

### Research Article

## Effects of Nitrogen Fertilization and Nitrogen Fixing Endophytic Bacteria Supplementation on Soil Fertility, N Uptake, Growth, and Yield of Sesame (*Sesamum indicum* L.) Cultivated on Alluvial Soil in Dykes

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The aim of this study was to determine the proper combination of nitrogen (N) fertilizer level and nitrogen fixing endophytic bacteria (NFEB) supplementation for the maximum grain yield of sesame cultivated in alluvial soil in dykes. The experiment followed a completely randomized block design with two factors. The first one was the levels of N fertilizer used, including 0, 50, 75, and 100% N of recommended fertilizer formula (RFF), and the other consisted of no bacteria applied, an individual strain of *Enterobacter cloacae* ASD-48 or *E. cloacae* ASD-21 applied, and their mixture, with 5 replicates. The results revealed that fertilizing with 100% N of RFF led to an enhancement of the plant height (16.8 cm), the chlorophyll a and b and their total content (6.45, 1.86, and 8.30  $\mu$ g mL<sup>-1</sup>), the number of capsules per plant (7.22 capsules plant<sup>-1</sup>), the total N uptake (126.5 mg N pot<sup>-1</sup>), and the grain yield (9.08 g pot<sup>-1</sup>), in comparison to no N fertilizer applied. Supplementation of two NFRB strains enhanced the soilconcentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, the total N uptake, and the grain yield. The treatment fertilized with 100% N of RFF plus an individual NFEB strain or their bacterial mixture had equivalent total N uptake to that the treatment fertilized with 100% N of RFF plus ASD-48 and ASD-21, either individually or in mixture, it increased by 2.39–8.56%, compared to that in the treatment fertilized with 100% N of RFF.

#### 1. Introduction

Sesame (*Sesamum indicum* L.) belongs to the family of Pedaliaceae. Sesame seeds are white, yellow, and black and contain great contents of oil, proteins, unsaturated fatty acids, vitamins, minerals, folic acids, and antioxidants [1, 2]. Nitrogen (N) is an essential nutrient that mostly limits the growth of crops [3, 4]. Moreover, fertilization with inorganic N plays an important role in agricultural production and

global food security. To be more specific, N fertilizer boosts growth, yield, P and K uptakes, and some other micronutrient contents [5, 6]. However, N use efficiency is low, and its overuse may result in many environmental issues, including greenhouse gases emission, and underground and surface water contamination due to the accumulation of nitrate concentration [7, 8]. For sesame cultivation, using N fertilizer in a dose from 46 to 100 kg N ha<sup>-1</sup> helps sesame to reach maximum yield [6]. Endophytic bacteria are promising to

enhance plants' growth and to perform biological control, and they have been used as biofertilizers, which are able to reduce the applied amount of chemical fertilizers and pesticides. Moreover, biofertilizers are cost-efficient and have smaller impacts on the environment in comparison to chemical fertilizers [9]. Furthermore, intensive crop farming combined with endophytic bacteria is able to fulfill the demand for a sustainable agriculture [10-12]. Among endophytic bacteria, those isolated from crops are found to provide to plants 47% N from the atmosphere, leading to an enhancement of plants' growth [8] and yield. In Vietnam, sesame is mainly cultivated in in-dyke alluvial soil in Can Tho, Dong Thap, and An Giang. Nevertheless, sesame yield can be decreased because of dykes used to control flooding. This leads to a reduction in nutrition in slurry. Therefore, this study was conducted in order to find out what level of N fertilizer plus NFEB was suitable for improving the growth, the yield, and the N uptake of sesame cultivated in alluvial soil in dykes.

#### 2. Materials and Methods

Time: the greenhouse experiment was conducted between September 2019 and March 2021.

Soil source: the soil used for cultivation was alluvial soil in dykes at the Agricultural Research and Experimental Farm, College of Agriculture, Can Tho University.

Sesame source: black sesame ADB1 used in this study was stored at the Department of Crop Science, College of Agriculture, Can Tho University.

Bacteria source: two NFEB strains of *Enterobacter cloacae* ASD-48 (MZ461952) and *E. cloacae* ASD-21 (MZ461951) were isolated from soil in the rhizosphere of upland crops in An Phu District, An Giang Province (our preliminary work).

Fertilizers: urea (46% N), superphosphate (16%  $P_2O_5$ ), and potassium chloride (60%  $K_2O$ ) were used.

A two-factor experiment was conducted following a completely randomized block design with 5 replicates, where a pot was considered as a replicate. Among the factors, the first one was the N fertilizer levels (0, 50, 75, and 100% N of recommended fertilizer formula, RFF), and the other was the supplementation of nitrogen fixing endophytic bacteria (NFEB) (no bacteria, an individual strain of ASD-48, an individual strain of ASD-21, and a mixture of both ASD-48 and ASD-21) for sesame in the Mekong Delta, Vietnam.

Sesame seeds preparation and bacterization: the black sesame seeds were first sanitized with 70% ethanol for 3 min and 1% sodium hypochlorite solution for 10 min and then washed twice with sterile distilled water. Black sesame seeds were incubated for 24 h under dark conditions for germination. Subsequently, for seed bacterization, 1,000 sesame seeds were mixed well with a 10 mL bacterial cell suspension of  $10^9$  cells mL<sup>-1</sup>, and others were mixed with 5 mL for each strain in a mixture for 1 h in a reciprocal shaker at 60 rpm. After 2 h of mixing, the seeds were taken out and dried under laminar airflow before planting. The control seeds were

similarly prepared in sterile distilled water. Two seeds were grown in each hole, so the NFEB density was roughly  $0.2 \times 10^8$  cells pot<sup>-1</sup>.

