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Original Article

Influence of *Trichoderma harzianum* and *Bacillus thuringiensis* with reducing rates of NPK on growth, physiology, and fruit quality of *Citrus aurantifolia*

Influência de *Trichoderma harzianum* e *Bacillus thuringiensis* na redução das taxas de NPK no crescimento, fisiologia e qualidade de frutos de *Citrus aurantifolia*

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Abstract

Continuous use of chemical fertilizers gradually shrinks the crop yield and quality, and these adverse effects can be reduced by adopting new sustainable practices such as the use of manure, biofertilizers, and nano fertilizers. Limited information is existed on the application of *Trichoderma harzianum* and *Bacillus thuringiensis* microbes to improve lemon seedlings growth, physiology, and fruit formation. Therefore, the current study is aimed to evaluate the effects of *T. harzianum* and *B. thuringiensis* microbes mixing with low levels of inorganic fertilizer (NPK) on the plant growth, development, and quality of *limau nipis* (key lemon) fruits. The lemon seedlings growing media were inoculated during transplanting with *T. harzianum* and *B. thuringiensis* at various NPK fertilizers under polybagged conditions. The seedlings were grown around eighteen (18) months after inoculation with biofertilizers followed by Randomized Complete Block Design (RCBD) with five (5) replications. The results showed that *T. harzianum* with 50 g of NPK treatment (T2) increased the seedling's height, branch number, leaf height, ground area, and absolute growth rate (AGR) plant height by 50.12%, 107.84%, 17.91%, 17.91%, 116.93%, and 56.02%, respectively, over the control treatment. The number of leaves (60.82%), leaf area (42.75%), stem diameter (27.34%), specific leaf area (SLA) (39.07%), leaf area index (LAI) (54.40%), and absolute growth rate for leaf number (73.86%), leaf area (306.85%) and stem diameter (46.8%) of lemon seedlings increased significantly with *B. thuringiensis* plus 50 g NPK treatment (T3). The applications of *B. thuringiensis* with 25 g NPK fertilizer treatment (T5) increased leaf fresh weight (LFW), leaf dry weight (LDW), leaf moisture content (LMC), specific leaf weight (SLW), leaf relative growth rate (RGR), and chlorophyll content by 96.45%, 56.78%, 13.60%, 24.76%, 45.45%, and 16.22%, respectively, over the control group. In addition, T5 treatment increased the fruits number, individual fruit weight, fruit diameter, fruit dimension, leaf total soluble solids (TSS), and fruit TSS content of lemon tress by 81.81%, 55.52%, 43.54%, 25.69%, 89.47%, and 70.78% compared to the control treatment. Furthermore, soil inoculation of *B. thuringiensis* significantly increased the pulp to peel ratio and juice content of lemon fruits. From this study, it can be concluded that soil inoculation of both *T. harzianum* and *B. thuringiensis* with 25-50% NPK during transplanting improved plant growth, physiology, and fruit quality of *limau nipis* trees.

Keywords: lemon, *Trichoderma harzianum*, *Bacillus thuringiensis*, biofertilizers, growth, fruit quality.

Resumo

O uso contínuo de fertilizantes químicos diminui gradualmente o rendimento e a qualidade das culturas, e esses efeitos adversos podem ser reduzidos com a adoção de novas práticas sustentáveis, como o uso de esterco, biofertilizantes e nanofertilizantes. A informação limitada existe sobre a aplicação de micróbios *Trichoderma harzianum* e *Bacillus thuringiensis* para melhorar o crescimento de mudas de limão, fisiologia e formação de frutos. Portanto, o presente estudo tem como objetivo avaliar os efeitos da mistura de micróbios *T. harzianum* e *B. thuringiensis* com baixo nível de fertilizante inorgânico (NPK) no crescimento, desenvolvimento e qualidade de frutos de *limau nipis* (limão-chave). Os meios de cultivo de mudas de limão foram inoculados durante o transplante com *T. harzianum* e *B. thuringiensis* em vários fertilizantes NPK sob condições de polybag. As mudas foram cultivadas em torno de 10 meses após a inoculação com biofertilizantes seguidas de delineamento em blocos completos

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randomizados (RCBD) com 5 repetições. Os resultados mostraram que *T. harzianum* com 50 g de tratamento NPK (T2) aumentou a altura de plântulas, número de ramos, altura de folha, área do solo e taxa de crescimento absoluto (AGR) em 50,12%, 107,84%, 17,91%, 17,91%, 116,93% e 56,02%, respectivamente, em relação ao tratamento controle. O número de folhas (60,82%), área foliar (42,75%), diâmetro do caule (27,34%), área foliar específica (SLA) (39,07%), índice de área foliar (IAF) (54,40%) e taxa absoluta de crescimento para número de folhas (73,86%), área foliar (306,85%) e diâmetro do caule (46,8%) das mudas de limão aumentaram significativamente com *B. thuringiensis* mais 50 g de tratamento NPK (T3). As aplicações de *B. thuringiensis* com 25 g de tratamento com fertilizante NPK (T5) aumentaram a massa fresca da folha (LFW), massa seca da folha (LDW), teor de umidade da folha (LMC), peso específico da folha (SLW), taxa de crescimento relativo da folha (RGR) e teor de clorofila em 96,45%, 56,78%, 13,60%, 24,76%, 45,45% e 16,22%, respectivamente, em relação ao grupo controle. Além disso, o tratamento T5 aumentou o número de frutos, peso individual do fruto, diâmetro do fruto, dimensão do fruto, sólidos solúveis totais foliares (SST) e teor de SST do fruto do limão em 81,81%, 55,52%, 43,54%, 25,69%, 89,47% e 70,78% em relação ao tratamento controle. Além disso, a inoculação no solo de *B. thuringiensis* aumentou significativamente a relação polpa/casca e o teor de suco de frutos de limão. A partir deste estudo, pode-se concluir que a inoculação no solo de *T. harzianum* e *B. thuringiensis* com 25-50% de NPK durante o transplante melhorou o crescimento das plantas, a fisiologia e a qualidade dos frutos de *limau nipis*.

Palavras-chave: lemon, *Trichoderma harzianum*, *Bacillus thuringiensis*, biofertilizantes, crescimento, qualidade de frutos.

1. Introduction

Key lemon or *limau nipis* (*Citrus aurantifolia*) are scattered in Southeast Asia, especially Malaysia. Trees have evergreen leaves leathery ovoid in shape, the margin serrate with sharp spines in the axils of the stalks. Bisexual flowers consist of five petals born in the leaf axile of lemon branches. Lemon fruit is a berry with an oval shape, its color turns from green into yellow during ripening, with a broad, low, and apical nipple, the pulp forms 8-10 segments containing acidic juice, the seeds are small oval white or yellowish-white in color some fruits are seedless (Mabberley, 2004). The fruit content can be a natural flavor and preservative added to different foods and salad, sauces, and baked foods. It is also used in the manufacture of soft drinks and the addition of the acidic taste of food products (González-Molina et al., 2010). As known, its juice is rich in vitamin C, which is fortified the immunity of the human body. Also, it is an important source of flavonoids, known as antioxidants, which remove free radicals that damage tissue cells within the body, regular eating of foods containing flavonoids leads to protection from cancer and cardiovascular diseases (Bondonno et al., 2019).

