

## EFFICACY OF SALT TOLERANCE BIO-PRODUCT NPISi ON SOIL PROPERTIES, GROWTH AND YIELD OF GREEN ONION IN TRAN DE DISTRICT, SOC TRANG PROVINCE

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ARTICLE INFO	ABSTRACT
Received: 30/12/2021	Cultivation of green onion has been developed widely in Tran De district, Soc Trang province, contributing to upgrade household income, however salt invasion has caused a decline in green onion production. The study aimed to determine the efficiency of salt tolerance bio-product NPISi (NPISi) on soil characteristics, growth and yield of green onion when grown on salt-affected soil in Tran De district, Soc Trang province. The experiment was carried out in a randomized complete block design with four treatments and four replicates of each treatment. Properties of soil chemistry and biology, growth and yield of green onion were collected. The result indicated that the treatment with NPISi or organic fertilizer (OF) had significantly higher values of $\text{NH}_4^+$ , $\text{NO}_3^-$ , $\text{P}_2\text{O}_5$ , nitrogen fixing bacteria, phosphorus, and silicate solubilizing bacteria in soil than in the positive control treatment with 100%NPK, moreover, the total nitrogen, phosphorus, potassium, and silicon concentration in green onion bulb were also higher than the control. The best performance of green onion was observed in treatment applied with both OF and NPISi. Additionally, the inoculant in NPISi product excellently grew, boosted green onion height and yield under salinity condition, especially when associated with OF, green onion yield was enhanced up to 56.0% as compared to the positive control treatment. Therefore, NPISi product is recommended to be used for crops grown on saline soils.
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### KEYWORDS

Green onion  
Nitrogen fixation bacteria  
Salt-affected soil  
Salt tolerance bio-product NPISi  
Soil property

## HIỆU QUẢ CỦA CHẾ PHẨM VI SINH CHỊU MẶN NPISi LÊN ĐẶC TÍNH ĐẤT, SINH TRƯỞNG VÀ NĂNG SUẤT CÂY HÀNH LÁ TẠI HUYỆN TRẦN ĐỀ, TỈNH SÓC TRĂNG

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THÔNG TIN BÀI BÁO	TÓM TẮT
Ngày nhận bài: 30/12/2021	Canh tác hành lá tại huyện Trần Đề, tỉnh Sóc Trăng đang có những bước tiến quan trọng góp phần gia tăng thu nhập nông hộ, tuy nhiên vấn đề xâm nhập mặn dẫn đến năng suất hành lá bị suy giảm. Nghiên cứu nhằm đánh giá hiệu quả của chế phẩm vi sinh chịu mặn NPISi (NPISi) lên đặc tính đất, sinh trưởng và năng suất hành lá tại Trần Đề, Sóc Trăng. Thí nghiệm được bố trí theo thể thức khối hoàn toàn ngẫu nhiên với 4 nghiệm thức và 4 lặp lại. Các chỉ tiêu về đặc tính hóa sinh học đất, sinh trưởng và năng suất hành lá được thu thập. Kết quả cho thấy nghiệm thức được bổ sung NPISi hoặc phân hữu cơ (PHC) giúp gia tăng các chỉ tiêu như hàm lượng $\text{NH}_4^+$ , $\text{NO}_3^-$ , $\text{P}_2\text{O}_5$ , mật số vi khuẩn cố định đạm, hòa tan lân và silic trong đất cũng như xu hướng gia tăng hàm lượng đạm, lân, kali và silic tổng số trong cây hành lá. Hiệu quả gia tăng tốt nhất khi có sự kết hợp giữa PHC và NPISi. Các đồng vi khuẩn trong chế phẩm NPISi phát triển, kích thích chiều cao và năng suất hành lá trong điều kiện nhiễm mặn, đặc biệt thúc đẩy năng suất lên đến 56,0% so với nghiệm thức bón 100%NPK khi có PHC. Do đó, chế phẩm NPISi được khuyến cáo sử dụng cho cây trồng trên đất nhiễm mặn.
Ngày hoàn thiện: 16/02/2022	
Ngày đăng: 23/02/2022	

### TỪ KHÓA

Chế phẩm vi sinh chịu mặn NPISi  
Đặc tính đất  
Đất nhiễm mặn  
Hành lá  
Vi khuẩn cố định đạm

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## 1. Introduction

Cultivation of green onion has been developed widely in Tran De district, Soc Trang province, contributing to upgrade household income, however salt invasion has caused a decline in green onion production [1], [2]. There were some methods to enhance tolerance ability, growth and yield of vegetables on salt-affected soil including amendment of gypsum ( $\text{CaSO}_4$ ), sulfuric acid, nitric acid, elemental sulfur to alleviate saline effect in soil [3]. The addition of humic acid,  $\text{NO}_3^-$ ,  $\text{Ca}^{2+}$ , K, P, salicylic acid, and silicon to salt-affected soil aimed at improving the salt tolerance vegetables [4]-[13]. Application of biofertilizer containing useful microorganisms mitigated salinity effects on vegetables and reduced soil salinization [14]. Moreover, the application of poultry manure, beneficial microorganisms as *Azotobacter* with nitrogen fixation ability, *Serratia* sp. with phosphate-solubilizing capability and *Pseudomonas* sp. effectively boosted NPK contents in bulb, growth and productivity of green onion in salt-affected soil [15]-[18]. However, it was not yet almost found studies that amendments of bacterial bio-products ameliorate soil characteristics as well as reinforce growth and yield of green onion on salt-affected soil. Therefore, this study was carried out to evaluate the efficiency of salt tolerance bio-product NPISi (NPISi) on soil properties, green onion height and yield over two consecutive crops on salt-affected soil.

