much more lower than those in dual culture plate testing for all the Trichoderma isolates tested. Our results were supported by Kucuk and Kivanc (2003), where the volatile metabolites inhibition effect of Trichoderma isolates found lower than non-volatile metabolites. In addition, the production of HCN by Trichoderma isolates was reported as an important antifungal feature for soil borne fungi pathogen management. This was due to the microbial produced cyanide can acts as a general metabolic inhibitor to avoid predation or competition without harming the host plant (Noori and Saud, 2012.). Moreover, hydrogen cyanide was also reported effectively in blocking the cytochrome oxidase pathway and which is highly toxic to microorganisms at picomolar concentrations (Manwar et al., 2011).

Plant growth-promoting properties such as auxins production and phosphate solubilizing are important in regulating plant and root growth in various crops. IAA produced by Trichoderma spp. were found important for root growth and to increase seedling quality (Gravel et al., 2007). Potential IAA-producing isolate (T61) was identified in the current study. This isolate is highly potential to be developed as plant growth promoter as it produced higher IAA concentration (93.75 μg mL⁻¹) as compared to those reported by Kotasthane et al. (2015) with only 30.08 μg mL⁻¹ and Salas-Marina et al. (2011) with 27 μg mL⁻¹. However, no P-solubilization activity was observed in NBRIP plate assay for all the Trichoderma isolates tested. This was in contrast with El-Katatny (2004), who reported that Trichoderma isolates are relatively good in P-solubilization.

The present study evaluated the growth promoting ability of Trichoderma isolates in rice seeds based on seed vigor index and seedlings dry biomass. It was observed that some Trichoderma isolates work as plant growth promoter and some even seen to be detrimental. The same observation was reported in association to the plant genetic background, the interaction with the Trichoderma isolate (Tucci et al., 2011) and the species tested. Therefore, the potential Trichoderma isolate as plant growth promoter in the current work cannot be generalized to all other planting materials. However, Trichoderma spp. are multifunctional plant symbionts for enhancing germination rate and increasing seedling lengths of pepper, bean, radish, tomato, pepper and cucumber (Avis et al., 2008; Oyarbide et al., 2001; Yossen et al., 2003). Moreover, the present investigation shows that the use of Trichoderma T61 exhibited the highest dry biomass of rice seedlings was explained in relation to the high IAA production. Gravel et al. (2007) demonstrated
Trichoderma induce plant Indole Acetic Acid (IAA) production and helps to improve root growth and seedling quality. The application of T. harzianum was reported to increase fresh shoot weight, root weight and/or root length in peas (Naseby et al., 2000), increase root area by 95%, root length by 75%, dry weight by 80%, shoot length by 45% and leaf area by 80% in cucumber when compared to the control (Yedidia et al., 2001). The mechanisms involved in the stimulation of plant growth by Trichoderma include interactions with plant roots similar to mycorrhizae, in which Trichoderma penetrates and colonizes root tissues without eliciting specific defense responses against the colonizing strain (Yedidia et al., 2001). However, in our study not all Trichoderma isolates enhance plant growth and is might be due to the suppression effect of the metabolite produced.

The effectiveness of Trichoderma spp. in controlling plant pathogens have been proven in many studies and through direct benefits of these fungi on plant growth and production (Avis et al., 2008). This was in agreement with our findings, where rice plants inoculated with Trichoderma spp. significantly reduce bakanae disease incidence and disease severity under greenhouse conditions. Various mechanisms were suggested to involve, such as induced systemic resistance in plants, antibiosis, competition for nutrients and/or space and mycoparasitism using lytic enzymes (Tseng et al., 2008). Lytic enzymes of Trichoderma such as cellulases, chitinases, glucanase and proteases are partially induced before direct contact with the host (Foreman et al., 2003). Besides, Trichoderma interaction with plant roots creates a sensitized state in the plant allowing it to respond more efficiently to subsequent pathogenic attack (Shoresh and Harman, 2008). Sharma (2011) demonstrated Trichoderma spp. as promising antagonist against F. fujikuroi by parasitized and lysed the mycelium of F. fujikuroi. Trichoderma spp. have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity (Harman et al., 2004). Therefore, the application of Trichoderma spp. in crop disease management is gaining more attention and the development of Trichoderma T40, T43, T45, T46, T50, T59 and T61 as biocontrol agents is potential to be an alternative in bakanae management.

CONCLUSION

The development of biological control approach of bakanae disease using Trichoderma spp. is potential to reduce the agrochemical usage. In the present study, the potential isolates (T40, T43, T45, T46, T50, T59 and T61) increased plant resistance against F. fujikuroi in rice variety MR219. However, further studies on plant defense mechanisms and disease suppression were suggested especially under natural field conditions.

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