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Effect of *Trichoderma* spp. on mustard growth (*Brassica juncea* L.)

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Abstrak: *Trichoderma* spp. dikenali sebagai kulat penggalak pertumbuhan tanaman dan terdapat beberapa kajian yang telah melaporkan bahawa *Trichoderma* ini mempunyai pelbagai mekanisme serta kesan yang positif dalam meningkatkan pertumbuhan pelbagai tanaman. Dalam kajian ini, keberkesanan *Trichoderma* spp. dalam menggalakkan pertumbuhan sawi telah dinilai secara eksperimen menggunakan kaedah secara rawak sepenuhnya di bawah keadaan rumah hijau. Anak benih sawi telah dikenakan dengan dua jenis rawatan iaitu rawatan *Trichoderma* spp. dan rawatan kawalan. Hasil kajian ini mendapati bahawa anak benih sawi yang dirawat dengan *Trichoderma* spp. telah menunjukkan peningkatan yang signifikan terhadap ketinggian pokok (<0.001) bilangan daun (0.001), panjang akar (0.007), berat akar segar (<0.001) dan berat akar kering (<0.001) berbanding anak benih sawi yang tidak dirawat dengan *Trichoderma* spp. (kawalan). Malah, rawatan *Trichoderma* spp. ini juga telah memberikan keputusan yang signifikan ke atas kandungan klorofil relatif sawi (0.028). Kajian ini menyimpulkan bahawa *Trichoderma* spp. dapat diaplikasikan sebagai agen penggalak pertumbuhan dalam pertanian sawi serta pada masa yang sama, penggunaan *Trichoderma* spp. juga akan membantu petani dalam meningkatkan agronomi negara.

Kata kunci: *Trichoderma* spp; Sawi (*Brassica juncea* L.); Tindakbalas Pertumbuhan

Abstract: *Trichoderma* spp. is also known as plant growth promoting fungi and some studies were reported that *Trichoderma* spp. have several mechanisms and positive effects to enhance plant growth. In this study, the effectiveness of *Trichoderma* spp. to promote *B. juncea* growth was evaluated experimentally using completely randomized design under greenhouse condition. *B. juncea* has been treated by two types of treatment namely *Trichoderma* spp. treatment and control treatment. This study indicated that the treated *B. juncea* with *Trichoderma* spp. significantly increased plant height (<0.001), leaf number (0.001), root length (0.007), root fresh weight (<0.001) and root dry weight (<0.001) compared to the untreated plants (control). In fact, the treatment of *Trichoderma* spp. also showed significant result on the chlorophyll relative content (0.028). The study concluded that *Trichoderma* spp. can be applied as potential growth promoting agent in *B. juncea* cultivation and at the same time, the use of *Trichoderma* spp. can assist the farmers in improving the agronomic state.

Keywords: *Trichoderma* spp; Mustard (*Brassica juncea* L.); Growth Response

1. Introduction

Brassica juncea breeders aim to make simultaneous improvement of agronomic performance, disease resistance and quality traits. Seed germinates within five days after sowing at 20-25°C. Under good conditions plants grow rapidly and leaves are harvestable after three weeks when plants have developed six to eight fully expanded leaves, but harvesting will start later when larger leaves are demanded for sale. *B. juncea* is self-fertile, but bees may aid by cross-pollination. Fruits develop rapidly and the seeds can be ready for harvesting within four weeks from flowering (Kaushik 2015).

Nowadays, the excessive use of chemical fertilizers and pesticides had created serious environmental pollution (Hermosa et al. 2012). The environmental pollution caused by excessive use and misuse of agrochemicals has led to considerable changes in people behavior towards the use of pesticides and agrochemicals in agriculture (Meena & Meena 2015). Hence, the strict regulations have been implemented on chemical pesticide and there is political pressure to prevent the most hazardous chemical from the market. Furthermore, the spread of plant diseases in natural ecosystem may rule out successful application of chemicals, because the correct amount of chemical used should be applied (Meena & Meena 2015). Consequently, some pest management researchers have focused on developing alternatives about synthetic chemicals for controlling pests and plant diseases. Among these alternatives, the biocontrol agents like *Trichoderma* spp. has been applied in agriculture which are free-living fungi that are common in soil and root ecosystems (Saldajeno et al. 2014).