Soil preparation: soil was collected at a depth of 0-30 cm, removed from plant residues, and allowed to dry naturally. Three kilograms of soil was weighed into each pot.

Bacteria preparation: the initial bacterial density in the form of the suspension was  $1 \times 10^{10}$  CFU mL<sup>-1</sup>. Liquid bacteria were poured into sesame soil at 0, 7, 14, 21, 28, 35, 42, and 49 days after planting (DAP), with 3 mL for each pot.

Sowing: in each hole of a tray, two sesame seeds were sown. After 7 days, two sesame plants were put into each pot.

Chemical fertilizers formula: the NPK fertilizers recommended for 1 hectare were 90 kg N-60 kg  $P_2O_5$ -60 kg  $K_2O$ . This formula was followed by Khuong et al. [13]. The reduction by 25% for each level is based on their uptake ability and also the effects of N fertilizer on growth and yield of sesame.

Soil sampling at harvest: a 20 cm soil push probe was used to collect soil at three random sites in a pot. The soil was then put into plastic bags and allowed to dry at room temperature. The soil was finally crushed through a 2.0 mm  $\times$  0.5 mm sieve for soil analysis.

2.1. Soil Analysis. All of the analytic methods used in this study were arranged by Sparks et al. [14] and were briefly described as follows.

pH<sub>H2O</sub> and pH<sub>KCl</sub> were extracted with a soil-water ratio of 1:2.5 and soil-KCl 1 M ratio of 1:2.5 and measured by a pH meter. The total nitrogen was turned into its inorganic forms by using a mixture of H<sub>2</sub>SO<sub>4</sub> saturated-CuSO<sub>4</sub>-Se with a ratio of 100-10-1, determined by the Kjeldahl method, and titrated by 0.01N H<sub>2</sub>SO<sub>4</sub>. The available nitrogen (NH<sub>4</sub><sup>+</sup>) was extracted by 2M KCl, indicated in color by a mixture of sodium nitroprusside, sodium salicylate, sodium citrate, sodium tartrate, sodium hydroxide, and sodium hypochlorite, and detected at 650 nm wavelength, while the available nitrogen (NO<sub>3</sub><sup>-</sup>) was also extracted by 2M KCl, indicated by a mixture of 0.5M HCl, vanadium (III) chloride, sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride, and detected at 540 nm wavelength. The soluble P was measured by the Bray II method, in which it was extracted from soil by 0.1 N HCl and 0.03 N NH<sub>4</sub>F with a soil-water ratio of 1:7, indicated in color by ascorbic acid in a spectrophotometer at 880 nm wavelength. The concentration of organic matter was determined by the Walkley-Black method, which used K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> to oxidize organic compounds and FeSO<sub>4</sub> 0.5 N to determine the excessive K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> content. The initial soil chemical properties of sesame were expressed in Table 1.

Bacterial density: according to Harada [15], bacterial density in the soil at harvest was determined by the relative counting method.

TABLE 1: Initial soil properties for sesame cultivation.

Property	Value	Unit
pH <sub>H2O</sub> (1:2.5)	$5.50\pm0.071$	_
pH <sub>KCl</sub> (1:2.5)	$4.01\pm0.057$	_
N <sub>total</sub>	$0.100\pm0.028$	%
P <sub>total</sub>	$0.110\pm0.007$	%
$\mathrm{NH_4}^+$	$10.2 \pm 1.00$	mg $\rm NH_4^+ kg^{-1}$
NO <sub>3</sub> <sup>-</sup>	$16.5 \pm 1.41$	$mg NO_3^- kg^{-1}$
P <sub>available</sub> (bray II)	$45.2 \pm 1.13$	mg P kg <sup>-1</sup>
Organic matter	$1.00\pm0.035$	% C

Values are mean of three replications; ±standard deviation.

2.2. Chlorophyll Concentration. The concentrations of chlorophyll a and b and their total were determined according to a method by Moran [16]. Leaves were collected at the 3rd position from the shoot tip at 44 DAP and were cleaned. Then, holes with  $1 \text{ cm}^2$  in the area were put on leaves by a tip head. The collected samples were transferred into tubes, and 3 mL of N,N-dimethylformamide was added later. The tubes were covered and placed in the dark for 24 h at 20°C. Finally, the liquid was measured by a spectrophotometer at wavelengths of 664 and 667 nm.

2.3. Analysis of N and P in Seeds, Stems, Leaves, and Capsule Peel. Methods for plant analysis were described by Walinga et al. [17] and were summarized as follows: an oxidative mixture of  $100 \text{ mL H}_2\text{SO}_4$  saturated, 18 mL water, and 6 g salicylic acid was used to break the structure of samples, including seeds, stems, leaves, and capsule peel. These samples were heated and added with  $\text{H}_2\text{O}_2$  until they were completely oxidized. Then, the inorganic solution was used to measure the amount of N and P. N was determined by the Kjeldahl method, and P was determined by the colorimetric method of phosphomolybdate blue complex made from ammonium molybdate, reduced by ascorbic acid, and measured at a wavelength of 880 nm.