The productivity of lemon plants is increased with organic matter containing soil and sunny periods but is reduced due to problematic soil, high rainfall, and humidity during the growing season (Ajuru et al., 2018). An imbalance in chemical fertilizer use can also cause negative impacts on the soil properties and microbial population, which may reduce plant growth, yield, and fruit quality. On the other hand, inorganic fertilizer is limited due to its high price, limited availability, and low benefit ratio (Ano and Agwu, 2005). Using the bio-fertilizers can be helped to achieve sustainability of farms, minimizing the use of mineral fertilizers for non-depletion of raw materials and increasing their price, increasing soil fertility and maintaining its natural structure, reducing concern about environmental risks, increasing crop production to provide food security, and improving the productivity of the trees (Akhtar and Siddiqui, 2009). Bio-fertilizers with chemicals increased the macronutrients content, organic carbon, nutrient availability, and improved soil pH (Ipsita and Singh, 2014). *Trichoderma harzianum* is one of the effective agents for controlling soil-borne pathogens that infect the roots of plants, and these fungi produce some compounds (antibiotics and others) that increase the resistance of the roots to pathogens and their effect. *Trichoderma spp* reduces the cost to the producers, so it is widely used to control diseases and safely for soil and plants, unlike chemical pesticides that harm the soil and human health, which promotes root and shoot development (Harman et al., 2004). Zin and Badaluddin (2020) stated that *Trichoderma spp* improves plant growth and defeats plant pathogenic fungi and bacteria growth. They also reported that *Trichoderma spp* secretes the secondary metabolites that suppress plant pathogenic microorganisms' growth and stimulate plant growth. The application of *Trichoderma spp* regulates the root architecture increases the growth of primary and lateral roots, resulting in nutrient uptake and accumulation in the plants (Cai et al., 2013; Yedidia et al., 2001). Cai et al. (2013) also stated that inoculation of *T. harzianum* strain SQR-T037 released the harzianolide secondary metabolite, which increased the seedling growth of tomato.

Bacillus thuringiensis is one of some microbes that improve plant growth which found in low numbers in the rhizospheric soil, and their effect on growth is a result of fixing N, dissolving P, and producing some growth regulators such as IAA and cytokines, which also produces inhibitory compounds for pathogens that are transmitted through the soil. The soil application of *Bacillus* with *Trichoderma spp* with basal fertilization increased the root growth and development, nutrient accumulation, and biomass content of strawberry plants (Eligio et al., 2006). Pırlak and Köse (2009) stated that soil application of Bacillus sp on the strawberry plants increased the fruits' number, TSS, and sugar content but decreased the fruit weight and pH of fruit juice TSS/acidity ratio. Banana seedlings inoculated with *Bacillus sp.* strains increased the adventitious root number, the length of pseudostem, the total biomass of the seedlings, and the leaf mineral content of banana plants (Jaizme et al., 2003). Bacillus, with other biofertilizers, may increase the auxin synthesis, which improved the plant growth through root development, increased the leaf area, shoot height, and enhanced the dry matter accumulation of the plants (Kavoo-Mwangi et al., 2013). Tomato seedlings treated with some strains of *B. thuringiensis* increased the root and shoot elongation, and the height of the plant's height and the fresh and dry matter content of the treated tomato plants increased significantly (Jiaheling et al., 2016). The application of biofertilizers increased the fruit set, fruit growth, TSS content, TSS: acidity ratio, and vitamin C content in the guava fruits (Dey et al., 2005).

As stated earlier, imbalance and constant use of inorganic fertilizers degrades soil properties, increases water and environmental pollution, and gradually shrinks the yield and quality of lemon fruits. Lemon trees do not develop a good structure root system, and sometimes fruit production is very low due to an imbalance in nutrient uptake and accumulation by a poor root system. Thus, suitable and well-balanced inorganic and biofertilizers need to apply to lemon trees for proper plant growth, fruit production, and quality improvement. The potential of *T. harzianum* and *B. thuringiensis* as biofertilizers on *limau nipis* tree's growth, development, and fruit quality improvement has not been exploited yet. In this study, we investigated the effectiveness of *T. harzianum* and *B. thuringiensis* in reducing rates of NPK fertilizers on morphology, physiology, and fruit quality on *limau nipis* trees. We also studied the growth analysis of lemon trees by the influence of these two microorganisms to determine the mechanism action of *T. harzianum* and *B. thuringiensis*. It is proposed that these two microbes can positively regulate the morphology, physiology, fruit formation, and fruit quality of *limau nipis*.

2. Materials and Methods

This study was conducted at the research farm of Bioresources and Food Industry Faculty at Universiti Sultan Zainal Abidin (UniSZA), Besut Campus, Besut, Terengganu, from February 2020 to December 2021. The current research was conducted to determine the effects of two bio-fertilizers (*T. harzianum* and *B. thuringiensis*) with reducing rates of NPK on growth development, physiological characteristics, and fruit quality in Key lime (*limau nipis*) seedlings under potted conditions. Eight treatments were applied to the same age (six months old) and shape of 40 healthy seedlings with five replicates planted in plastic bags (35 cm × 25 cm) containing 50% garden soil + 50% organic soil (plant residue-based organic soil) as growing medium (Table 1). Seedlings were planted under a drip irrigation system at the rate of five minutes every 12 hours in the first month, and the rate was gradually increased later according to age and need. For weeding, it was done manually every two weeks. A Randomized Complete Block Design (RCBD) was used for arranging the differential investigated treatments. The treatments were added as follows:

To prepare the treatment combinations 10 g of *Trichoderma* or *Bacillus* powder was considered as 100% as 100 g microbe per seedling may create negative impacts on plant growth and development of *limau nipis* (Table 1). The amount of microbial powder and treatment combinations were selected according to the study of Dheware and Waghmare (2009). They used 10 g microbial powder for the citrus trees. In this study, *T. harzianum* and *B. thuringiensis* were inoculated once to the root zone in each seedling during transplanting of the seedlings in polybags, while NPK fertilizers were applied twice, the first after a week from transplanted seedlings and the second was after one month from the first addition. The response of *limau nipis* seedlings to two biofertilizers and NPK application rates were measured and recorded as the following data:

2.1. Morphological parameters

Six months (6) old *limau nipis* seedlings were used in this study to investigate the effects of two biofertilizers. The experiment was started in February 2020 and after six months (6) from inoculation, the morphological and physiological parameters were measured 2 times 3 months interval.

The height (cm) of lemon seedling was measured by meter tab, leaf, and branch numbers were accounted for manually, and the vernier caliper measured stem diameter (cm). The following equation estimated the leaf area (cm^2) of lemon trees; leaf area = leaf length × mean of the width of the leaf.