Trichoderma spp. was suggested as a Plant Growth Promoting Fungi (PGPF) due to their ability to produce siderophores, phosphate-solubilizing enzymes and phytohormones (Doni et al. 2013). *Trichoderma* can be found in many ecosystems and some strains have the ability to inhibit plant pathogens, mainly in the soil or plant roots, through high antagonistic and mycoparasitic potential (Viterbo & Horwitz 2010). Besides, *Trichoderma* spp. also has been applied against phytopathogen fungi and some strains are able to produce metabolites in promoting plant growth (Hoyos-Carvajal et al. 2009). *Trichoderma* species also have diverse beneficial effects on development and plant growth, which help in increasing the proliferation of secondary roots, leaf area, shoot length, dry weight and crop yield (Hermosa et al. 2013; Mukherjee et al. 2013).

Some of the previous researches showed positive result in using *Trichoderma* spp. on productivity and plant growth such as maize (Akladios & Abbas 2014), tomato (Jamal Uddin et al. 2016), chili (Islam et al. 2011), cabbage and red beet (Topolovec-Pintaric et al. 2013) and bean (Hoyos-Carvajal et al. 2009) compared to untreated plants. Anand & Ashok (2015) reported in their research that root length and length of aerial parts (LAP) of treated bean have better growth than control plant.

Therefore, this kind of research will be applied on mustard (*B. juncea*) to observe its growth because there is still no research on *B. juncea* growth. In fact, its growth factor also makes this plant suitable to be applied in this research because it only takes a short period to grow compared to other plants like peppers or carrots. Furthermore, *B. juncea* is more resistant against heat and drought stress than *B. rapa* and *B. napus* (Woods et al. 1991). Thus, this research is relevant to study the positive effect of *Trichoderma* spp. on physiology and *B. juncea* growth. As a result, the productivity and plant growth can be improved through the application of biocontrol agents in agriculture and at the same time, we can help the farmers in improving their state agronomy. This research was conducted to examine the effect of *Trichoderma* spp. on *B. juncea* growth in 30 days based on plant height, leaf number, root length, root fresh weight, root dry weight and chlorophyll content.

2. Materials and Methods

2.1 Fungus culture

Trichoderma asperellum SL2 was obtained from the Laboratory of 3160, School of Biosciences and Biotechnology, Faculty of Sciences and Technology, Universiti Kebangsaan Malaysia, Bangi. This fungus was subcultured on potato dextrose agar (PDA). Cultures that were incubated for seven days at a temperature of 30°C were kept at a temperature of 4°C as culture stock. While, for the preparation of spore suspension, the fungus was incubated for 15 days. After incubation, the spores were harvested from plates by adding 10 ml of sterile water and the spores were transferred immediately to an Erlenmeyer flask containing sterilized distilled water. Spore concentration was adjusted to 10⁷ spores/ml based on hemocytometer counts.

2.2 *B. juncea* seed inoculation and seedlings preparation

Seeds were surface-sterilized by soaking in ethanol 70% for 30 min, followed by soaking in 5% sodium hypochlorite for 30 min, and then washing with sterilized distilled water. A total of 15 seeds were treated with *Trichoderma* spores by soaking in 10^7 spores/ml suspension for one hour and another 15 seeds were soaked in sterilized distilled water for one hour which served as the control.

The seeds for respective trials, treatment (T, with *Trichoderma* inoculation) and control (C, without inoculation), were grown separately for seven days under greenhouse condition (temperature 30 ± 4 °C, light intensity of 320 ± 3 μ mol, humidity $80 \pm 3\%$, and photoperiod of 11 h 11 m 17 s \pm 9 s) in seedling trays containing a mixture of sterilized soil, sterilized sand and sterilized compost. Then, five day-old *B. juncea* seedlings were transplanted singly in 9 \times 12 inch polyethylene plastic bags containing a mixture of 500 g sterilized soil, 500 g sterilized sand, and 500 g sterilized compost as the growth medium. Water was given carefully and maintained at 2 cm level from soil surface. The soils were kept moist with no standing water allowed and actively aerated by physically disturbing and breaking-up the soil surface once per week.

2.3 Measurement of *B. juncea* growth

B. juncea growth components were measured 30 days after transplanting. Plant height (cm) was measured from ground level to the tip of the longest leaf and leaf number was counted for each treatment and control. For *B. juncea* root length (cm) and root fresh weight (g) measurements, *B. juncea* were extracted out carefully from the soil. Root length was measured from the base of the stem to the longest root using a ruler while the root fresh weight was measured using digital scales. *B. juncea* root dry weight (g) measurement was done after *B. juncea* roots were dried in the oven at a temperature of 65 °C for seven days.