## 2. Methodologies

### 2.1. Experimental design

The experiment was conducted as a completely randomized block design with four treatments and four replicates each one in Vien Binh ward, Tran De district, Soc Trang province. The area of salt-affected soil around 480 m<sup>2</sup> was divided into 16 plots, and each plot had 30 m<sup>2</sup> (2 m x 15 m = 30 m<sup>2</sup>). The study lasted in two consecutive crops involving the first crop (12/2020-02/2021), and the second crop (3/2021-4/2021). The treatments were listed as follows:

**Table 1.** List of the treatments carried out in the field condition

No.	Treatment	Abbreviation
T1	Recommended NPK	NPK
T2	Recommended NPK + salt tolerance bio-product NPISi (75 kg.ha <sup>-1</sup> )	NPISi
T3	Recommended NPK + organic fertilizer (2 t.ha <sup>-1</sup> )	OF
T4	Recommended NPK + OF + NPISi (75 kg.ha <sup>-1</sup> )	OF+NPISi

The organic fertilizer Con Ca of ADC company containing 22.0% organic matter, 5.0% total nitrogen, 2.0% humic acid, 2.0%  $\text{P}_2\text{O}_5$ , 2.0%  $\text{K}_2\text{O}$ , 4.0% Ca, 2.17% Mg, 4.0% S, 200 ppm Zn, 200 ppm Fe, 200 ppm Mn, 200 ppm Cu, and 1500 ppm B was used in the study and basal fertilized once in the soil preparation time.

The salt tolerance bio-product NPISi containing four isolates comprising *Bacillus aquimaris* KG6-3, *Burkholderia* sp. BL1-10, *Bacillus megaterium* ST2-9, and *Ochrobactrum ciceri* TCM\_39 obtained abilities of nitrogen fixation, phosphate solubilization, IAA synthesis, and silicate solubilization, respectively, with the number of the bacterial cell around 10<sup>9</sup> CFU.g<sup>-1</sup> bio-product [19], [20]. NPISi was amended at 15, 22, 29, and 36 days after seedling with a total dose of 75 kg.ha<sup>-1</sup> by mixing sand and spreading evenly on the surface of block. Weed was controlled by handwork methods. Insects and diseases were managed by the IPM method, simultaneously extreme limitation of plant protection products.

### 2.2. The collected parameters

#### 2.2.1. Soil chemical and biological properties of green onion cultivation soil in Tran De district, Soc Trang province

At the end of the experiment, the soil sample was collected at 0-20 cm depth in many sites, and then mixed in a bigger soil sample in each plot. The soil sample was analyzed parameters as follows:

- pH and EC (electrical conductivity): pH and EC were determined by 744 pH Meter-Metrohm and EC Schott model 960, respectively. Extract solution with ratio of soil: water = 1.0: 2.5. Soil solution was shaken at 150 rpm in two hours, after that centrifuged at 2000 rpm in 3 min.

- Total nitrogen ( $N_t$ ): soil sample was digested by a mixture of  $CuSO_4$ , Se, and  $K_2SO_4$ , then total nitrogen concentration was evaluated via the Kjeldahl method [21].

- Total phosphorus ( $P_t$ ): soil sample was digested in a mixture of  $H_2SO_4$  and  $HClO_4$ , and total phosphorus content was determined at 880 nm wavelength [22].

- Total potassium ( $K_t$ ): soil sample was digested by mixture of  $H_2SO_4$  and  $HClO_4$ , and total potassium content was determined by atomic absorption spectroscopy [23].

- Available nitrogen concentration  $NH_4^+$ -N and  $NO_3^-$ -N: available nitrogen concentration  $NH_4^+$ -N and  $NO_3^-$ -N in soil were adopted by [24], and examined at 650 nm and 540 nm, respectively by spectrophotometer.

- Soluble phosphorus ( $P_s$ ): soil sample was extracted with  $NaHCO_3$  0.5 M, pH 8.5 (ratio of soil: solution = 1:20), and shaken in 30 min, then filtered through filter papers. The extract solution was evaluated at 880 nm wavelength [25].

- Exchangeable potassium ( $K_{ex}$ ): soil sample was extracted with  $BaCl_2$  0.1 M and determined by atomic absorption spectroscopy [26].