2.2. Growth characteristics

Leaf height (LH) is the distance between the soil surface and the node corresponding to the leaf. Ground area (GA) means the total ground plant area covered by the plant (canopy area), and it is expressed as cm^2. An electronic balance (ML 204, Mettler Toledo company, Switzerland) was used to measure the leaf fresh weight after collecting the leaves from the treated and untreated plants. After measuring the fresh weight, leaves were placed into an oven at 80°C until achieving a constant dry weight. Leaf dry weight is also measured by using an electronic balance. Leaf moisture content was measured and expressed on a wet basis (Equation 1).

$$\text{Leaf Moisture Content}\left(\%\right)=\frac{\text{Ww}-\text{Wd}}{\text{Ww}}\times 100 \qquad (1)$$

Where Ww is wet weight and Wd is the dry weight of the leaf.

2.3. Growth analysis of lemon seedlings

Specific Leaf Area (SLA) is the ratio of plant leaf area and leaf dry weight and is expressed in cm^2g^{-1} (Kvet et al., 1971). The photosynthesis surface of the plants is positively correlated with SLA but final yield production is not only determined by the SLA. The following equation measured specific leaf area (Equation 2):

$$\text{Specific Leaf Area}\left(\text{SLA}\right)=\frac{\text{Leaf area}}{\text{Leaf dry weight}} \qquad (2)$$

Specific Leaf Weight (SLW) represents the ratio between leaf dry weight and leaf area, and this ratio is expressed as $g\ cm^{-2}$ as reported by Pearce et al. (1968). The accumulation of dry matter inside the leaves increases if the specific leaf weight per unit leaf area increases, which reflects the yield positively and the following equation measures it (Equation 3):

$$\text{Specific Leaf Weight}\left(\text{SLW}\right)=\frac{\text{Leaf dry weight}}{\text{leaf area}} \quad (3)$$

The relative Growth Rate (RGR) of the leaf is the rate of increment of dry matter per unit of biomass per time unit (a day) and expressed as unit dry weight /unit dry weight /unit time (g g^{-1} day^{-1}), it is calculated as (Equation 4):

$$\text{Relative Growth Rate}\left(\text{RGR}\right)=\frac{\text{DM2}-\text{DM1}}{\text{t2}-\text{t1}}\times\frac{1}{\text{DM1}} \quad (4)$$

Where DM_2 is the dry matter yield at time t_2 and DM_1 is the dry matter yield at t_1. t2 - t1, represent the difference between the two times assessed by Tylova-Munzarova et al. (2005).

Absolute Growth Rate (AGR) of plant height: AGR is the mean total growth per unit of time, this rate of absolute growth includes the value of biomass between a specified period, it was calculated by using this equation according to Williams (1946), which express as cm day^{-1} (Equation 5)

$$\text{Absolute Growth Rate for plant height}\left(\text{AGR}\right)=\frac{\text{h2}-\text{h1}}{\text{t2}-\text{t1}} \quad (5)$$

Whereas h2 and h1represent the plant height at times t2 and t1, respectively. AGR for leaf number, leaf area, and stem diameter was also estimated using the same absolute growth rate equation (Williams, 1946).

Leaf Area Index (LAI): LAI can be expressed by the ratio of total leaf area and plant ground area (Williams, 1946). The total plant leaf area of a plant was calculated by counting the total number of leaves and multiplying with the individual leaf area of the plant; the following equation estimated it (Equation 6):

$$\text{Leaf Area Index}\left(\text{LAI}\right)=\frac{\text{Total leaf area of a plant}}{\text{Ground area occupied by the plant}} \quad (6)$$

2.4. Physiological characteristics of lemon trees

Junior-Pam chlorophyll fluorometer was used for measuring the chlorophyll fluorescence in the leaves of lemon trees. For measuring the chlorophyll fluorescence, the meter was connected to a special computer unit by USB cable and installed with WinControl-3 software. It was represented in lower fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), and photosynthetic yield (Fv/Fm). Leaf chlorophyll fluorescence and photosynthetic yield of treated and untreated lemon trees were measured according to the methods described by Khandaker et al. (2017). A portable chlorophyll meter (SPAD-502 Minolta Japan) was used to measure the chlorophyll content in the leaves. The SPAD meter was calibrated about 15 minutes before use. The leaf clip was fixed in clean mature leaf tissue, and then chlorophyll content was shown within two seconds (Khandaker et al., 2018). Leaf stomatal conductance (mmol /m^2 s) was measured with a handheld leaf porometer according to the methods described by Jamaludin et al. (2020).

2.5. Fruit development and quality measurements

The *limau nipis* fruits were harvested 4 months after flowering from the treated and untreated plants. Fruit growth and developmental characteristics (fruit weight, fruit diameter, fruit dimension, and pulp to peel ratio were measured and recorded after harvesting the fruits from the treated and controlled lemon trees. The fruit weight of the lemon was measured and recorded by using an electronic weighing balance after harvesting (Model: Mettle PJ3000, Japan). Fruit diameter and fruit dimension were measured by using a vernier caliper. A fruit Juicer extracted fruit juice content, and the fruits were collected for each treatment, then washed and put in the fruit juicer; the juice extractor (Citrus Juicer, NS-2000E-6, China) with reamer extractors (RE) was used to extract lemon juice. The fruits of lemon were put automatically into the juicer, cut in half, and the halved fruits were pressed onto the automatic self-reversing reamer (Li et al., 2021).

Leaf total soluble solids (TSS) is the percentage of soluble solids present in a pure sap or juice of a leaf, and it represents as Brix index (Magwaza and Opara, 2015). Leaf TSS was measured by hand refractometer according to the steps described by Buang et al. (2018), where after collecting leaves samples for different treatments, they were cleaned with distilled water and dried by air, and leaf veins were removed. The samples were ground and for each 1g sample added 1 ml of distilled water was to make juice and two drops of leaf juice were placed on the refractometer sensor. Fruit TSS content was also evaluated with a hand refractometer (Atago, Japan). After collecting lemon fruits for each treatment, two drops of lemon juice were put into the refractometer sensor to read and record the data as the same leaf TSS content according to the procedure mentioned by Buang et al. (2018).

2.6. Statistical analysis

The obtained data of this study were analyzed using SPSS software (version 20.0; SPSS Inc). The numerical data related to the growth and development of lemon trees were taken as means and standard deviations. One-way ANOVA was used to compare variables between the control treatments with other treatments, and data is considered statistically significant when P-value is less than 0.05. Then, the Duncan Multiple Range Test (DMRT) was run to identify which treatment significantly differed from the control treatment.

3. Results and Discussion

In this current study, different morpho-physiological, growth, yield, and quality characteristics were evaluated under different rates of two biofertilizers and NPK fertilizers. At different rates, two microbes *T. harzianum* and *B. thuringiensis* were inoculated in the soil during the transplanting of lemon seedlings (Table 1). This study showed that lemon tree growth, morphological, physiological, and yield characteristics were affected significantly by different treatments of microbes as biofertilizers.

