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Potential of *Trichoderma* spp. as plant growth promoter on Okra (*Abelmoschus esculentus*)

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Okra (*Abelmoschus esculentus*) is one of the important vegetable crops in Malaysia. However, some seeds used to grow this plant are not competent. Besides, over-reliance on synthetic fertilizers also disrupt the nutrient supplying capacity, thus making the soils becoming infertile. The genus *Trichoderma* consists of many species that have the potential as plant growth promoters and indirectly helps in the reduction of chemical fertilizers consumption. Therefore, this experiment was carried out with the objective of determining the effectiveness of *Trichoderma* that could affect the growth of okra via seed treatment and soil application. Okra seeds were coated with fungi suspension of *T. harzianum, T. atroviride* and *T. hamatum* respectively, supplemented with arabic gum as a carrier. Germination of seeds was recorded after seven days. Among tested fungal suspension, we examined the promise of fungi as fungal inoculants, applied singly and in combination as a consortium, to improve the plant height, germination and root length. The results showed seed-treated with *T. atroviride* had the highest germination rate of 90% compared to other treatments.The relationship among the fungal activities and the nutrients dynamics in soils and okra morphological traits were also assessed. The abundance of minerals (phosphorus, calcium, magnesium, sodium and iron) in *T. harzianum*-treated soil compared to other soil treatments proved that *T. harzianum* increases the solubilisation activity in the soil. These findings support that the *Trichoderma* treatment had the capability for plant growth promotion on okra.

Keywords: *Trichoderma* spp., okra, plant growth promoter, biofertilizer.

INTRODUCTION

Okra (*Abelmoschus esculenthus*) belongs to the family Malavaceae and is commonly known as 'bendi' in Malaysia. It is native to tropic and subtropic countries. It is cultivated for its leaves, fruits, seeds, floral parts, and stems (Matthew et al. 2018). Recent reports suggest that okra seeds could be a source of oil and protein (Moosavi et al. 2018; Torkpo et al. 2009). Multiple studies showed that okra seeds contain a protein of 26% (average) (Male and Surendra, 2017; Oyelade et al. 2003; Sami et al. 2013). However, some seeds used to grow this plant are not competent and they need a lot of fertilizer to complete the crop cycle which takes about three to four months.

Heavy reliance on chemical fertilizers and pesticides in modern agriculture is worsened. The need to increase agricultural productivity has led to this and caused severe environmental pollution (Sureshra et al. 2016). The chemical residues produced are difficult to degrade, accumulative and very harmful to plants, animals and humans.

These chemicals also disrupt the nutrient supplying capacity, thus making the soils becoming infertile (Manjunath et al. 2016). The continuous nutrient mining while using chemical fertilizer leads to an ever-increasing gap between depletion and replenishment leaving the soil hungrier and proves unsustainable (Ghosh, 2004). Nutrients deficiencies in the soil are one of the limiting factors in food production. Current studies had been done on multiple mycorrhizal fungi including *Trichoderma* spp. as potential biofertilizers and biopesticides to maintain high production with low environmental impact.

Trichoderma spp. are currently widely studied for their benefits in agriculture, whether in disease control or higher yield production (Mukhtar et al. 2012), even under saline conditions (Contreras-Cornejo et al. 2009). *Trichoderma* spp. have developed several mechanisms that result in better performance in plant resistance to disease and plant growth activities (Agent et al. 2020). Nagaraju et al. (2012) reported that *T. atroviride, T. harzianum* and *T. virens* has been used as an alternative to chemical pesticide. The discoveries of *Trichoderma* spp. secondary metabolites can be developed as new biofertilizers or biopesticides instead of the use of chemical fertilizer and pesticides. Therefore, this experiment were performed with the intention of determining the effectiveness of three Trichoderma species, which could have an impact on okra growth through seed treatment and soil application.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted under a aroung 40% shade black net to avoid heavy rain affect the samples, using a completely randomized design. The okra seeds without cracks or other visible deformations were selected and sterilized with 70% alcohol for two minutes and Chlorox for three minutes. The seeds are then rinsed with water and let dry in the air.