- Soluble silicon ( $Si_s$ ): soil sample was digested in a mixture of  $Na_2CO_3$  ( $10\text{ g.L}^{-1}$ ) and 50 mL  $NH_4NO_3$  ( $16\text{ g.L}^{-1}$ ), then shaken at 60 rpm in an hour, kept in 5 days [27]. After that, soluble silicon content will be determined as Molybdenum Blue Colorimetric method [28]. The method for determining soluble Si content in liquid medium is described as follows. An aliquot of 1 mL of the sample will be transferred into 50 mL falcon then adding 2.5 mL ammonium acetate 20%, 1 mL ammonium molybdate 0.3 M, vortex 5 sec, left the fixed sample in 5 min for stabilization, then, added 0.5 mL tartaric acid 20%, 0.5 mL reducing solution, 2 mL acetic acid 20%, after that, put stationary sample at laboratory condition for another 60 min and finally, samples will be measured by spectrophotometer with 815 nm.

- Number of microorganisms in soil: Microbial solution will be diluted with dilution coefficient 10, mix sample well in a vortex. A defined amount ( $100\text{ }\mu\text{L}$ ) of microbial suspension in  $10^{-2}$ ,  $10^{-4}$  and  $10^{-6}$  will be spread on Tryptone Soya Agar (TSA), Potato Dextrose Agar (PDA), Starch Agar, Burk Agar, National Botanical Research Institute's phosphate growth medium (NBRIP) Agar, Soil Extract Agar (SEA) medium surface to determine the number of bacteria, fungi, actinomyces, nitrogen fixation bacteria, phosphate-solubilizing bacteria, and silicate-solubilizing bacteria in soil, respectively. After that, all of the samples will be incubated in a  $30^\circ\text{C}$  incubator for 24 hours then finally count the number of colonies exhibited on agar medium for determining the microbial number in liquid medium (CFU/mL) [29]-[32].

- Bacterial survivability in NPISi after two consecutive green onion crops in the field condition: bacterial community in soil was extracted DNA by CTAB 3.0% [33], then did PCR with primer pair 341F-GC/534R and thermal cycle as 1 cycle ( $94^\circ\text{C}$  in 3 min), 29 cycles ( $94^\circ\text{C}$  in 20 sec;  $55^\circ\text{C}$  in 45 sec;  $72^\circ\text{C}$  in 45 sec) and 1 cycle ( $72^\circ\text{C}$  in 7 min). Then, PCR product was analyzed DGGE Denaturing Gradient Gel Electrophoresis (DGGE) gel with acrylamide 8.0% in Tris Base Acetate (TAE) 1X at 45V,  $60^\circ\text{C}$ , in 16 hours on vertical electrophoresis system (Dcode, Biorad). Finally, DGGE gel dyed Ethium Bromide was examined by Gel Logic 1500.

### 2.2.2. Height, nutrient uptake ability and yield of green onion plant

- Plant height: plant height was measured from the soil surface to the highest top of green onion in  $0.5\text{ m}^2$  cell.

- Total silicon concentration in bulb: bulb of green onion was dried at 70°C in 48 hours, and sieved through 0.5 mm sieve. Then a defined aliquot of 0.1 g sample was transferred to centrifuged tube 50 mL, next 3 mL NaOH 50% was transferred to tube, sterilized at 120°C in 20 min. After that, the sample was calibrated to 50 mL volume with distilled water. Finally, total silicon concentration in the bulb was evaluated by the Molybdenum Blue Colorimetric method [28].

- Total NPK concentration in bulb: bulb of green onion was digested with concentrated H<sub>2</sub>SO<sub>4</sub> and salicylic acid. After that, total nitrogen, phosphorus, and potassium concentration were determined via Kjeldahl, spectrometer, and spectroscopy method.

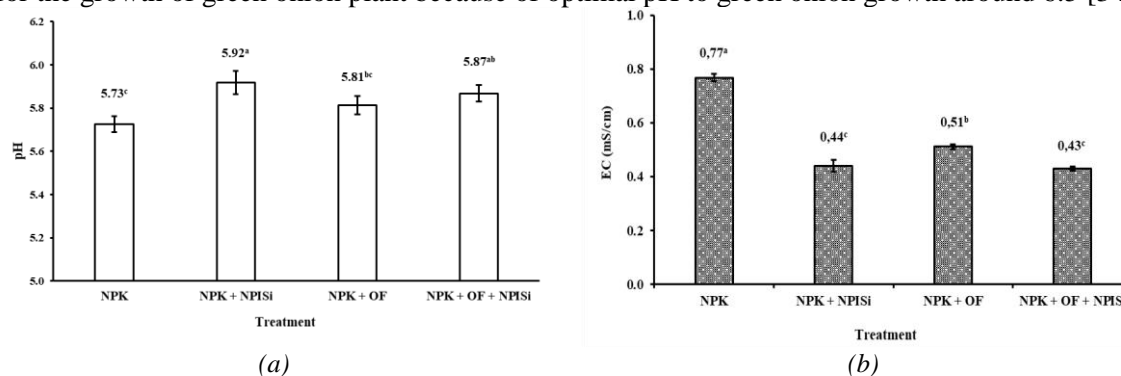
- Yield of green onion plant: (1) crop 1: green onion plant was harvested in the 50 day after seedling period, and collected three plots each block, then, evaluated fresh yield of green onion plant (t.ha<sup>-1</sup>); (2) crop 2: green onion plant was harvested 30 days after the seedling period due to salinity of irrigation water extremely high (6.49 mS.cm<sup>-1</sup>), so the study could not last anymore. One hundred of green onion bundle was randomly collected in each block, then, fresh yield of green onion plant (t.ha<sup>-1</sup>) evaluated was based on plant density (20 cm x 15 cm).