3.1. Morphological characteristics

The data regarding the morphological and growth characteristics of *limau nipis* seedling as affected by two biofertilizers (*T. harzianum* and *B. thuringiensis)* with a reducing rate of NPK fertilizers are presented in Table 2. The seedling's growth and developmental characteristics of lemon were affected significantly by applying the two biofertilizers with reducing NPK fertilizers (Table 2).

The highest plant height (146.00 cm) and branch number per plant (26.50) were recorded in T2. The control treatment yielded the lowest plant height (97.25 cm) and branch number (12.75) (Table 2). The leaf number and leaf area of lemon trees were also significantly affected by the application of biofertilizers and reduced rates of NPK (Table 2). Table 2 showed that the highest leaf number per plant (369.5) was recorded in T3 treatment followed by T5, T4, T2, T1, T6, and T7 with a value of 325.50, 309.50, 288.30, 283.30, 240.30 and 230.50, respectively. The highest leaf area (42.87 cm^2) was observed in T3 treatment followed by T4, T5, T2, T7, T1, and T6 with a value of 39.82 cm^2, 37.90 cm^2, 36.12 cm^2, 34.78 cm^2, 33.70 $cm^{2,}$ and 33.30 cm^2, respectively. Whereas, the lowest leaf number (229.75) and leaf area (30.03 cm^2)were also recorded in the control treatment (Table 2). In this study, the biofertilizers and reduced rates of NPK treatments did not significantly affect the stem diameter of lemon trees (Table 2). The higher stem diameter of lemon seedlings was recorded in the T3 treatment with a value of 1.56 cm, and the control treatment produced the lowest stem diameter of lemon seedlings (Table 2).

Table 1. Treatment application and arrangement of this current study.

Treatment	Title
T0	Without fertilization (as control)
T1	NPK 100% (equivalent to 100 g NPK)
T2	*T. harzianum* 50% (equivalent 5 g) + NPK 50%
T3	*B. thuringiensis* 50% (equivalent 5 g) + NPK 50%
T4	*T. harzianum* 75% (7.5 g) + NPK 25%
T5	*B. thuringiensis* 75% (7.5 g) + NPK 25%
T6	*T. harzianum* 100% (10 g)
T7	*B. thuringiensis* 100% (10 g)

3.2. Leaf morphological and growth characteristics

The two biofertilizers with a reducing rate of NPK produced significant effects on leaf morphology and growth characteristics of *limau nipis* seedlings in this study (Table 3). The highest value of leaf height (19.75 cm) was observed in T2 followed by T4, T1, T5, T6, and T7 treatments with a value of 19.25 cm, 18.50 cm, 17.25 cm, 16.50 cm, and 14.25 cm, respectively. While the lowest leaf height (12.00 cm) was observed in T3 (*B. thuringiensis* 50% (5 g) + NPK 50%) (Table 3). The ground area of *limau nipis* trees was also significantly affected by the two biofertilizers with a reducing rate of NPK. The highest value of ground area (7764.0 cm^2) was recorded in T2 followed by T1, T3, T4, T5, T6 and T7 treatments with a value of 6656.0 cm^2, 5327.0 cm^2, 5314.0 cm^2, 4944.0 cm^2, 4938.0 cm^2, and 3684.0 cm^2, respectively. The control plants (T0) gave the lowest ground area with a value of 3579.0 cm^2 (Table 3). Leaf fresh, leaf dry weight, and leaf moisture content of lemon trees were affected significantly by the treatments in the study. The highest value of leaf fresh weight (1.55 g) and dry weight (0.49g) were recorded in T5, followed by treatments T1, T7, and T2. While the lowest leaf fresh & dry weight was recorded in control treatment T0 (Table 3). Leaf moisture content was highest in the T1 treatment with 71.76%, while the control treatment produced the lowest leaf moisture content (59.81%).

3.3. Leaf growth analysis

The specific leaf area (SLA), specific leaf weight (SLW), and leaf relative growth rate (RGR) data were measured and presented in Figures 1-3). The results showed that lemon trees' SLA, SLW, and RGR were significantly affected

Table 2. The influence of biofertilizers mixing with different levels of NPK on growth and morphological characteristics in *limau nipis (Citrus aurantifolia)*.

Treatment	Plant height (cm)	No. of branches/ plant	No. of leaves/ plant	Leaf area (cm^2)	Stem diameter (cm)
T0	97.25^{b}	12.75^{c}	229.75^{d}	30.03^{b}	1.225^{a}
T1	106.00^{b}	19.25^{b}	283.30^{bc}	33.70^{ab}	1.405^{a}
T2	146.00^{a}	26.50^{a}	288.30^{b}	36.12^{ab}	1.413^{a}
T3	107.50^{b}	23.75^{ab}	369.50^{a}	42.87^{a}	1.560^{a}
T4	113.00^{b}	26.25^{a}	309.50^{b}	39.82^{ab}	1.450^{a}
T5	117.00^{b}	25.50^{a}	325.50^{ab}	37.90^{ab}	1.340^{a}
T6	116.30^{b}	24.50^{a}	240.30^{cd}	33.30^{ab}	1.330^{a}
T7	105.30^{b}	22.75^{ab}	230.50^{d}	34.78^{ab}	1.327^{a}

Means within the same column of the table followed by a different letter differ significantly according to DMRT at P ≤ 0.0.5. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

Table 3. Effect of two biofertilizers mixing with different levels of NPK on leaf morphological and growth characteristics in *limau nipis (Citrus aurantifolia)*.

Treatment	Leaf height (cm)	Ground area ($cm^{2)}$	Leaf fresh wt. (g)	Leaf dry wt. (g)	Leaf moisture content (%)
T0	16.75^{ab}	3579.0^{c}	0.79^{d}	0.31^{c}	59.81^{b}
T1	18.50^{a}	6656.0^{ab}	1.29^{b}	0.36^{b}	71.76^{a}
T2	19.75^{a}	7764.0^{a}	1.05^{c}	0.36^{b}	65.48^{a}
T3	12.00^{b}	5327.0^{ab}	0.91^{c}	0.32^{c}	65.56^{a}
T4	19.25^{a}	5314.0^{bc}	1.25^{b}	0.43^{a}	65.40^{a}
T5	17.25^{ab}	4944.0^{bc}	1.55^{a}	0.49^{a}	67.95^{a}
T6	16.50^{ab}	4938.0^{bc}	0.99^{c}	0.32^{c}	67.67^{a}
T7	14.25^{ab}	3684.0^{c}	1.12^{bc}	0.42^{a}	62.52^{ab}

Means within the same column of the table followed by a different letter differ significantly according to DMRT at $P \leq 0.0.5$. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