2.4. Agronomic Parameters. The agronomic parameters were determined on both plants in a pot at 51 DAP in mean values.

Plant height (cm) was measured from the ground to the peak of a sesame plant.

The number of leaves per plant (leaf plant<sup>-1</sup>) was counted as green leaves on a plant.

Leaf length (cm) was measured between the two tips of the fifth leaf pair from the top of a plant.

Leaf width (cm) was measured at the middle of the fifth leaf pair from the top of a plant.

2.5. Dry Biomass. Dry seeds: fresh seeds were dried at 70°C for 72 h to reach unchanged biomass; from which, dry weight could be weighed.

Stems, leaves, and capsule peel: fresh stems, leaves, and capsule peel were dried at 70°C for 72 h to reach unchanged biomass; from which, dry weight could be weighed.

2.6. Nutrients Uptake. Nitrogen uptake in seeds was the concentration of N in seeds  $\times$  dry weight of seeds.

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Nitrogen uptake in stems, leaves, and capsule peel was the concentration of N in stems, leaves, and capsule peel- $\times$  dry weight of stems, leaves, and capsule peel.

Total N uptake was the sum of N uptake in seeds, stems, leaves, and capsule peel.

A similar calculation was applied to P uptake in seeds, stems, leaves, and capsule peel, as well as total P uptake.

2.7. Yield Components and Grain Yield. The number of capsules per pot (capsules) was determined by counting all of the capsules on both plants in a pot. The data were calculated for each plant.

The other parameters were determined randomly on capsules:

The number of rows per capsule (rows) was determined by counting the rows on a capsule.

The number of seeds per row (seeds) was determined by counting all the seeds in a row.

Capsule length (cm) was determined by measuring from both ends of a capsule.

Capsule diameter (cm) was determined by measuring at the middle of the capsule, where the diameter was the largest.

1,000-seed weight (g) was determined by weighing the weight of 1,000 filled seeds in a capsule.

Sesame yield (g pot<sup>-1</sup>): the weight of fresh seeds as well as their humidity after harvesting was measured. Then, the yield was calculated by converting the weight at a humidity of 8.0%.

2.8. Statistical Analysis. The Microsoft Excel version 2013 was used to proceed the data. The variance analysis was carried out by ANOVA to compare the differences in the means of treatments which was tested by Duncan at a significant level of 5% in SPSS software version 13.0.

#### 3. Results

3.1. Influences of the Combination of N Fertilization and NFEB Supplementation on the Soil Properties of Alluvial Soil in Dykes. Under the effects of both N fertilizer levels and NFEB supplementation, values of  $pH_{H2O}$ ,  $pH_{KCl}$ , total N concentration, and soluble P concentration in soil changed insignificantly at the 5% level. The results in  $pH_{H2O}$  and  $pH_{KCl}$  fluctuated from 5.15 to 5.25 and from 4.09 to 4.26, respectively, for all N fertilizer levels, while for NFEB supplementation, the values were 5.20–5.25 and 4.12–4.22. The total N and soluble P content in the soil had an average value of 0.17% and 58.1 mg P kg<sup>-1</sup> for both N fertilizer levels and NFEB supplementation (Table 2).

According to Table 2, the  $NH_4^+$  concentration in the soil had no significant difference among N fertilizer levels, ranging from 13.4 to 15.1 mg  $NH_4^+$  kg<sup>-1</sup>. On the other hand, for NFEB supplementation,  $NH_4^+$  concentration was significantly different at 5% among treatments. In detail, treatments supplemented with an individual strain of ASD-48, ASD-21, or their mixture had equivalent amounts of  $NH_4^+$ , that is, 14.0, 15.3, and 15.0 mg  $NH_4^+$  kg<sup>-1</sup>, respectively, which were higher than that of treatments

Factors		pH <sub>H2O</sub> 1:2.5	pH <sub>KCl</sub> 1:2.5	N <sub>total</sub>	$\mathrm{NH_4}^+$	NO <sub>3</sub> <sup>-</sup>	P <sub>available</sub>	Bacterial density
		_	_	%	mg NH <sub>4</sub> <sup>+</sup> kg <sup>-1</sup>	mg NO <sub>3</sub> <sup>-</sup> kg <sup>-1</sup>	mg P kg <sup>-1</sup>	x10 <sup>5</sup> CFU g <sup>-1</sup> DSW
	0	5.23	4.14	0.18	13.5	15.8 <sup>d</sup>	56.2	1.57 <sup>b</sup>
Nitrogen fertilizer	50	5.23	4.26	0.17	13.7	17.2 <sup>c</sup>	57.0	1.59 <sup>b</sup>
levels (A) (%)	75	5.25	4.19	0.18	13.4	19.0 <sup>b</sup>	60.3	1.77 <sup>a</sup>
	100	5.19	4.09	0.17	15.1	20.8 <sup>a</sup>	58.9	$1.87^{a}$
	No-NFEB	5.20	4.17	0.17	11.4 <sup>b</sup>	15.7 <sup>c</sup>	57.5	0.02 <sup>c</sup>
NFEB inoculants (B)	ASD-48	5.25	4.17	0.17	$14.0^{a}$	17.4 <sup>b</sup>	57.6	2.12 <sup>b</sup>
$(8 \times 10^7 \mathrm{CFU} \mathrm{g}^{-1} \mathrm{DSW})$	ASD-21	5.25	4.22	0.17	15.3 <sup>a</sup>	$17.0^{b}$	60.3	$2.07^{b}$
0	Mixture	5.21	4.12	0.18	15.0 <sup>a</sup>	22.5 <sup>a</sup>	57.0	2.58 <sup>a</sup>
	F (A)	ns	ns	ns	ns	*	ns	*
Significant differences	F (B)	ns	ns	ns	*	*	ns	*
	F (A*B)	ns	ns	ns	*	*	ns	*
CV (%)		1.81	4.86	18.1	26.6	9.69	21.6	15.1

TABLE 2: Effects of nitrogen fertilizer levels and nitrogen fixing endophytic bacteria supplementation, *Enterobacter* spp., on improvement of alluvial soil fertility in dykes cultivated sesame at harvest.