Source of Potential Fungi

The *Trichoderma* spp. (*T. harzianum, T. hamatum* and *T. atroviride*) were obtained from stock collection of UniSZA. It was prepared in powder form with a concentration of 10^7 spores/mL. There were five treatments with 15 replications each. The five treatments include control, *T. harzianum, T. hamatum, T. atroviride* and consortium. The consortium is a combination of three tested fungi which is *T. harzianum, T. atroviride* and *T. hamatum*. The solution mixture is consisting of 10 mL of distilled water, 4 g of Arabic gum as an adhesive and 3 g *Trichoderma* granule. For the consortium solution, the 3 g of *Trichoderma* granules were made up of 1 g from three *Trichoderma* spp. used. Arabic gum and water as were used as control.

Experimental procedure

Determination of germination and growth rate of okra seed-treated with different *Trichoderma* spp.

The seeds were coated with each treatment of *Trichoderma* spp. solution for 15 minutes. The seed was then dried on a plastic plate. There were 50 seeds in each treatment. The Germination Rate was calculated as in Swain et al. (2018);

$$Germination\ rate\ (\%) = \frac{Number\ of\ germinated\ seeds}{Number\ of\ seeds\ on\ each\ tray} \times 100$$

Germination of coated seeds was compared with untreated control. Germinated seeds were recorded after seven days. From the 50 seeds tested, the best 15 seedlings were selected to observe its growth for 42 days. The plant height, number of leaves, root length and number of fruits were recorded.

Determination of growth rate of okra planted in treated soil with different *Trichoderma* spp.

Before planting, 15 polybags containing soil mixture (2:1) were treated with *Trichoderma* solution. The solution consisted of 200 mL water with 10 g *Trichoderma* granules (spore suspension 10^7/ml). The solutions were water (control), *T. harzianum, T. atroviride, T. hamatum* and consortium. The okra seeds were sterilized before sowing using 70% alcohol for two minutes and Chlorox for three minutes then rinsed with water. The seeds are then sown to the polybag after seven days of prepping the soil with *Trichoderma* granules. For the next 14 days, the germination rate were calculated using the previous formula. The germination of seeds was compared with the seed-untreated soil. The growth of the okra plant was also observed for five weeks after planting. The growth parameters involved the plant height and number of leaves were collected weekly, while root length, root mass and leaf area were measured at the end of experiment.

Analysis of available nutrients in treated soil using ICP-OES

Available nutrients in the treated soil were analysed after six weeks. The analysis was to determine the presence of P, K, Mg, Al, Fe and Ca. The nutrients were extracted by doubled acid then determined by Thermo-Scientific ICAP 7000 series Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES).

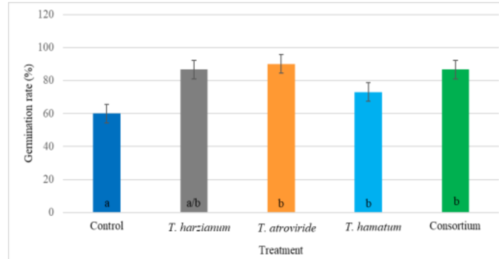
Data Analysis

The plant height, number of leaves, root length, root mass and the concentration of nutrients were analysed using one-way analysis of variance (ANOVA). The analysis was performed using IBM SPSS Statistics Software. The significant difference was determined by Dunnet's Multiple Range Test at the significance level of 0.05.

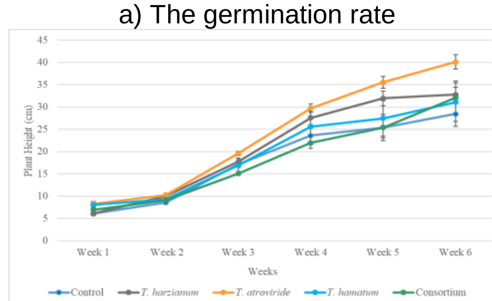
RESULTS AND DISCUSSIONS

Determination of germination and growth rate of okra seed-treated with different *Trichoderma* spp.