### 3. Results and discussions

#### 3.1. The efficiency of salt tolerance bio-product NPISi on soil chemical and biological properties cultivated green onion plant over two consecutive crops in Tran De district, Soc Trang province

##### 3.1.1. pH and EC in soil

pH in the soil at the end of the experiment varied from 5.73 to 5.92 (Fig. 1a). The treatment with NPISi or OF led to increasing pH in the soil. Especially the treatment with NPISi boosted better pH in soil than the treatment with OF. In details, the treatment with NPK+NPISi had pH peaked at 5.92 was significantly higher than as compared with the treatment without NPISi ( $p < 0.05$ ), however was non-significantly different as compared to the treatment with NPK+OF+NPISi (pH 5.87). In short, the application of NPISi reinforced pH as a beneficial trend for the growth of green onion plant because of optimal pH to green onion growth around 6.5 [34].



**Figure 1.** pH (a) and EC in soil (b) of the treatments in the field condition in Tran De district, Soc Trang province (4/2021)

Fig. 1b showed EC in the soil of the treatments fluctuated in 0.43-0.77 mS.cm<sup>-1</sup>, and the treatments had significant differences in comparison with each other ( $p < 0.05$ ). Among them, the treatment with NPK+NPISi and NPK+OF+NPISi owned the lowest EC. The treatment with recommended NPK obtained the highest EC peaked at 0.77 mS.cm<sup>-1</sup>. Although EC in soil was approximately 0.43-0.77 mS.cm<sup>-1</sup>, which not yet caused significantly deleterious effects for growth of green onion, however EC of irrigation water for green onion plant in crop 2 was extremely high (6.49 mS.cm<sup>-1</sup>) due to that period time was dried season and irrigation water was salt-affected. Moreover, soil used for the study had compositions including sand 52.4%, loam

34.0%, and clay 13.7%. Due to sand being the dominant component in the soil, it could not retain salt in salt-affected water, hence would not lead to detrimental effects on green onion plant. Concurrently, after irrigation, salt-affected water moved into and accumulated in furrows as a salt dizzy layer on soil surface (Fig. 2). In summary, amendment of NPISi alleviated EC in soil up to 44.2% as opposed to the control treatment. This was one out of convenient conditions for growth and yield of vegetables in general and green onion plant on salt-affected soil in specific [35].



**Figure 2.** Salt dizzy layer on soil furrow surface in the field condition in Tran De district, Soc Trang province

### 3.1.2. $\text{NH}_4^+$ , $\text{NO}_3^-$ , $\text{P}_2\text{O}_5$ , exchangeable $\text{K}^+$ and soluble silicon concentration in soil cultivated green onion at the end of the experiment

Concentration of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$ , exchangeable  $\text{K}^+$  ( $\text{K}_{\text{ex}}$ ) and soluble silicon ( $\text{H}_4\text{SiO}_4$ ) in soil cultivated green onion at the end of the experiment of the treatments ranged in 6.96-7.54  $\text{mg.kg}^{-1}$ , 34.9-38.8  $\text{mg.kg}^{-1}$ , 57.5-70.9  $\text{mg.kg}^{-1}$ , 0.44-0.48  $\text{meq/100 g}$ , and 96.0-98.1  $\text{g.kg}^{-1}$ , respectively (Table 1). Simultaneously, it was found that there were statistically significant differences among the treatments, but the treatments had exchangeable  $\text{K}^+$  and soluble silicon ( $\text{H}_4\text{SiO}_4$ ) concentration in soil was statistically non-significant different from each other ( $p > 0.05$ ).

**Table 1.** Concentration of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$ , exchangeable  $\text{K}^+$  and soluble silicon ( $\text{H}_4\text{SiO}_4$ ) in the soil at the end of the experiment in Tran De district, Soc Trang province (4/2021)

Treatment	Concentration				
	$\text{NH}_4^+$ ( $\text{mg.kg}^{-1}$ )	$\text{NO}_3^-$ ( $\text{mg.kg}^{-1}$ )	$\text{P}_2\text{O}_5$ ( $\text{mg.kg}^{-1}$ )	$\text{K}_{\text{ex}}$ ( $\text{meq/100 g}$ )	$\text{H}_4\text{SiO}_4$ ( $\text{g.kg}^{-1}$ )
NPK	6.96 <sup>c</sup>	34.9 <sup>b</sup>	57.5 <sup>c</sup>	0.44	98.1
NPK+NPISi	7.33 <sup>b</sup>	38.3 <sup>a</sup>	63.9 <sup>b</sup>	0.45	96.5
NPK+OF	7.24 <sup>b</sup>	38.5 <sup>a</sup>	70.5 <sup>a</sup>	0.46	96.0
NPK+OF+NPSi	7.54 <sup>a</sup>	38.8 <sup>a</sup>	70.9 <sup>a</sup>	0.48	97.4
F	*	*	*	ns	ns
CV (%)	3.20	4.9	9.0	6.47	5.5