In a column, the different superscripts indicate significant differences at 5% (\*, P < 0.05), and without letter indicates no significant difference according to Duncan's post hoc test at 5% level; NFEB: nitrogen fixing endophytic bacteria; No-NFEB: no applied nitrogen fixing endophytic bacteria; Mixture: both ASD-48 and ASD-21; CV: coefficient of variation; DSW: dry soil weight.

supplemented with no bacteria, whose available N content was  $11.4 \text{ mg NH}_4^+ \text{ kg}^{-1}$ .

The concentration of  $NO_3^-$  in soil was significantly different at 5% among treatments, influenced by changes in N fertilizer levels and NFEB supplementation. Treatments fertilized with 50, 75, and 100% N of RFF had  $NO_3^-$  content of 17.2, 19.0, and 20.8 mg  $NO_3^-$  kg<sup>-1</sup>, respectively. The treatment fertilized with no N fertilizer had the lowest concentration with 15.8 mg  $NO_3^-$  kg<sup>-1</sup>. Regarding NFEB, the treatment supplemented with the NFEB mixture peaked in  $NO_3^-$  concentration at 22.5 mg  $NO_3^-$  kg<sup>-1</sup>. Then, treatments supplemented with an individual strain of ASD-48 and ASD-21 had  $NO_3^-$  concentrations that were equivalent to each other's, 17.4 and 17.0 mg  $NO_3^-$  kg<sup>-1</sup>, respectively, but higher than that in the treatment supplemented with no bacteria, 15.7 mg  $NO_3^-$  kg<sup>-1</sup> (Table 2).

For the density of nitrogen fixing bacteria, both the N fertilizer factor and the NFEB factor had significant effects at 5%. The bacterial densities at N fertilizer levels of 75 and 100% N of RFF were statistically equal to each other, and valued at 1.77 and  $1.87 \times 10^5$  CFU g<sup>-1</sup> DSW, respectively, higher than those in the treatments fertilized with 0 and 50% N of RFF, whose results were 1.57 and  $1.59 \times 10^5$  CFU g<sup>-1</sup> DSW. The highest bacterial density was recorded in the treatment supplemented with the NFEB mixture  $(2.58 \times 10^5$  CFU g<sup>-1</sup> DSW). Treatments supplemented with an individual strain of NFEB ASD-48 and ASD-21 resulted in 2.12 and  $2.07 \times 10^5$  CFU g<sup>-1</sup> DSW), respectively, higher than that in the treatment supplemented with no bacteria applied  $(0.02 \times 10^5$  CFU g<sup>-1</sup> DSW) (Table 2).

#### 3.2. Influences of the Combination of N Fertilization and NFEB Supplementation on Biomass, N Concentration, and N Uptake in Sesame Cultivated on Alluvial Soil in Dykes

3.2.1. Biomass in Dry Seeds, Stems, Leaves, and Capsule Peel of Sesame. Biomass in dry seeds, stems, leaves, and capsule

peel of sesame changed significantly at 5% according to N fertilizer levels and NFEB supplementation. The biomass values in plant parts rose along with an increase of N fertilizer levels of 50, 75, and 100% N of RFF and were correspondingly 4.04, 5.33, and 6.13 g  $\text{pot}^{-1}$  in seeds and 2.55, 3.12, and 3.83 g pot<sup>-1</sup> in stems, leaves, and capsule peel. The treatment fertilized with no N fertilizer had the smallest biomass in seeds and stems, leaves, and capsule peel (1.56 and  $0.83 \text{ g pot}^{-1}$ ). Regarding the other factor, the treatments supplemented with the individual strain of ASD-21 or with the bacterial mixture had the highest and equivalent biomass to each other in stems, leaves, and capsule peel, 4.46 and  $4.55 \text{ g pot}^{-1}$ , respectively, higher than  $4.29 \text{ g pot}^{-1}$  in the treatment supplemented with the individual strain of ASD-48. The biomass in dry stems, leaves, and capsule peel was lowest in the treatment supplemented with no bacteria  $(3.75 \text{ g pot}^{-1})$ . For biomass in dry seeds, treatments supplemented with an individual strain of ASD-48 and ASD-21 and their mixture had statistically equal results, all of which were higher than that in the treatment supplemented with no bacteria,  $2.37 \text{ g pot}^{-1}$  (Table 3).