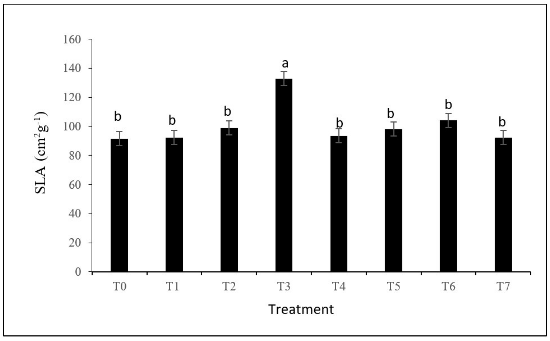

Figure 1. Effect of two biofertilizers mixing with different level of NPK on specific leaf area of *limau nipis*. Error bars indicate ± S. E. Different small case letters in mean value bars represent statistical difference at 5% level. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

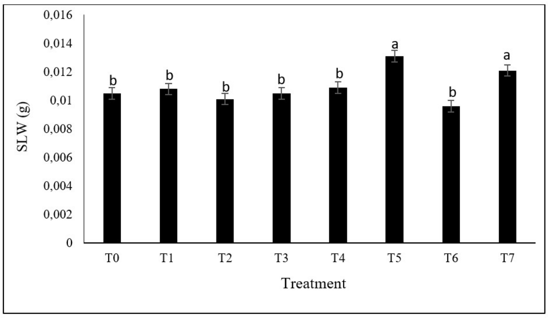

Figure 2. Effect of two biofertilizers mixing with different level of NPK on specific leaf weight of *limau nipis*. Error bars indicate ± S. E. Different small case letters in mean value bars represent statistical difference at 5% level. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

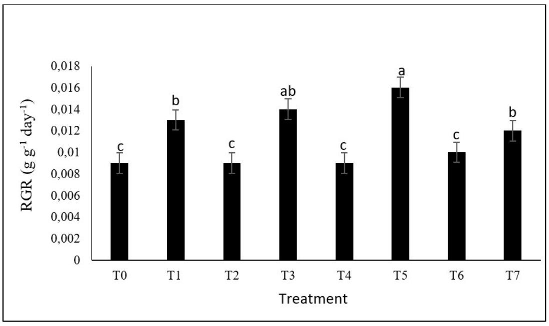

Figure 3. Effect of two biofertilizers mixing with different level of NPK on relative growth rate (RGR) of *limau nipis*. Error bars indicate ± S. E. Different small case letters in mean value bars represent statistical difference at 5% level. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%; T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

by applying the two biofertilizers with reducing NPK fertilizers (Figures 1-3).

The highest value of SLA (132.93 cm^2g^{-1}) was recorded when lemon trees were treated with T3 followed by T6, T2, and T7 treatments, while the lowest value was recorded when T0 treated trees with a value (91.18 cm^2g^{-1}) (Figure 1). For SLW, the highest value (0.0131 g cm^{-2}) was recorded in T5 followed by T7 with a value of 0.0121 g cm^{-2} and other treatments, while the lowest value (0.0075 g cm^{-2}) was recorded when trees were treated by T3 (Figure 2). As for leaf RGR, the results showed that when lemon trees treated with T5 gave the best value (0.016 g g^{-1} day^{-1}), followed by T1 and T7, while the lowest value (0.009 g g^{-1} day^{-1}) was given by T0 (Figure 3).

3.4. Plant growth analysis

Table 4 represents the data regarding the absolute growth rate (AGR) of plant height, leaf number, leaf area, stem diameter, and leaf area index of treated and untreated *limau nipis* trees. The results showed that the AGR of plant height, number of leaves, and leaf area of lemon trees were affected significantly by the different applications of the two biofertilizers with reducing NPK fertilizers (Table 4).

The result indicates that the AGR of plant height was the highest in the T2 treatment with a value of 0.44 cm day^{-1}, whereas the lowest absolute growth was found in the control treatment (0.28 cm day^{-1}) (Table 4). The highest value for AGR of leaf number (1.5330 cm day^{-1}) was recorded in T3 followed by T5 (1.3700 cm day^{-1}), while the lowest value (0.8800 cm day^{-1}) was recorded in the control treatment (Table 4). The highest value of AGR of leaf area (0.07125 cm day^{-1}) was recorded by T3 followed by T2, while the lowest value (0.0175 cm day^{-1}) was recorded by control treatment (Table 4). For the leaf area index, the results revealed that T3 gave the highest value (2.98), followed by T5 (2.50), whilst (T2) gave the lowest value (1.34) (Table 4). In this study, the absolute growth rate of stem diameter was not significantly affected by applying the two biofertilizers with a reducing rate of NPK (Table 4). The highest value of AGR of stem diameter (0.003675 cm day^{-1}) was recorded in T3 followed by T4, while the lowest value was recorded in the control treatment (0.0025 cm day^{-1}) (Table 4).

3.5. Physiological characteristics of lemon trees

The result indicates that lower fluorescence (F0) and chlorophyll content (SPAD unit) were affected significantly by the biofertilizer treatments (Table 5). However, maximum fluorescence (Fm), photosynthetic yield (Fv/Fm), and stomatal conductance of lemon trees were not affected significantly by the treatments of biofertilizers with inorganic fertilizers (Table 5).

The highest value of lower fluorescence (f0) (412.5) and maximum fluorescence (Fm) (1842) were read in T6, followed by T3, while control treatment T0 read the lowest content of chlorophyll fluorescence (F0) (348.5) and T1 gave the lowest content of chlorophyll fluorescence (Fm) (1710) (Table 5). The highest photosynthetic yield (Fv/Fm) was read in T4 with a value of 0.79, followed

Table 4. Effect of two biofertilizers mixing with different levels of NPK on plant growth analysis (AGR and leaf index) in *limau nipis (Citrus aurantifolia).*

Treatment	AGR of plant height (cm day^{-1})	AGR of leaf number (cm day^{-1})	AGR of leaf area (cm day^{-1})	AGR of stem diameter (cm day^{-1})	Leaf area index (LAI)
T0	0.28^{b}	0.88^{f}	0.017^{b}	0.0025^{a}	1.93^{b}
T1	0.29^{b}	1.27^{c}	0.033^{ab}	0.0035^{a}	1.56^{b}
T2	0.44a	1.18^{d}	0.060^{ab}	0.0036^{a}	1.34^{b}
T3	0.31^{b}	1.53^{a}	0.071^{a}	0.0036^{a}	2.98^{a}
T4	0.29^{b}	1.27^{c}	0.057^{ab}	0.0035^{a}	2.33^{a}
T5	0.30^{b}	1.37^{b}	0.054^{ab}	0.0025^{a}	2.50^{a}
T6	0.31^{b}	0.93^{e}	0.028^{ab}	0.0032^{a}	1.70^{b}
T7	0.29^{b}	0.91^{ef}	0.041^{ab}	0.0027^{a}	2.23^{ab}

Means within the same column of the table followed by a different letter differ significantly according to DMRT at P ≤ 0.0.5. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