2.4 Chlorophyll content analysis

Chlorophyll was measured 30 days after transplanting by selecting 15 *B. juncea* plants from each treatment. A total of 0.1 g of leaf had been cut into ~2 mm pieces was placed in a test tube, to which 20 ml of 80% acetone was added. This mixture was homogenized by a vortex and then incubated for 48 hours in the dark. Concentrations of chlorophyll a and chlorophyll b were analyzed using an UV spectrophotometer at wavelengths of λ 663 nm and at λ 645 nm, respectively. Chlorophyll a and b were calculated according to the equations below, were expressed as mg/g fresh leaf weight (Shibghatallah et al. 2013).

$$C_{\text{chl-a}} = 12.7 A_{663} - 2.69 A_{645}$$

$$C_{\text{chl-b}} = 22.9 A_{645} - 4.68 A_{663}$$

Chlorophyll content was also determined by using a SPAD 502 Plus Chlorophyll Meter (Konica Minolta Ltd.). Three different *B. juncea* leaves were chosen randomly from each *B. juncea* plant. The leaves were clipped in a SPAD meter, and readings were recorded carefully.

2.5 Statistical analysis

All data were statistical analyzed by using independent t-test. The significance effect of the treatment was determined using significance level ($\alpha=0.05$). Regression relationship was determined using the data analysis SPSS software version 23.

3. Results

3.1 *B. juncea* plant growth performance

This research focused the important role of *T. asperellum* SL2 on *B. juncea* growth for 30 days and the result showed the treated *B. juncea* with *T. asperellum* SL2 is significant and have better growth compared to untreated plant (control) (Table 1).

Table 1. Effect of *T. asperellum* SL2 on *B. juncea* growth (30 Days Growth)

Treatment	Plant height (cm)	Root length (cm)	Leaf number	Root fresh weight (g)	Root dry weight (g)
<i>Trichoderma</i> spp.	36.06 (4.38)	13.35 (3.27)	8 (0.88)	0.407 (0.179)	0.048 (0.020)
Control	30.07 (3.14)	9.58 (3.76)	7 (0.62)	0.151 (0.079)	0.018 (0.011)
<i>t</i> -value	4.304	2.936	3.833	5.076	5.103
<i>p</i> -value ($\alpha=0.05$)	0.000	0.007	0.001	0.000	0.000

Standard deviation are given in parentheses (n=30).

All means are significantly different between treatments at $p < 0.05$.

The height of *B. juncea* plants treated with *Trichoderma* spp. (36.06 cm) was better than control plants (30.07 cm) and there was a significant difference in plant height between these treatments ($t_{28} = 4.304$, $p < 0.001$) (Table 1). Significant increase in root length was observed for the *Trichoderma* spp. treated *B. juncea* plants, registering root length at 13.35 cm while the mean for control was recorded at 9.58 cm only, $t(28) = 2.936$, $p = 0.007$ (Table 1). Besides, significantly value for leaf number was also observed for *Trichoderma* spp. treated *B. juncea* plants and the differences of leaves size for these treatments can be observed in Figure 1. The value for leaf number was eight for the *Trichoderma* spp. treated plants while for the control, leaf number was seven, $t(25.033) = 3.833$, $p = 0.001$ (Table 1).



Figure 1. Leaves size of *T. asperellum* SL2 inoculated *B. juncea* plants (left) is larger than control (right).

Besides, root fresh weight of *B. juncea* plant treated with *Trichoderma* spp. was more better (0.407 g) compared to control plant (0.151 g). In fact, both of these treatments showed significant difference ($t_{19.262} = 5.076$, $p < 0.001$). Root dry weight was found to be significantly increased for the *Trichoderma* spp. treated *B. juncea* plants at 0.048 g while the root dry weight for control was 0.018 g only, $t(21.453) = 5.103$, $p < 0.001$ (Table 1). The differences of growth between the *Trichoderma* spp. treated *B. juncea* plants and untreated plants (control) can be seen clearly as shown in Figure 2.



Figure 2. The use of *T. asperellum* SL2 (left) resulting in better growth of *B. juncea* compared to control (right).