3.2.2. N Concentration in Dry Seeds, Stems, Leaves, and Capsule Peel of Sesame. N concentration in stems, leaves, and capsule peel of sesame varied significantly at 5% under the effects of N fertilizer levels and NFEB supplementation. At 100% N of RFF, the N concentration in stems, leaves, and capsule peel peaked at 0.49%. The treatment fertilized with 75% N of RFF came in second at 0.43%, and the lowest results were 0.41 and 0.45% in the treatments with 0 and 50% N of RFF, respectively. In the case of applying NFEB in soil, the treatments supplemented with the individual strain of ASD-21 or the bacterial mixture of both ASD-21 and ASD-48 had statistically the same amount of N in stems, leaves, and capsule peel (0.49 and 0.47%, resp.), higher than the treatments supplemented with the individual strain of ASD-48 or without bacteria (0.40 and 0.42%, resp.) (Table 3).

			Dry biomass		Nitrogen content		Nitrogen uptake	
Factors		Seed	Stem, leaf, and capsule peel	Seed	Stem, leaf, and capsule peel	Seed	Stem, leaf, and cansule neel	Total
			$(g pot^{-1})$		(%)		$(mg pot^{-1})$	
	0	$0.83^{d}$	1.56 <sup>d</sup>	2.85 <sup>c</sup>	0.41 <sup>c</sup>	23.8 <sup>d</sup>	6.50 <sup>d</sup>	$30.3^{d}$
	50	2.55 <sup>c</sup>	$4.04^{c}$	$3.07^{\mathrm{b}}$	$0.43^{\rm bc}$	78.1 <sup>c</sup>	17.3 <sup>c</sup>	95.5 <sup>c</sup>
Nitrogen Ierulizer levels (A) (%)	75	$3.12^{b}$	$5.33^{\mathrm{b}}$	$3.09^{\mathrm{b}}$	$0.45^{\mathrm{b}}$	96.6 <sup>b</sup>	24.1 <sup>b</sup>	$120.7^{b}$
	100	$3.83^{a}$	6.13 <sup>a</sup>	$3.30^{a}$	$0.49^{a}$	$126.6^{a}$	$30.1^{a}$	$156.7^{a}$
	No-NFEB	$2.37^{b}$	3.75 <sup>c</sup>	2.85 <sup>c</sup>	$0.42^{\rm b}$	69.6 <sup>c</sup>	$16.4^{\mathrm{b}}$	86.0 <sup>c</sup>
NFEB inoculants (B)	ASD-48	$2.64^{a}$	4.29 <sup>b</sup>	$3.17^{ab}$	$0.40^{\rm b}$	$84.8^{\mathrm{ab}}$	$17.2^{b}$	$102.0^{b}$
$(8 \times 10^7 \text{ CFU g}^{-1} \text{ DSW})$	ASD-21	$2.62^{a}$	$4.46^{a}$	$3.05^{\mathrm{b}}$	$0.49^{a}$	$80.5^{\mathrm{b}}$	$22.4^{\mathrm{a}}$	$102.9^{b}$
	Mixture	$2.70^{a}$	4.55 <sup>a</sup>	$3.24^{\mathrm{a}}$	$0.47^{a}$	$90.2^{a}$	$22.0^{a}$	$112.3^{a}$
	F (A)	*	*	*	*	*	*	*
Significant differences	F (B)	*	*	*	*	*	*	×
)	$F(A^*B)$	*	*	*	*	*	*	*
CV (%)		10.2	4.57	8.29	10.1	13.6	11.5	11.6
In a column, the different superscripts in endophytic bacteria; No-NFEB: no appl	dicate significant c lied nitrogen fixin	lifferences at g endophyti	: 5% (*, <i>P</i> < 0.05), and without letter ind c bacteria; Mixture: both ASD-48 and	licates no sig l ASD-21; CV	nificant difference according to Duncar 7: coefficient of variation; DSW: dry s	n's post hoc te oil weight.	est at 5% level; NFEB: nitro	ge

Table 3 shows that the N concentration in seeds differed significantly at 5% among treatments, affected by N fertilizer levels and NFEB supplementation. The treatment fertilized with 100% N of RFF had the highest N concentration in seeds with 3.30%. Then, the treatments fertilized with 50 and 75% N of RFF possessed equivalent contents of N, which were 3.07 and 3.09%, respectively, higher than 2.85% in the treatment fertilized with no N fertilizer. In addition, in treatments supplemented with bacteria, the one supplemented with the individual strain of ASD-48 had N concentration in seeds of 3.17%, equivalent to treatments supplemented with the individual strain of ASD-21 or the one supplemented with their mixture, whose N concentration values were 3.05 and 3.24%, respectively. Specifically, the treatment supplemented with the bacterial mixture had a dominant N content in seeds compared to the treatment supplemented with the individual strain of ASD-21. The lowest N concentration was found in the treatment supplemented with no bacteria, at 2.85%.