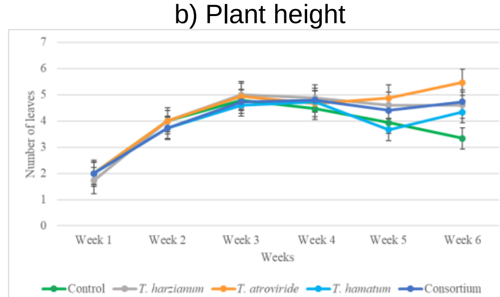
The effect of seed-treated with formulation from the isolates of *T. harzianum, T. atroviride* and *T. hamatum* were assessed individually and in combination. The result showed that the germination rate of seed-treated okra with *T. atroviride, T. hamatum* and consortium were significantly increased over control ($p<0.05$). Among the samples, *T. atroviride* exhibited 90% germination followed equally by *T. harzianum* and consortium with 86.67% respectively while untreated (Control) showed 60% germination. This may be due to the ability of *Trichoderma* spp. to enhance the production of phytohormones like auxin, gibberellins, cytokinins, vitamins and solubilizing minerals (Sain & Pandey, 2018). The number of leaves, the number of okra fruit and the plant root length showed a significant difference between other treatments when treated with *T. atroviride* (five leaves, one fruit and 23.2cm) compared to control ($p>0.05$). Afzal et al. (2013) reported that *Trichoderma* can suppress the growth of soil-borne pathogen and improved the root length. Promotion of root in plants are contributed by the production of auxin by *Trichoderma* (Contreras-Cornejo et al. 2009).



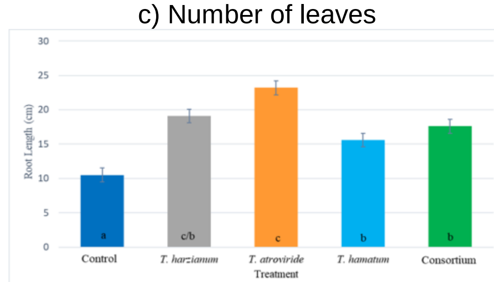
a) The germination rate



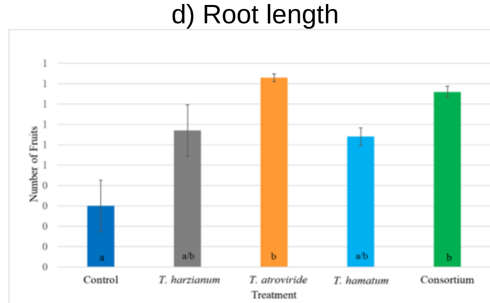
b) Plant height



c) Number of leaves



d) Root length



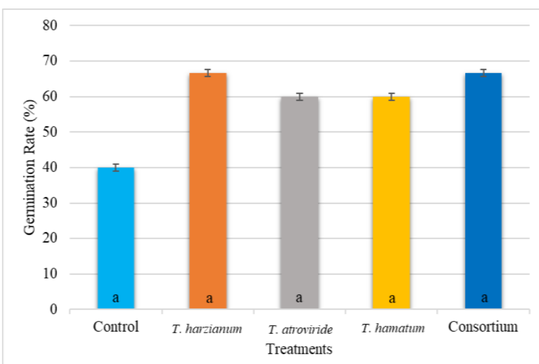
e) The number of fruits

Figure 1: Effect of *Trichoderma*-treated seed on (a) germination rate, (b) plant height, (c) number of leaves, (d) root length and (e) number of fruits. (Means with the same letters are not significantly different at p>0.05)

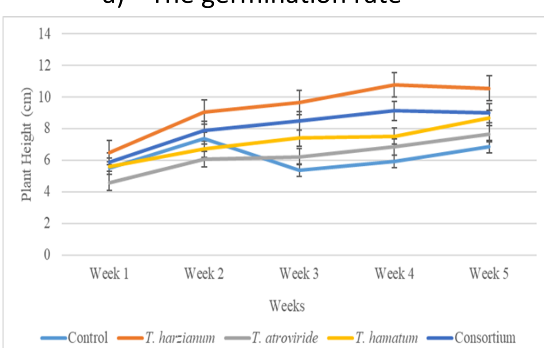
Determination of growth rate of okra planted in treated soil with different *Trichoderma* spp.