3.2 Chlorophyll content

The general assessment from this experiment is that the inoculation of *B. juncea* with *Trichoderma* spp. was significantly increased the chlorophyll contents of *B. juncea* plants (Table 2).

Table 2. Effect of *T. asperellum* SL2 on Chlorophyll Contents in *B. juncea* (30 Days Growth)

Treatment	Chlorophyll relative (SPAD)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)
<i>Trichoderma</i> spp.	25.4 (1.435)	0.612 (0.105)	0.265 (0.046)
Control	23.3 (3.122)	0.530 (0.077)	0.284 (0.033)
<i>t</i> -value	2.367	2.462	1.124
<i>p</i> -value ($\alpha=0.05$)	0.028	0.020	0.271 (ns)

Standard deviation are given in parentheses (n=30).

ns; not significant.

The chlorophyll relatives of *B. juncea* plants that measured by SPAD meter was high in *B. juncea* plants inoculated with *Trichoderma* spp. (25.4) compared to control treatment (23.3). There was a significant difference in chlorophyll relative between *Trichoderma* spp. treated *B. juncea* plants and untreated plants (control) which is $t(19.661) = 2.367$, $p = 0.028$ (Table 2). Additionally, the application of *Trichoderma* spp. to *B. juncea* plants significantly influenced the chlorophyll a of the *B. juncea* plants (Figure 3). Inoculation of *B. juncea* plants with *Trichoderma* spp. significantly increased the chlorophyll a of *B. juncea* plants (0.612 mg/g) and better than control plants that only contain 0.53 mg/g of chlorophyll a, $t(28) = 2.462$, $p = 0.020$ (Table 2). However, there was no significant difference in chlorophyll b for the *Trichoderma* spp. treated *B. juncea* plants (Figure 3), $t(28) = 1.124$, $p = 0.271$. This reading of chlorophyll a and chlorophyll b were obtained by using the other method than SPAD. In many cases, destructive chlorophyll determinations are done in most plants and the use of chemical extraction like acetone has dominated the protocol. Such a method may produce a misleading result because of pigment degradation by acetone. Alternatively, destructive sampling can be avoided by using SPAD-502, a portable meter that determines the relative amount of chlorophyll by measuring the transmittance of the leaf in two wavelength reactions (Ramlan et al. 1999).

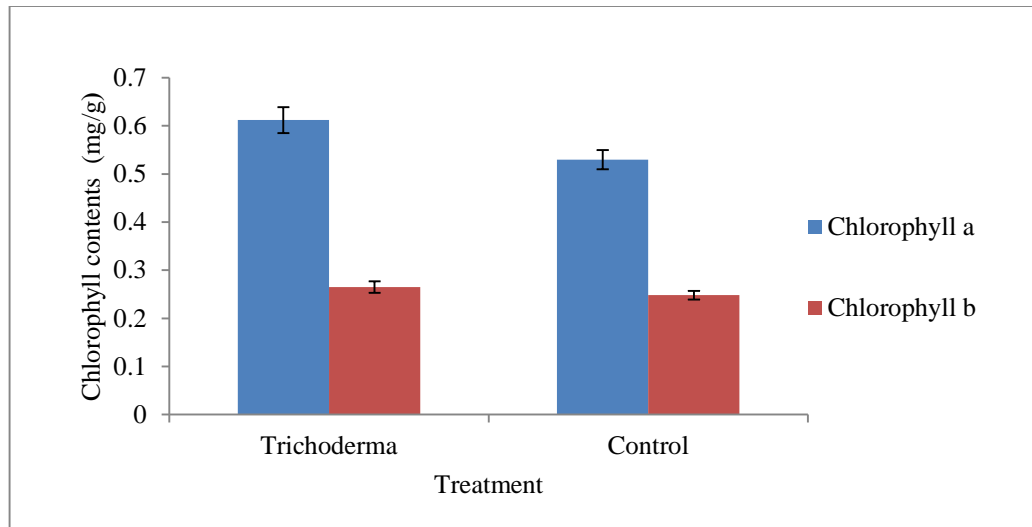


Figure 3. *B. juncea* inoculated by *T. asperellum* SL2 showed high chlorophyll content compared to control.