Table 5. Effect of two biofertilizers mixing with different levels of NPK on physiological characteristics in *limau nipis (Citrus aurantifolia).*

Treatment	Lower fluorescence (F0)	Maximum fluorescence (Fm)	Photosynthetic yield (Fv/Fm)	Stomatal conductance (mmol/m^2 s)	Chlorophyll content SPAD value
T0	348.5^{b}	1720^{a}	0.77^{a}	112.1^{a}	44.38^{e}
T1	358.5^{b}	1710^{a}	0.79^{a}	115.0^{a}	49.53^{d}
T2	374.3^{ab}	1768^{a}	0.78^{a}	131.0^{a}	52.95^{bcd}
T3	412.0^{a}	1835^{a}	0.77^{a}	128.2^{a}	53.85^{bc}
T4	361.5^{b}	1788^{a}	0.79^{a}	147.4^{a}	53.30^{bcd}
T5	367.8^{b}	1731^{a}	0.78^{a}	131.5^{a}	57.05^{b}
T6	412.5^{a}	1842^{a}	0.78^{a}	129.8^{a}	64.18^{a}
T7	354.8^{b}	1735^{a}	0.77^{a}	144.4^{a}	51.58^{cd}

Means within the same column of the table followed by a different letter differ significantly according to DMRT at P ≤ 0.0.5. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

by T2 and other treatments, although their differences were not statistically significant at the 5% level (Table 5). The highest value of stomatal conductance (147.4 mmol /m^2 s) was observed in T4, followed by T7. The lowest stomatal conductance value (112.1 mmol /m^2 s) was recorded in the control treatment (Table 5). In this study, chlorophyll content (SPAD value) was significantly affected by applying the two biofertilizers with a reducing rate of NPK in limau nipis (Table 5). The chlorophyll content was highest in T6 (64.18 SPAD), followed by T5, and the lowest SPAD value (44.38) was read in untreated control plants (T0) (Table 5).

3.6. Fruit growth and quality parameters

Table 6 represents the lemon fruit's growth and development data as affected by *T. harzianum* and *B. thuringiensis* with reduced rates of NPK fertilizers. The results showed that fruit number, fruit weight, fruit diameter, pulp to peel ratio, and fruit juice content of lemon trees were significantly affected by soil application of the two biofertilizers with reducing rates of NPK fertilizers (Table 6).

In this study, the highest fruit number per plant (20 fruits), individual fruit weight (63.78 g), and fruit diameter (5.675 cm) were recorded in the T5 treatment from the first harvest, while the control treatment (T0) gave the lowest value of fruit number per plant (11), fruit weight (41.013 gm) and fruit diameter (3.957 cm) (Table 6). Different treatments also affected the fruit pulp to peel ratio and fruit juice percentage of lemon. The highest value of pulp to peel ratio (8.750) and fruit juice (50.04%) was recorded in T7, whilst the lowest value of pulp to peel ratio (4.493), and fruit juice (30.26%) was recorded in the control treatment (Table 6).

3.7. Leaf and fruit TSS content

The leaf and fruit TSS content of limau nipis trees as affected significantly by T. harzianum and B. thuringiensis with a reducing rate of NPK fertilizers are present in Figures 4-5.

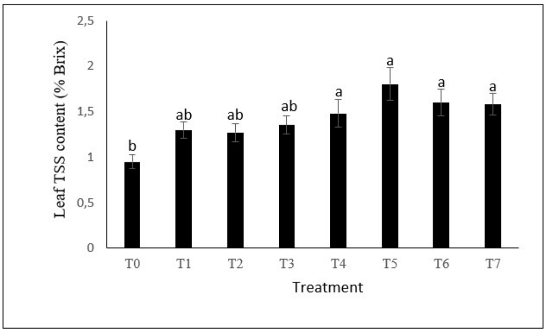

Figure 4. Effect of two biofertilizers mixing with different level of NPK on leaf TSS content of Key lemon (*Limau nipis*). Error bars indicates ±SE. Different letters in the bar graph represent the statistically significant at 5% level. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

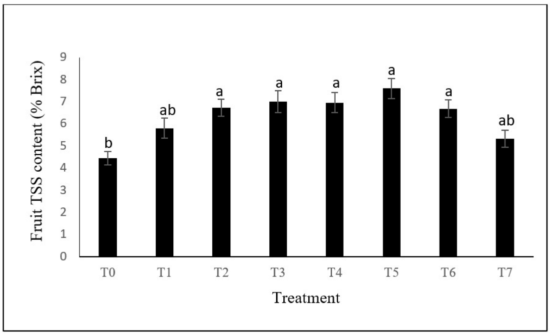

Figure 5. Effect of two biofertilizers mixing with different level of NPK on fruit TSS content of Key lemon (*Limau nips*). Error bars indicates ±SE. Different letters in the bar graph represent the statistically significant at 5% level. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

Table 6. Effect of two biofertilizers mixing with different levels of NPK on fruiting and fruit quality in *limau nipis (Citrus aurantifolia).*

Treatment	No fruits/plant	Fruit weight (gm)	Fruit diameter (cm)	Fruit dimension	Pulp to peel ratio	Fruit Juice %
T0	11.00^{c}	41.013^{e}	3.957^{d}	5.375^{bc}	4.493^{f}	30.26^{e}
T1	14.00^{b}	48.22^{d}	4.512^{c}	4.975^{bc}	5.450^{e}	38.46^{d}
T2	17.00^{a}	51.40^{cd}	4.515^{c}	5.325^{bc}	5.330^{e}	43.20^{bcd}
T3	15.00^{b}	52.88^{c}	4.700^{bc}	5.905^{b}	6.700^{cd}	39.66^{cd}
T4	18.00^{a}	58.10^{b}	4.850^{b}	6.075^{ab}	6.238^{d}	44.10^{bc}
T5	20.00^{a}	63.78^{a}	5.675^{a}	6.750^{a}	7.250^{bc}	46.04^{ab}
T6	19.00^{a}	62.93^{a}	4.657^{bc}	5.655^{bc}	7.665^{b}	49.40^{a}
T7	17.00^{a}	62.93^{a}	4.900^{b}	5.668^{bc}	8.750^{a}	50.04^{a}

Means within the same column of the table followed by a different letter differ significantly according to DMRT at P ≤ 0.0.5. T0, control; T1, NPK 100% (100 g); T2, *T. harzianum* 50% (5g) + NPK 50%; T3, *B. thuringiensis* 50% (5g) + NPK 50%; T4, *T. harzianum* 75% (7.5g) + NPK 25%; T5, *B. thuringiensis* 75% (7.5 g) + NPK 25%, T6, 100% *T. harzianum* (10 g); T7, 100% *B. thuringiensis* (10 g).

Our results showed that the leaf TSS and fruit TSS content were significantly affected by the treatment of biofertilizers and reduced NPK fertilizers (Figures 4-5). T5 treatment produced the highest amount of leaf TSS content (1.80), followed by T6 (1.60) and T4 (1.48) % Brix. The lowest value of TSS (0.95) was recorded in the leaves of control plants (Figure 4).