3.2.3. N Uptake in Stovers of Sesame and Its Total. According to Table 3, N uptake in seeds, stems, leaves, and capsule peel had significant differences at 5% among treatments, affected by N fertilizer levels and NFEB supplementation. The uptake in these stovers rose steadily with the N fertilizer levels: 17.3, 24.1, and 30.1 mg N pot<sup>-1</sup> in stem, leaves, and capsule peel and 78.1, 96.6, and 126.6 mg N pot<sup>-1</sup> in seeds with N fertilizer levels of 50, 75, and 100% N of RFF. The treatment fertilized with 0% N of RFF resulted in the lowest N uptake in stems, leaves, and capsule peel and in seeds, 6.50 and 23.8 mg N pot<sup>-1</sup>, respectively. For the NFEB factor, treatments supplemented with the individual strain of ASD-21 or with the mixture of both ASD-21 and ASD-48 had insignificantly different N uptake in stems, leaves, and capsule peel, 22.4 or 22.0 mg N pot<sup>-1</sup>, respectively, higher than that in the treatment supplemented with the individual strain of ASD-48 or with no bacteria, 17.3 or 6.50 mg N pot<sup>-1</sup>. N uptake in seeds in the treatment supplemented with the individual strain of ASD-48 was 84.8 mg N pot<sup>-1</sup>, equivalent to that in treatments supplemented with the individual strain of ASD-21 or with the bacterial mixture, where the results were 80.5 and 90.2 mg N pot<sup>-1</sup>, respectively. However, the treatment supplemented with the mixture of the two NFEB strains had statistically better N uptake than the treatment supplemented with the individual strain of ASD-21. The N uptake in sesame seeds was the lowest in the treatment supplemented with no bacteria  $(69.6 \text{ mg N pot}^{-1}).$ 

The total N uptake in sesame varied significantly at 5% under the influence of differences in N fertilizer levels and NFEB supplementation. In detail, according to the N fertilizer levels at 50, 75, and 100% N of RFF, the total N uptake was 95.5, 120.7, and 156.7 mg N pot<sup>-1</sup>, respectively. The lowest total N uptake was in the treatment fertilized with no N fertilizer, with 30.3 mg N pot<sup>-1</sup>. Moreover, the treatment supplemented with the bacterial mixture possessed the highest total N uptake (112.3 mg N pot<sup>-1</sup>). Then, the treatments supplemented with an individual strain of ASD-48 or ASD-21 had equivalent uptakes, 102.0 or 102.9 mg N pot<sup>-1</sup>,

respectively. The treatment supplemented with no bacteria had the lowest uptake, with  $86.0 \text{ mg N pot}^{-1}$  (Table 3).

The interactions between N fertilizer levels and NFEB supplementation led to significantly different total N uptake. The treatment fertilized with 100% N of RFF had a total N uptake value of 124.4 mg N pot<sup>-1</sup>, which was statistically equal to that in the treatment fertilized with 75% N of RFF plus NFEB individually or dually: 120.8, 125.2, and 125.5 mg N pot<sup>-1</sup>, respectively. Additionally, the treatments fertilized with 100% N of RFF plus an individual strain of ASD-48 or ASD-21 had equivalent total N uptake, which was 157.3 or 162.1 mg N pot<sup>-1</sup>, respectively. Moreover, at 100% N of RFF, the treatment supplemented with the combination of both NFEB strains had the highest N uptake, 183.3 mg N pot<sup>-1</sup> (Figure 1).

3.3. Influences of the Combination of N Fertilization and NFEB Supplementation on the Growth of Sesame Cultivated on Alluvial Soil in Dykes. At harvest, the plant height and the number of leaves of sesame were significantly influenced at 5% by both N fertilizer levels and NFEB supplementation. The plant height and the leaf number increased following the rise of levels of N fertilizer, with heights of 40.2, 43.5, and 46.7 cm and 12.6, 14.3, and 15.2 leaves  $plant^{-1}$  corresponding to 50, 75, and 100% N of RFF. The shortest plants and the fewest leaves were found in the treatment fertilized with no N fertilizer, 30.0 cm and 8.93 leaves plant<sup>-1</sup>. In the treatment supplemented with both NFEB strains, both plant height and leaf number peaked (41.7 cm and 13.8 leaves plant<sup>-1</sup>). In the treatments supplemented with an individual strain of either ASD-48 or ASD-21, plant height and leaves number were 39.8 or 40.7 cm and 13.1 or 12.4 leaves  $plant^{-1}$ , respectively, higher than those in the treatment supplemented with no bacteria (38.1 cm and 11.7 leaves  $plant^{-1}$ ) (Table 4).

The leaf size was significantly different at 5% in both length and width, caused by the differences in N fertilizer levels and NFEB supplementation. Treatments fertilized with 50, 75, and 100% N of RFF had leaf sizes of 6.26, 6.79, and 7.41 cm in length and 3.47, 3.94, and 4.64 cm in width. Simultaneously, the treatment fertilized with no N fertilizer had leaf length and width of 4.64 and 2.72 cm, respectively. In the treatment supplemented with an individual strain of ASD-48, ASD-21, or their combination, leaf size fluctuated around 6.34–6.49 cm in length and 3.72–3.85 cm in width, bigger than the leaf size in the treatment with no bacteria, which was 5.89 cm long and 3.36 cm wide (Table 4).

The chlorophyll a and chlorophyll b concentrations in sesame changed dynamically at 5% among treatments. In detail, chlorophyll a and chlorophyll b in sesame leaves increased with N fertilizer levels of 50, 75, and 100% N of RFF, valued at 9.59, 11.9, and 12.5  $\mu$ g mL<sup>-1</sup> and 2.71, 3.31, and 3.40  $\mu$ g mL<sup>-1</sup>, respectively, all higher than those in the treatment fertilized with no N fertilizer, whose results were 6.05 and 1.54  $\mu$ g mL<sup>-1</sup>. The treatment supplemented with the bacterial mixture had the highest chlorophyll a content (10.9  $\mu$ g mL<sup>-1</sup>). The second-highest chlorophyll a level was found in the treatments supplemented with an individual



FIGURE 1: Effects of nitrogen fertilizer levels and nitrogen fixing endophytic bacteria supplementation, *Enterobacter* spp., on improvement of N uptake in sesame at harvest on alluvial soil in dykes.