For the germination rate of okra seed-treated compared soil-treated okra, it showed a lower rate by 30% (between treatment of *T. atroviride*) in soil-treated okra. The much lower germination rate also may have affected by low soil temperature and excessive soil moisture because the soil-treated test had been done around December to January where the rainy season occurred. This is supported by Conway et al. (2001), who reported delayed or inconsistent okra seed emergence is due to environmental factors such as low temperature and excess moisture in the soil.

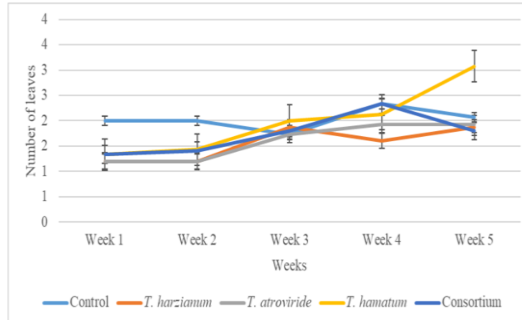
There is no significance difference in the number of leaves, the root length and root mass in every treatment (p>0.05). However, the leaf area when treated with *T. atroviride, T. hamatum and* consortium showed a slightly significant higher compared to control (p>0.05). Production of harzianolide by *T. harzianum* may be a contributing factor to the highest leaf area recorded in this study.



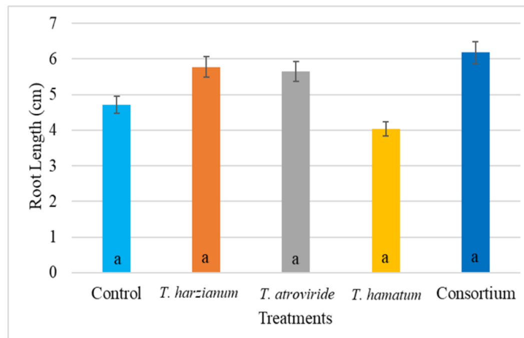
a) The germination rate



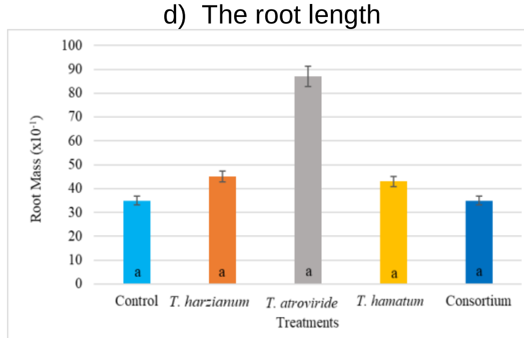
b) The plant height



c) The number of leaves



d) The root length



f) The root mass

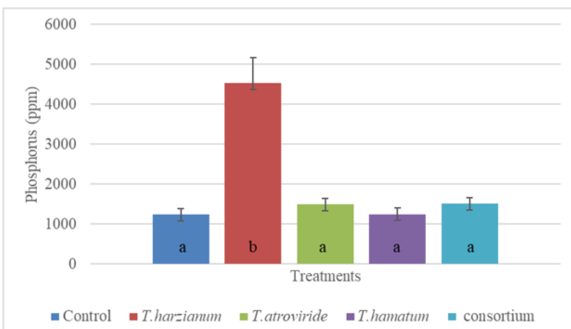
Figure 2: Effect of *Trichoderma*-treated soil on (a) germination rate, (b) plant height, (c) number of leaves, (d) root length, (e) root mass and (f) leaf area. (Means with the same letters are not significantly different at p>0.05)

Analysis of nutrients content in soil-treated with *Trichoderma* spp.

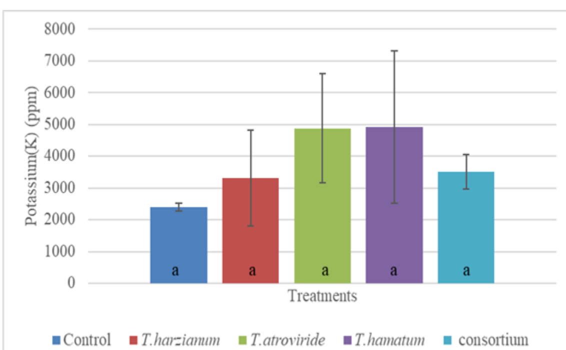
Nutrient content in soil is important for development in plant growth. Bononi et al. (2020) reported *Trichoderma* spp. has the capability of phosphorus-solubilizing that converted organic phosphorus into inorganic phosphorus and make it readily available to the soil for plant uptake. That's explained the presence of phosphorus (P) content in the *Trichoderma* spp.-treated soil is significantly higher when treated with *T.*

harzianum (4527 ppm) compared to other soil treatment (Control: 1240 ppm; *T. hamatum*-treated soil: 1251 ppm; *T. atroviride*-treated soil: 1490 ppm; Consortium: 1515 ppm).