The highest amount of fruit TSS content (7.60% Brix) was recorded in T5, followed by T4 (6.67% Brix), and the lowest value (4.45% Brix) was recorded in the control treatment (Figure 5).

4. Discussion

In this study, the effects of two beneficial microbes *T. harzianum* and *B. thuringiensis* with reducing rates of NPK were investigated on the *limau nipis* growth, physiology, and fruit quality under polybagged conditions. Our results showed that key lemon growth and developmental characteristics were affected significantly by these two beneficial microbes. Similar types of findings on other lemon varieties have also been reported by several researchers. The shoot length and leaf area of Eureka lemon trees were increased by added biofertilizers and farmyard manure with a little rate of NPK fertilizers (Ennab, 2016). Khehra and Bal (2014) also reported that the biofertilizers with chemical fertilizer led to improvements in vegetative growth like stem height, the diameter of the stem, and the canopy diameter of lemon trees. This study showed that T2 treatment increased the seedling's height (50.12%) and the number of branches (107.84%) over the control group. The number of leaves, leaf area, and stem diameter of *limau nipis* seedlings was 60.82%, 42.75%, and 27.34% higher in T3 treatment compared to the control. In addition, El-Khawaga and Maklad (2013) reported that applying *Azotobacter chroococcum* and *Bacillus sp.* with inorganic N led to an increase in vegetative growth in orange trees compared to trees fertilized by nitrogen only. This increase in vegetative growth might be due to the addition of biofertilizers increasing the content of suitable nutrients and soil enzymes activity, which reflect on planting roots improving and leads to increasing the vegetative growth as mentioned by Lal and Dayal (2014) and Barakat et al. (2012).

Biofertilizers with reducing rates of NPK was significantly affected leaf height, ground area, leaf fresh and dry weight, and leaf moisture content of *limau nipis* trees. Leaf height and ground areas were 17.91% and 116.93% higher in T2 treatment, while, the leaf fresh weight, leaf dry weight, and leaf moisture content were 96.20%, 58.06, and 14.60% higher in T5 treatment compared to the control plants. These results agree with many previous studies; hence, Mohammad et al. (2010) reported that leaf characteristics of pumpkins increased with the added organic and biofertilizer fertilizers in the soil. Soil application with biofertilizers significantly affected Basil's leaf fresh and dry matter (Jahan et al., 2013). Application with biofertilizers and organic manure with reduced chemical fertilizers increased Eureka lemon leaves' fresh and dry weight (Ennab, 2016). Maybe the beneficial microorganisms increased plant growth by producing plant growth hormones, provoking metabolic activities of roots, and supplying biologically fixed nitrogen to the plants. Increasing numbers of active microorganisms in the soil increase the biodegradation process and suitable nutrients, promoting the shoot and leaves formation in seedlings (Ingham, 2005; Oludele et al., 2019).

Results of this study indicated that lemon leaf's SLA, SLW, and RGR were significantly affected by the two biofertilizers treatments with NPK treatments. The leaf's SLA was 44.52% higher at T3 treatment, on the other hand, SLW and RGR were 24.76% and 77.78% higher at T5 treatment compared to the control plants. Al-Freeh et al. (2019) reported that Oat plants treated with biofertilizers gave a higher rate of RGR in the two seasons under study. Also, Kumari et al. (2018) reported that biofertilizers with NPK gave a higher value of RGR in baby corn plants. Biofertilizers work on the development of root structure and increase the flow rate of root xylem as a result of increasing and providing absorption of water and suitable nutrient elements, which leads to a difference in growth rates from plants not treated with biofertilizers (Al-Bayati et al., 2013; Azarpour et al., 2014). Our current study reported that inoculation of *T. harzianum* increased the seedling's height (50.12%), branch number (107.84%), ground area (116.93%), and AGR of lemon trees (56.02%) over the control. Hyakumachi and Kubota (2003) also stated that *T. harzianum* is a plant growth-promoting fungi that produce secondary metabolites and create a suitable environment for plant growth. *Trichoderma* fungi produce auxin and auxin-related substances, which induce root and shoot development (Contreras-Cornejo et al., 2014). Indole Acetic Acid (IAA) and kinetin regulates plant growth by controlling cell division and enlargement, tissue differentiation, morphogenesis and abscission of the plant parts (Moneruzzaman et al., 2010; Ljung, 2013). *Trichoderma spp* produces harzianic acid at the root cellular level, which stimulates the xylem transport in plants and accumulates more Fe (III), and regulates the roots and shoot growth of the plant (Yedidia et al., 2001; Vinale et al., 2013). Yedidia et al. (2001) also reported that soil inoculation *of T. harzianum* increased the concentration of macro and micronutrients in the inoculated roots and the shoots. This elevated level of nutrients due to soil inoculation of *Trichoderma spp* may play a significant role in the plant growth of *limau nipis*. *Trichoderma spp* also increases the rate of organic matter decomposition, leading to higher nutrient availability and absorption in the root zone soil. *T. harzianum* produces cell wall degrading enzymes cellulase, β-glucosidase, exoglucanase, and endoglucanase, which stimulate the decomposition rate of organic matter and increase the rate of plant growth (Ahmed et al., 2009).

The absolute growth rate (AGR) of guava rootstocks was increased by adding organic and biofertilizers with inorganic fertilizers (Veras et al., 2016). Application with bio and organic fertilizers in the soil improves the water holding capacity of root zone soil and increases the nutrient absorption by plant roots, promoting the absolute growth rate of baby spinach (Parwada et al., 2020). On the other hand, Jahan et al. (2013) mentioned that biofertilizers increased leaf area index (LAI) in Basil plants, the same result was mentioned by Kumari et al.

(2018), who found that a significant increment in baby corn leaf area index was applied with *Agrobacterium sp*. followed by *Trichoderma sp.* during the period of study. Also, Ye et al. (2020) reported that organic fertilizer application increased the leaf area index (LAI) of pear jujube trees. Al-Freeh et al. (2019) recorded a significantly increasing effect in LAI of oat plants (*Avena sativa* L.) using mineral fertilizers and biofertilizers. In this study, the positive effects of two microbes on the absolute growth rate of lemon trees may be due to an elevated level of internal plant hormone, well-developed root structure, and high dry matter accumulation. The application of microorganisms increases the soil nitrogen content, stimulates the production of plant hormones, protects the roots from the fungal pathogen, which leads to an increase in roots surface area, which absorbs more water and mineral nutrients from the soil and reflects in an increase in leaves number and size, which lead to increase in LAI (Sivasakthi et al., 2014). Similar positive impacts of biofertilizers on trees growth and development were reported by Shirkhani and Nasrolahzadeh (2016) and Nooni (2018).