TABLE 4: Effects of nitrogen fertilizer levels and nitrogen fixing endophytic bacteria supplementation, *Enterobacter* spp., on growth of sesame cultivated on alluvial soil in dykes at harvest.

Factors		Plant	Number of leaves	Leaf length	Leaf width	Chl a (µg	Chl b (µg	Chl a+b
Factors		height (cm)	per plant (leaf)	(cm)	(cm)	$mL^{-1}$ )	$mL^{-1}$ )	$(\mu g m L^{-1})$
	0	30.0 <sup>d</sup>	8.93 <sup>d</sup>	4.64 <sup>d</sup>	2.72 <sup>d</sup>	6.05 <sup>d</sup>	1.54 <sup>d</sup>	7.60 <sup>d</sup>
Nitrogen fertilizer levels (A)	50	40.2 <sup>c</sup>	12.6 <sup>c</sup>	6.26 <sup>c</sup>	3.47 <sup>c</sup>	9.59 <sup>c</sup>	2.71 <sup>c</sup>	$12.2^{c}$
(%)	75	43.5 <sup>b</sup>	$14.3^{b}$	6.79 <sup>b</sup>	3.94 <sup>b</sup>	11.9 <sup>b</sup>	3.11 <sup>b</sup>	15.0 <sup>b</sup>
	100	46.7 <sup>a</sup>	15.2 <sup>a</sup>	7.41 <sup>a</sup>	4.64 <sup>a</sup>	12.5 <sup>a</sup>	3.40 <sup>a</sup>	15.9 <sup>a</sup>
	No- NFEB	38.1 <sup>d</sup>	11.7 <sup>d</sup>	5.89 <sup>b</sup>	3.36 <sup>b</sup>	9.38 <sup>c</sup>	2.45 <sup>b</sup>	11.8 <sup>c</sup>
NFEB inoculants (B) $(9 \times 10^7 \text{ CEU s}^{-1} \text{ DEW})$	ASD-48	39.8 <sup>c</sup>	13.1 <sup>b</sup>	6.49 <sup>a</sup>	3.72 <sup>a</sup>	9.93 <sup>b</sup>	2.83 <sup>a</sup>	12.8 <sup>b</sup>
$(8 \times 10 \text{ CFU g} \text{ DSW})$	ASD-21	$40.7^{b}$	12.4 <sup>c</sup>	6.34 <sup>a</sup>	3.85 <sup>a</sup>	9.87 <sup>b</sup>	2.63 <sup>ab</sup>	12.4 <sup>b</sup>
	Mixture	41.7 <sup>a</sup>	13.8 <sup>a</sup>	6.39 <sup>a</sup>	3.83 <sup>a</sup>	10.9 <sup>a</sup>	2.85 <sup>a</sup>	13.8 <sup>a</sup>
	F (A)	*	*	*	*	*	*	*
Significant differences	F (B)	*	*	*	*	*	*	*
	F (A*B)	*	*	ns	ns	*	ns	*
CV (%)		2.23	7.10	10.1	11.0	6.74	14.6	6.75

In a column, the different superscripts indicate significant differences at 5% (\*, P < 0.05), and without letter indicates no significant difference according to Duncan's post hoc test at 5% level; Chl: chlorophyll; NFEB: nitrogen fixing endophytic bacteria; No-NFEB: no applied nitrogen fixing endophytic bacteria; Mixture: both ASD-48 and ASD-21; CV: coefficient of variation; DSW: dry soil weight.

strain of ASD-48 or ASD-21, whose results were equivalently 9.93 and 9.87  $\mu$ g mL<sup>-1</sup>, respectively. The treatment supplemented with no bacteria had the lowest result of 9.38  $\mu$ g mL<sup>-1</sup>. The chlorophyll b content in the treatment supplemented with the individual strain of ASD-21 was 2.63  $\mu$ g mL<sup>-1</sup>, equivalent to that in the treatments supplemented with no bacteria, with the individual strain of ASD-48 and the bacterial mixture whose results were correspondingly 2.45, 2.83, and 2.85  $\mu$ g mL<sup>-1</sup>. Surprisingly, in the treatments supplemented with the bacterial mixture, chlorophyll b concentrations were equivalent to each other and higher than that in the treatment supplemented with no bacteria (Table 4).

In Table 4, treatments affected by N fertilizer levels and NFEB supplementation resulted in significantly different

total chlorophyll concentrations at 5%. The treatment fertilized with 100% N of RFF had the highest total chlorophyll (15.9  $\mu$ g mL<sup>-1</sup>). The treatment fertilized with 75% N of RFF outweighed the one fertilized with 50% N of RFF in total chlorophyll, with 15.0 compared to 12.2  $\mu$ g mL<sup>-1</sup>. The treatment fertilized with 0% N of RFF had the lowest total chlorophyll (7.60  $\mu$ g mL<sup>-1</sup>). For NFEB supplementation, in the treatments supplemented with the NFEB mixture, the total chlorophyll peaked at 13.8  $\mu$ g mL<sup>-1</sup>. The total chlorophyll content in the treatments supplemented with an individual strain of ASD-48 or ASD-21 was statistically equivalent, 12.8  $\mu$ g mL<sup>-1</sup> compared to 12.4  $\mu$ g mL<sup>-1</sup>. However, the treatment supplemented with no bacteria had the lowest total chlorophyll (11.8  $\mu$ g mL<sup>-1</sup>).