Notes: \*significant difference at level 5% by Tukey's test; in same column numbers followed by same letters was non-significant at level 5%; ns: significant difference at level 5%

More specifically, the treatment with NPISi, OF, or OF+NPISi obtained higher  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$  concentration in soil than the control with recommended NPK. The treatment with NPK+OF+NPISi tended to have the highest  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$  concentration in soil peaked at 7.54, 38.8, and 70.9  $\text{mg.kg}^{-1}$ , respectively. The treatment with NPK possessed a tendency of the lowest  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$  concentration in soil bottomed out at 6.96, 34.9, and 57.5  $\text{mg.kg}^{-1}$ , respectively ( $p < 0.05$ ). The treatment with NPK+NPISi and NPK+OF had a trend that  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$  concentration in soil was non-significant different from each other. The result was in agreement with the past study indicating that the use of bio-fertilizer raised  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$  concentration in soil, however exchangeable  $\text{K}^+$  concentration in soil was non-significant different [36], [37]. Moreover, the studies about the use of bio-fertilizer to climb soluble silicon content in soil were not found. In short, utilization of OF and NPISi augmented  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$  concentration in soil.

### 3.1.3. Number of microorganisms in the soil

The number of microorganisms in the soil of the treatments indicated Table 2 showed that there were statistically significant differences in the number of bacteria, fungi, nitrogen fixation bacteria, phosphate-solubilizing bacteria, and silicate-solubilizing bacteria in soil ( $p < 0.05$ ), but the treatments had number of actinomyces in soil fluctuated in 5.23-5.29  $\log_{10}$  CFU.g<sup>-1</sup> and was non-significant different in comparison with each other.

**Table 2.** Number of microorganisms in soil (4/2021) at the end of the experiment in Tran De district, Soc Trang province (4/2021)

Treatment	Number of microorganisms in soil ( $\log_{10}$ CFU.g <sup>-1</sup> )					
	Bacteria	Fungi	Actinomyces	Nitrogen fixation bacteria	Phosphate-solubilizing bacteria	Silicate-solubilizing bacteria
NPK	5.68 <sup>d</sup>	3.84 <sup>a</sup>	5.29	4.59 <sup>b</sup>	3.95 <sup>bc</sup>	3.48 <sup>b</sup>
NPK+NPISi	6.38 <sup>b</sup>	3.59 <sup>b</sup>	5.24	4.75 <sup>a</sup>	4.01 <sup>ab</sup>	3.79 <sup>a</sup>
NPK+OF	6.22 <sup>c</sup>	3.60 <sup>b</sup>	5.23	4.65 <sup>b</sup>	3.83 <sup>c</sup>	3.58 <sup>b</sup>
NPK+OF+NPISi	6.54 <sup>a</sup>	3.88 <sup>a</sup>	5.28	4.76 <sup>a</sup>	4.13 <sup>a</sup>	3.85 <sup>a</sup>
F	*	*	ns	*	*	*
CV (%)	5.39	3.92	1.61	1.83	3.16	4.48

Notes: \*significant difference at level 5% by Tukey's test; in same column numbers followed by same letters was non-significant at level 5%; ns: significant difference at level 5%

Bacterial density in the soil of the treatments ranged from 5.68 to 6.54  $\log_{10}$  CFU.g<sup>-1</sup>, and was significantly different as compared to each other ( $p < 0.05$ ). The treatment with NPK+OF+NPISi had the highest bacterial density in soil peaked at 6.54  $\log_{10}$  CFU.g<sup>-1</sup>, while the treatment with NPK owned the lowest bacterial density in soil reached 5.68  $\log_{10}$  CFU.g<sup>-1</sup> ( $p < 0.05$ ).

The number of fungi in the soil of the treatments varied in 3.59-3.88  $\log_{10}$  CFU.g<sup>-1</sup>. It was found that fungal density in the soil of the treatments was statistically significantly different from each other. The treatment with NPK+OF+NPISi and NPK had the highest fungal density in soil around 3.84-3.88  $\log_{10}$  CFU.g<sup>-1</sup>, and was non-significant different each other, however were significantly higher fungal density in soil than the others.

The treatment with NPISi obtained a higher number of nitrogen fixation bacteria, phosphate-solubilizing bacteria, and silicate-solubilizing bacteria in soil than the treatments without NPISi ( $p < 0.05$ ). In detail, the treatment with NPK+OF+NPISi possessed a number of nitrogen fixation bacteria, phosphate-solubilizing bacteria, and silicate-solubilizing bacteria in soil peaked at 4.76, 4.13, and 3.85  $\log_{10}$  CFU.g<sup>-1</sup> ( $p < 0.05$ ), simultaneously was significantly higher than the treatment with NPK and NPK+OF had a number of nitrogen fixation bacteria, phosphate-solubilizing bacteria, and silicate-solubilizing bacteria in soil ranged in 4.59-4.65, 3.83-3.95, and 3.48-3.58  $\log_{10}$  CFU.g<sup>-1</sup> ( $p < 0.05$ ). Moreover, it was not found any significant difference between the treatment with NPK and NPK+OF.