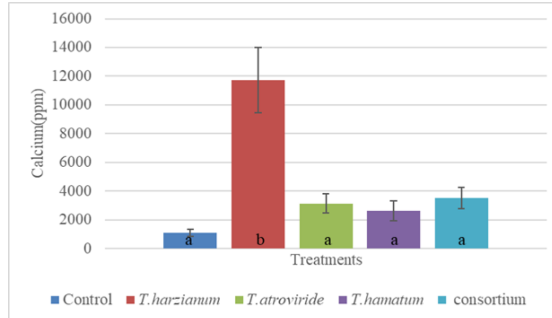
The amount of calcium (Ca), magnesium (Mg), iron (Fe), and manganese (Mn) are significantly higher when treated with *T. harzianum* (11718 ppm, 4558 ppm, 13774 ppm, 729 ppm) compared to other soil treatment (*T. atroviride*-treated soil: 3151 ppm, 937 ppm, 3917 ppm, 145 ppm; *T. hamatum*-treated soil: 2363 ppm, 744 ppm, 6249 ppm, 177 ppm; consortium: 3501 ppm, 904 ppm, 4263 ppm, 160 ppm; and control: 1099 ppm, 2967 ppm, 7889 ppm, 549 ppm). The dissolution of these micronutrients contributed to the soil able to improved colonization of roots and provided better performance in plant metabolism (Jangir et al. 2017).

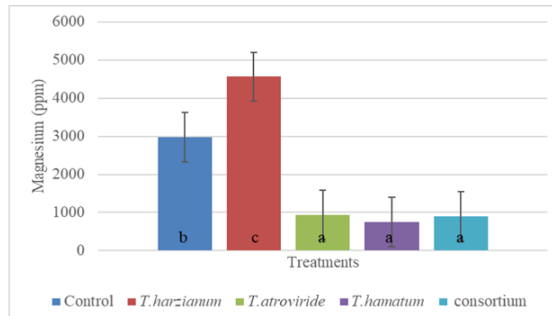


a) Phosphorus



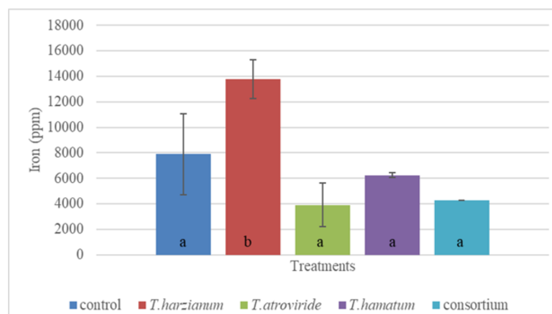
b) Potassium





d) Calcium

d) Magnesium



e) Iron

Figure 3: Nutrient availability of (a) phosphorus, (b) potassium, (c) calcium, (d) magnesium, (e) iron and (f) manganese in soil-treated with *Trichoderma* spp. and control. (Means with the same letters are not significantly different at P>0.05)

CONCLUSION

Based on this study, it showed that plant growth promotion by seed inoculation show effects on plant height, germination, root length and a number of fruits. However, soil application with *Trichoderma* spp. showed a slightly greater effect on plant height, germination, root length and root mass compared to control. The abundance of minerals such as phosphorus, calcium, sodium, magnesium, iron and manganese in *T. harzianum*-treated soil increases the solubilization activity in the soil compared to other soil treatments proved. Both findings support that the *Trichoderma* treatment had the capability of plant growth promotion.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

SNHML performed experiments and data analysis. MMK, MHS, MNL and NAB designed experiments and reviewed the manuscript. All authors read and approved the final version.

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