Our results reported that the *T. harzianum* with NPK fertilizer significantly increased the leaf chlorophyll content (44.61%) and chlorophyll fluorescence (F0) (18.36%) over the control plants. Photosynthetic yield (Fv/Fm) and SPAD value of wheat leaves under osmotic stress were increased significantly with the addition of biofertilizers (Sharifi et al., 2017). Mohammed et al. (2010) reported that biofertilizers better affected leaf chlorophyll content on pear trees. Soil inoculation of biofertilizers with chemical fertilizer affects rice growth and yield by increasing leaf area and leaf chlorophyll content (Naher et al., 2018). Arefe et al. (2013) also reported that biofertilizers positively affected chlorophyll content (SAPD value) in basil leaves. In this study, stomatal conductance was positively affected by biofertilizer treatments with NPK. The addition of microbes with inorganic fertilizer may increase the mineral nutrients and water absorptions by improving the root system, thus affecting the leaf stomatal conductance of lemon trees. Organic fertilizer application increased chlorophyll content, stomatal conductance, and net photosynthetic rate of pear jujube trees (Ye et al., 2020). Osman and El-Rhman (2010) clarified that biofertilizers gave the highest value of leaf total chlorophyll content of fig trees. Biofertilizers produce plant growth hormones (auxin) and organic acids that promote plant growth and enzyme activities and increase chlorophyll content in plant leaves (Panhwar et al., 2015).

Our results showed that the two biofertilizers' fruit growth and quality characteristics of *limau nipis* were significantly improved with reduced rates of NPK fertilizers. Our study's findings agree with the results of Ennab (2016), who reported that biofertilizers and farmyard manure with NPK doses improved fruit quality like fruit number, the weight of fruit, and fruit dimension of Eureka lemon trees. Also, Todeschini et al. (2018) reported that soil inoculation of beneficial fungi and bacteria improved fruit quality in strawberries. Dheware and Waghmare (2009) mentioned that biofertilizers with NPK increased the number of fruits and weight of fruits in sweet orange. Hadole et al. (2015) reported that the Nagpur mandarin tree was affected by biofertilizers plus NPK, where the yield increased by 50% more than the control treatment. Improved fruit growth and quality could be ascribed to the constant supply of nutrients, especially potassium, higher concentrations of soil enzymes, and growth-promoting substances produced by soil-applied microorganisms, which may have aided in the biosynthesis and translocation of carbohydrates into the fruit (Thejaswini et al., 2022). It has been reported that biofertilizers application increased the level of endogenous auxins hormone in treated plants (Contreras-Cornejo et al., 2014). These elevated levels of auxins in the fruit can promote the sink potential of the fruits, which is positively correlated with the fruit growth rate (Khandaker and Boyce, 2016).

Our results showed that soil inoculation of *B. thuringiensis* increased the number of fruits (81.81%), fruit weight (55.52%), fruit diameter (43.54%), fruit dimension (35.69%), pulp to peel ratio (94.87%) and fruit juice content (65.36%) compared to the control group. Soil inoculation of *Bacillus spp* increases the growth and biomass of roots, shoots, and leaves (Ashraf et al., 2004; Radhakrishnan and Lee, 2016), synthesis of plant growth regulators (IAA, GAs, Cytokinins, and Spermidines) (Xie et al., 2014; Radhakrishnan and Lee, 2016), and elevate the levels of photosynthetic pigments, sugars, amino acids, proteins, and mineral nutrients in plants (Kang et al., 2014; Radhakrishnan and Lee, 2016) and increase fruit yield (Dursun et al., 2010). *Bacillus spp* converts the complex of macronutrients to an available form for uptake and accumulation by plant roots, thus enhancing the growth and biomass of the plants (Kuan et al., 2016). The nitrogenase (nifH) gene of the *Bacillus spp* produces enzyme nitrogenase which can fix atmospheric N_2 and supply to plants for stimulation of plant growth and yield (Ding et al., 2005; Kuan et al., 2016). Bacillus spp synthesis plant growth regulators enhance cell division and cell elongation during fruit set and development (Xie et al., 2014; Radhakrishnan and Lee, 2016). *Bacillus spp* also secretes the enzyme ACC deaminase, which inhibits the synthesis of ethylene in plants and enhances the growth of the plants (Xu et al., 2014; Pourbabaee et al., 2016). The enzyme ACC deaminase in plant cells breaks the 1-amino-1 cyclopropane carboxylic acid (ACC) into ammonia (NH_3), and α-ketobutyrate ($C_4H_6O_3$), and cross-talk between ACC deaminase and IAA inhibit the ethylene (C_2H_4) production, thereby stimulating the growth and development of plant (Glick, 2014). Ismail et al. (2015) reported that fruit quality can be improved only at the production level, but after harvest, the fruits can only maintain their quality. Our results reported that biofertilizers treatment increased the leaf and fruit TSS content *limau nipis*. Fruit TSS content of Eureka lemon trees is significantly affected by biofertilizers and farm manure which reduce the rate of NPK (Ennab, 2016). It was reported earlier that soil application of biofertilizers increased the fruit TSS content of guava trees (Dutta et al., 2014). Ye et al. (2020) also stated that applying organic fertilizer improved the total soluble solids content and fruit quality of Jujube. Increasing TSS content in leaves and fruits may be due to improved net photosynthetic rates and plant growth, which increases the accumulation of photosynthates and nutrients, and transfer accumulates to fruits causing improved fruit quality (Naik and Babu, 2007).

The solubilization of mineral nutrients, synthesis of plant growth regulators and secondary metabolites, and enzyme secretions from *T. harzianum* and *B. thuringiensis* confirm their biofertilizer effects on lemon trees towards improving growth physiology, and fruit quality of *limau nipis*.

5. Conclusion

To overcome the shortcomings associated with chemical fertilizer-based citrus fruit production, the *Trichoderma harzianum* and *Bacillus thuringiensis* were applied as biofertilizers to improve the growth, physiological characteristics, and fruit quality of limau nipis plants. From the above results, we conclude that biofertilizers treatments, particularly T2 (*T. harzianum* 50% + NPK 50%), T3 (*B. thuringiensis* 50% + NPK 50%), and T5 (*B. thuringiensis* + 75% NPK 25%), improved the growth, physiology and fruit quality of *limau nipis*. *T. harzianum* with 50 g NPK increased the plant height, number of branches, ground area, and absolute growth rate of lemon trees. *B. thuringiensis* with 50 g NPK increased the number of leaves, leaf area, specific leaf area, AGR for leaf area and number of leaves, and leaf area index of lemon trees. Furthermore, application of *B. thuringiensis* with 25 g NPK increased leaf fresh and dry weight, leaf moisture content, specific leaf weight, leaf relative growth rate, number of fruits, fruit weight, fruit diameter, fruit dimension, and TSS content of leaf and fruit of limau nipis trees. As a result, it is concluded that soil inoculation of *B. thuringiensis* with 25-50 g NPK could enhance the morpho-physiological, growth, and fruit quality parameters of *limau nipis* under polybagged conditions.

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