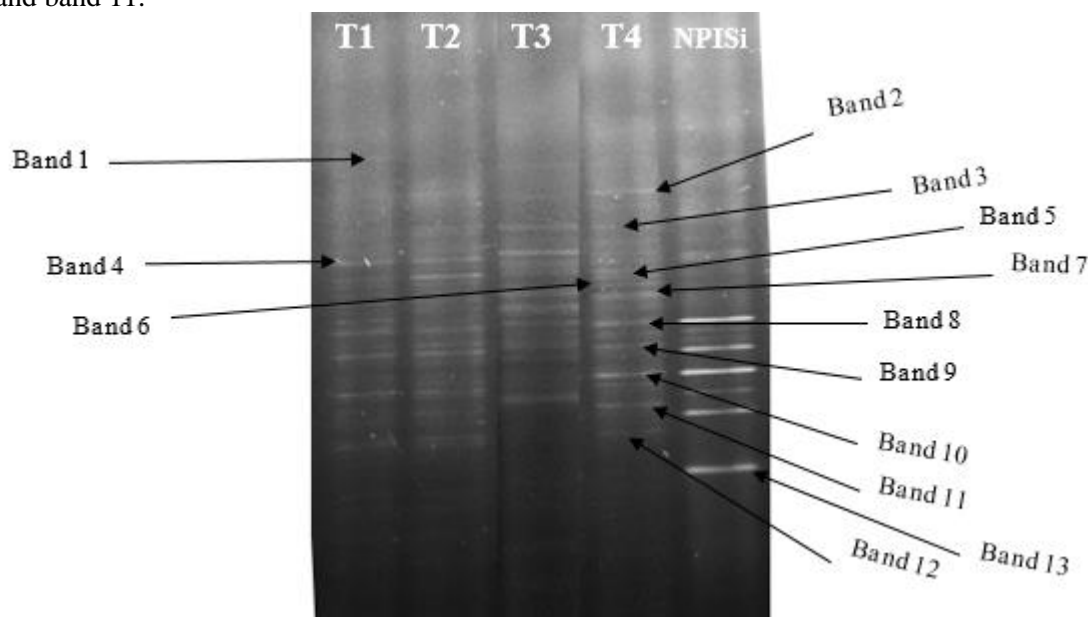
In general, the result agreed with the previous studies that showed that the application of bio-fertilizer with beneficial bacteria boosted useful bacterial density in soil [38]. However, the studies about correlations among effective bacteria, fungi and actinomyces were not almost found.

To sum up, application of NPISi enhanced the number of total bacteria, and beneficial bacteria in soil including nitrogen fixation bacteria, phosphate-solubilizing bacteria, and silicate-solubilizing bacteria. Furthermore, amendment of OF without NPISi has not yet boosted useful microbial density in soil over two consecutive crops on salt-affected soil in the field condition.

### 3.1.4. Bacterial survivability in salt tolerance bio-product NPISi in soil cultivated green onion plant over two consecutive crops

After two consecutive crops, a number of bacterial species of the treatments NPK, NPK+NPISi, NPK+OF, and NPK+OF+NPISi was shown via a number of bands reached at 19,

19, 16, and 19, respectively (Fig. 3). The treatment with OF had a lower number of the band (16 bands) than the others. Moreover, the treatment with NPK+OF+NPISi had a higher light-intensity of the band than the others at the same band positions. This meant that application of NPISi or OF+NPISi not yet enhanced the number of bacterial species in soil over two consecutive crops as compared to the control (recommended NPK). Besides, the treatment with NPK+OF+NPISi boosted number of specific bacteria in soil (band 2, band 7, band 8, band 9, band 10, and band 11). However, utilization of OF mitigated the number of bacterial species as compared to the control but reinforced bacterial density of three species involving band 3, band 4, and band 11.



**Figure 3.** DGGE profile of bacterial communities in soil cultivated green onion plant of the treatments in Tran De district, Soc Trang province (4/2021)

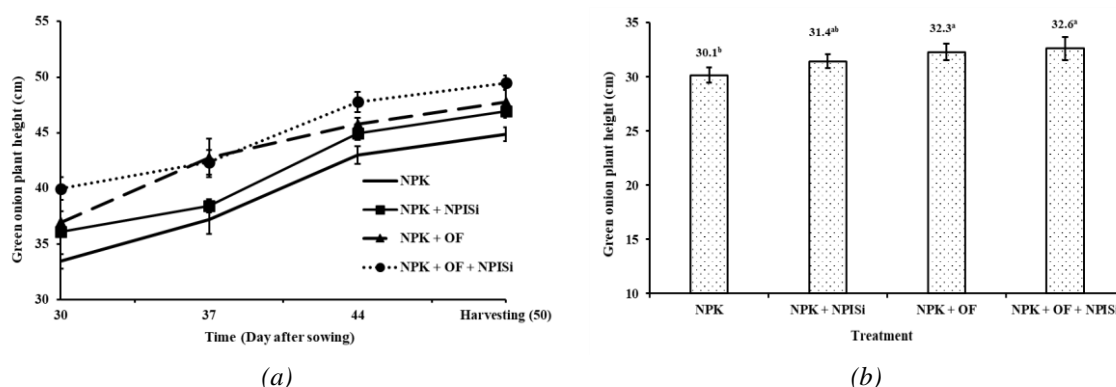
\*Note: NPISi: bio-product NPISi; T1: NPK; T2: NPK + NPISi; T3: NPK + OF; T4: NPK + OF + NPISi