4. Discussion

Although *Trichoderma* spp. is mainly known as a biocontrol agent, it also acts as plant growth promoting fungi (Hermosa et al. 2012; Lorito et al. 2010; Shores et al. 2010). *Trichoderma* spp. is able to increase plant resistance under suboptimal growth conditions, efficiency of nutrient uptake, photosynthetic efficiency, development of roots and above-ground plant parts, increase root hair formation and enhance deeper rooting (Lorito et al. 2010; Shores et al. 2010). Therefore, this research has been conducted to study the ability of *Trichoderma* spp. in enhancing *B. juncea* growth and the results revealed that the *Trichoderma* spp. treatments highly significantly increased *B. juncea* growth and better compared to control treatments (Table 1).

4.1 *B. juncea* plant growth performance

Plant height of *Trichoderma* spp. inoculated *B. juncea* plants was higher compared to control plant (Table 1). The ability of *Trichoderma* spp. to produce phytohormones like indole-3-acetic acid (IAA) and gibberellic acid (GA₃) is the key factor in the increase in *B. juncea* plant height as reported by Chowdappa et al. (2013). *B. juncea* which were treated with *Trichoderma* spp. also have better nutrient uptake as suggested by Saba et al. (2012). Better nutrient uptake will enhance the physiological processes within the *B. juncea* plants treated with *Trichoderma* spp. leading to good growth performance.

Leaf number was significantly increased in *Trichoderma* spp. treated *B. juncea* plants compared to control (Table 1). The enhancement of leaf number by *Trichoderma* spp. was made possible because of the ability of the *Trichoderma* spp. to act through several mechanism and induce resistance to a variety of abiotic stresses, including water deficit, temperature, salt and osmotic stress (Najam et al. 2014). Furthermore, Bae et al. (2009) have reported that *Trichoderma hamatum* has significantly increased the ability of cocoa plant to tolerate water deficit and increase cocoa root growth. In this research, these mechanisms are believed to be contributing factors that led to higher leaf number.

The root length of *B. juncea* plants treated with *Trichoderma* spp. significantly increased compared to control (Table 1). Furthermore, the fresh weight and dry weight of *B. juncea* root plants treated with *Trichoderma* spp. were also significant greater than control. Akladios & Abbas (2014) reported that the increase of root size resulted into the increase of shoot size, which translates into the increase of shoot biomass production indicating a beneficial effect of inoculation on plant growth and development. *Trichoderma* spp. facilitates root colonization of their hosts by the production and regulation of hormonal signals (Saldajeno et al. 2014). For example, *Trichoderma* strains that promote plant growth are found to produce the plant hormones auxin (Hoyos-Carvajal et al. 2009) which promotes root growth (Samolski et al. 2012). Auxin-induced modifications in root architecture (e.g. increased number of root hair), increases total absorptive surface of the roots, thereby facilitating nutrient uptake resulting to increased plant growth (Samolski et al. 2012). Even, it will also increase plant productivity and the yields of reproductive organ (Najam et al. 2014). The presence findings also in agreement with previous research by Akladios & Abbas (2014) that revealed the role of *Trichoderma harzianum* significantly increased maize root length compared to control plants due the production of indole-3-acetic acid (IAA). Some *Trichoderma* isolates are known to produce ACC deaminase (ACCD), which reduces the availability of the ACC necessary for ET biosynthesis, which might result in plant root growth (Viterbo et al. 2010) and they have reported that *T. asperellum* is able to promote canola seedling root elongation via ACC deaminase (ACCD) activity.

4.2 Chlorophyll content

In this study, the results revealed that the *Trichoderma* spp. treatments significantly increased the chlorophyll relative content of *B. juncea* plants as compared to control (Table 2). Photosynthesis is the most essential physiological process in plants. The findings from our experiments showed *T. asperellum* SL2 inoculation positively enhanced chlorophyll content, which in turn improves the photosynthesis processes in plants. Doni et al. (2016) showed that *Trichoderma* spp. in rice increased root size resulted into increase shoot size, which translates into increasing in the shoot biomass, these were resulted in increasing of chlorophyll content. There are also difference contents in chlorophyll a and chlorophyll b between both of the treatments (Table 2). It is because chlorophyll a generally present in almost double the quantity of chlorophyll b (Srivastava & Prasad 2010). Chlorophyll a is the green pigment which is responsible for the absorption of light, providing energy for oxygenic photosynthesis while chlorophyll b is the green pigment which is responsible for collecting light energy and passing into chlorophyll a during photosynthesis (Lakna 2017).

4. Conclusions

The present study concludes that the treated *B. juncea* plants with *Trichoderma* spp. resulted in better growth compared to untreated plants.

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