Isolates in NPISi in corresponding with bands 4, 8, 9, 10, and 11 were survival over two consecutive crops in two treatments including NPK+NPISi and NPK+OF+NPISi. It was not almost reported the study about the diversity of bacterial communities in salt-affected soil cultivated green onion plants when applied bio-fertilizer. In short, bacteria in NPISi were efficiently survived and grew in salt-affected soil cultivated green onion plants in Tran De district, Soc Trang province over two consecutive crops. Especially, application NPISi+OF stimulated bacterial density of special species in soil, concurrently assisted isolates in NPISi to grow better.

### 3.2. The efficacy of salt tolerance bio-product NPISi on height, nutrient absorption ability, and yield of green onion plant over two consecutive crops in Tran De district, Soc Trang province

#### 3.2.1. Plant height

Fig. 4a and 4b showed green onion plant height of the treatments indicated an upwards movement and ranged from 30.1 cm to 49.5 cm. The treatment with NPK+OF+NPISi had the highest plant height ( $p < 0.05$ ), followed by the treatment with NPK+OF and NPK+NPISi owned plant height was equal at all the sampling periods ( $p > 0.05$ ). The control treatment obtained the lowest plant height at any sampling time. The result of the study corresponded to the previous studies which showed that the use of bio-fertilizer improved growth of green onion [39], [40].



**Figure 4.** Green onion plant height of the treatments in the field condition crop 1 (a), and crop 2 (b) in Tran De district, Soc Trang province (12/2020-02/2021)

In general, the application of sole OF or NPISi considerably enhanced plant height. It was excellently noticeable that the combination of OF+NPISi outstandingly ameliorated plant height and stabilized the efficiency over all the observed periods.

### 3.2.2. Concentration of total nitrogen, phosphorus, potassium and silicon in green onion bulb

Content of total nitrogen ( $N_t$ ), phosphorus ( $P_t$ ), potassium ( $K_t$ ) and silicon ( $S_t$ ) in green onion bulb in crop 1 fluctuated in 4.56-4.75%, 1.75-1.93%, 6.47-6.78%, and 7.15-7.28%, respectively. The treatment with NPK+OF+NPISi and NPK+OF had a higher tendency of  $N_t$  and  $P_t$  concentration in bulb than the others. Additionally, the treatments had concentration of  $K_t$  and  $S_t$  in bulb was non-significant different each other in the crop 1.

**Table 3.** Concentration of total nitrogen, phosphorus, potassium and silicon in green onion bulb of the treatments in crop 1 in Tran De district, Soc Trang province (02/2021)

Treatment	Concentration			
	$N_t$ (%N)	$P_t$ (% $P_2O_5$ )	$K_t$ (% $K_2O$ )	$S_t$ (g.kg <sup>-1</sup> )
NPK	4.56 <sup>b</sup>	1.75 <sup>b</sup>	6.47	7.15
NPK+NPISi	4.61 <sup>b</sup>	1.79 <sup>b</sup>	6.57	7.22
NPK+OF	4.72 <sup>a</sup>	1.86 <sup>ab</sup>	6.78	7.20
NPK+OF+NPISi	4.75 <sup>a</sup>	1.93 <sup>a</sup>	6.63	7.28
F	*	*	ns	ns
CV (%)	1.84	4.78	3.75	2.80

Notes: \*significant difference at level 5% by Tukey's test; in same column numbers followed by same letters was non-significant at level 5%; ns: significant difference at level 5%

Table 4 presented that concentration of total nitrogen ( $N_t$ ), phosphorus ( $P_t$ ), potassium ( $K_t$ ) and silicon ( $S_t$ ) in green onion bulb of the treatments in crop 2 varied in 2.96-3.29%, 1.19-1.30%, 3.76-4.34%, and 7.89-10.4%, respectively. The treatment with NPISi, OF, and OF+NPISi had significantly higher  $N_t$ ,  $P_t$ ,  $K_t$ , and  $S_t$  content in green onion bulb than the control treatment (recommended NPK). Moreover, the treatment with NPK+NPISi obtained statistically higher  $P_t$  concentration than most of the other treatments.

Utilization of OF, NPISi or OF+NPISi highly boosted total nitrogen, potassium and silicon concentration in green onion bulb. The increase of nutrient content in a plant can be due to the enhancement of the biological  $N_2$ -fixation and/or production of organic acids to solubilize P, K and Si and/or production of certain growth-promoting substances, which positively affect root development and consequently their function in the uptake of both water and nutrients. As a result, this supported green onion to grow and develop better under salt-affected soil. This result was similar to previous studies, which indicated that utilization of bio-fertilizer climbed concentration of nutrients in green onion bulb as compared to the control [41], [42].



**Table 4.** Concentration of total nitrogen, phosphorus, potassium and silicon in green onion bulb of the treatments in crop 2 in Tran De district, Soc Trang province (4/2021)

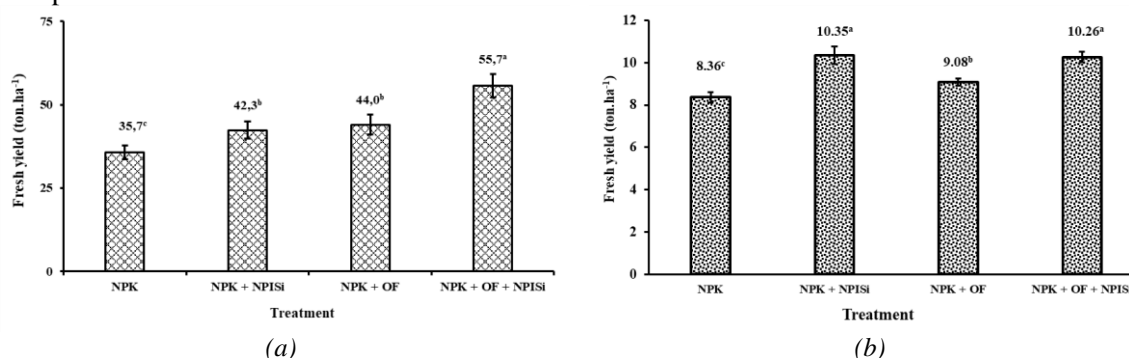
Treatment	Concentration			
	N <sub>t</sub> (%N)	P <sub>t</sub> (%P <sub>2</sub> O <sub>5</sub> )	K <sub>t</sub> (%K <sub>2</sub> O)	Si <sub>t</sub> (g.kg <sup>-1</sup> )
NPK	2.96 <sup>b</sup>	1.20 <sup>b</sup>	3.76 <sup>b</sup>	7.89 <sup>b</sup>
NPK+NPISi	3.29 <sup>a</sup>	1.30 <sup>a</sup>	4.34 <sup>a</sup>	10.4 <sup>a</sup>
NPK+OF	3.21 <sup>a</sup>	1.19 <sup>b</sup>	4.31 <sup>a</sup>	10.3 <sup>a</sup>
NPK+OF+NPISi	3.26 <sup>a</sup>	1.25 <sup>ab</sup>	4.25 <sup>a</sup>	10.4 <sup>a</sup>
F	*	*	*	*
CV (%)	4.32	4.01	5.66	2.80

Notes: \*significant difference at level 5% by Tukey's test; in same column numbers followed by same letters was non-significant at level 5%; ns: significant difference at level 5%

To sum up, the application of OF boosted total nitrogen, phosphorus, potassium and silicon content in green onion bulb, especially N<sub>t</sub> and P<sub>t</sub> concentration extremely raised in combination with NPISi.

### 3.2.3. Fresh yield of green onion

Fig. 5 indicated fresh yield of green onion plant of the treatments over two consecutive crops that fresh yield ranged in 35.7-55.7% (crop 1), and 8.36-10.4% (crop 2). The treatment with NPISi had a higher fresh yield than the others. The control obtained the lowest fresh yield ( $p < 0.05$ ). As a consequence, application of NPISi or OF reinforced fresh yield of green onion 18.5-23.8%, and 8.61-23.2%, respectively as compared to the positive treatment (recommended NPK). Especially, the combination of OF+NPISi enhanced the yield of green onion up to 56.0% as compared to the control.



**Figure 5.** Fresh yield of green onion plant of the treatments in the field condition crop 1 (a), and crop 2 (b) (12/2020-4/2021)

It was extremely noticeable in the second crop that the treatments with NPISi had a higher fresh yield of green onion than the treatment with OF, this is due to isolates in NPISi were adapted and developed a large amount of bacterial density to support salt tolerance, growth and yield ability of green onion plant. The same findings were reported by some authors who presented that amendment of the bio-fertilizer enhanced weight of green onion [40], [43], [44]. However, there was not any study about the application of bio-fertilizer to increase salt tolerance, growth and yield of green onion on salt-affected soil.

To sum up, amendment of sole NPISi or combined OF+NPISi boosted yield of green onion on salt-affected soil in Tran De district, Soc Trang province. Especially, application of NPISi containing beneficial bacteria as nitrogen fixation, phosphate-solubilizing, IAA synthesis, and silicate-solubilizing bacteria stimulated more efficient nutrient uptake of green onion plant than only utilization of OF.

#### 4. Conclusions

Treatment with NPISi or OF application enhanced significantly higher values of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{P}_2\text{O}_5$ , the number of nitrogen fixing bacteria, phosphorus, and silicate solubilizing bacteria in soil than in the positive control treatment with 100%NPK, moreover, treatment with NPISi or OF application induced a higher value level of the total nitrogen, phosphorus, potassium, and silicon concentration in green onion bulb as compared to the control. The best performance of green onion was observed in treatment applied with both OF and NPISi. Additionally, the inoculant in NPISi product excellently grew, boosted green onion height and yield under salinity condition, especially when associated with OF, green onion yield was enhanced up to 56.0% as compared to the positive control treatment. Therefore, NPISi product is recommended to be utilized for crops grown on saline soils.

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