				Yield co	mponents			
Factors		Number of capsules per plant	Capsule length	Capsule diameter	Number of rows per capsule	Number of seeds per row	Weight 1,000 seeds	Grain yield
		Ċapsule plant <sup>-1</sup>	cm	cm	Rows capsule <sup>-1</sup>	Seed row <sup>-1</sup>	ad	g pot <sup>-1</sup>
	0	3.88 <sup>d</sup>	1.91 <sup>c</sup>	1.12 <sup>b</sup>	6.53°	8.88 <sup>d</sup>	2.43	2.72 <sup>d</sup>
Nitrogen fertilizer	50	7.50 <sup>c</sup>	$2.16^{\mathrm{b}}$	$1.22^{a}$	$7.07^{b}$	11.1 <sup>c</sup>	2.48	9.11 <sup>c</sup>
levels (A) (%)	75	$9.44^{\mathrm{b}}$	$2.24^{\mathrm{b}}$	$1.24^{\mathrm{a}}$	7.45 <sup>a</sup>	$12.2^{b}$	2.47	$9.80^{\rm b}$
	100	$11.1^a$	$2.42^{a}$	$1.24^{\mathrm{a}}$	$7.62^{a}$	$13.2^{a}$	2.49	$11.8^{a}$
	No-NFEB	7.08 <sup>c</sup>	2.13 <sup>b</sup>	$1.13^{b}$	6.72 <sup>c</sup>	$10.7^{\mathrm{b}}$	2.45	$7.37^{b}$
NFEB inoculants (B)	ASD-48	7.75 <sup>b</sup>	$2.24^{a}$	$1.22^{a}$	$7.22^{ab}$	$11.7^{a}$	2.50	8.75 <sup>a</sup>
$(8 \times 10^7 \text{ CFU g}^{-1} \text{ DSW})$	ASD-21	$7.78^{\mathrm{b}}$	$2.12^{b}$	$1.23^{\rm a}$	7.15 <sup>b</sup>	$11.5^{a}$	2.44	$8.50^{a}$
)	Mixture	$9.32^{a}$	$2.23^{a}$	$1.25^{a}$	$7.58^{a}$	11.5 <sup>a</sup>	2.48	$8.85^{a}$
	F (A)	*	*	*	*	*	us	*
Significant differences	F(B)	*	×	*	*	*	ns	×
)	$F(A^*B)$	*	*	ns	ns	ns	ns	*
CV (%)		7.12	6.15	5.86	8.24	10.0	4.44	6.60
In a column, the different super endophytic bacteria; No-NFEB	scripts indicate sig no applied nitrog	nificant differences at 5% (*, gen fixing endophytic bacte	, <i>P</i> < 0.05), and without le ria; Mixture: both ASD-	etter indicates no s -48 and ASD-21; 0	ignificant difference accorc CV: coefficient of variation	ling to Duncan's post hc n; DSW: dry soil weigh	oc test at 5% level; NFEI t.	B: nitrogen fixing

TABLE 5: Effects of nitrogen fertilizer levels and nitrogen fixing endophytic bacteria supplementation, Enterobacter spp., on yield components and grain yield of sesame cultivated on alluvial



FIGURE 2: Effects of nitrogen fertilizer levels and nitrogen fixing endophytic bacteria supplementation, *Enterobacter* spp., on improvement of sesame yield cultivated on alluvial soil in dykes.

#### 3.4. Influences of the Combination of N Fertilization and NFEB Supplementation on the Yield Components and the Yield of Sesame Cultivated on Alluvial Soil in Dykes

3.4.1. Yield Components. According to the results in Table 5, the number of capsules per plant changed significantly at 5% with different N fertilizer levels and NFEB supplementation. Treatments fertilized with N fertilizer had the number of capsules ranging from 7.50 to 11.1 capsules plant<sup>-1</sup>, while in the treatment fertilized with no N fertilizer, the number of capsules was the smallest (3.88 capsules plant<sup>-1</sup>). Along the same lines, the treatment supplemented with bacteria had approximately 7.75–9.32 capsules plant<sup>-1</sup>, more than the 7.08 capsules plant<sup>-1</sup> in the treatment supplemented with no bacteria.

The number of rows per capsule varied significantly at 5% among N fertilizer levels and NFEB supplementation. In detail, in the treatments fertilized with 75 and 100% N of RFF, the number of rows was equivalently 7.45 and 7.62 rows capsule<sup>-1</sup>, higher than that in the treatment fertilized with 50% N of RFF (7.07 rows capsule<sup>-1</sup>). Besides, the treatment fertilized with no N fertilizer had the lowest number of rows per capsule (6.53 rows capsule<sup>-1</sup>). Moreover, in the treatments supplemented with an individual strain of ASD-48 and ASD-21 or their mixture, the number of rows was in a range of 7.15–7.58 rows capsule<sup>-1</sup>, higher than that in the treatment supplemented with no bacteria (6.72 rows capsule<sup>-1</sup>) (Table 5).

The number of seeds per row significantly varied at 5% among treatments, affected by both factors. The number of seeds per row rose as N fertilizer levels increased from 50 to 75 to 100% N of RFF, from 11.1 to 12.2 to 13.2 seeds  $row^{-1}$ , while in the treatment fertilized with no N fertilizer, the

result was the lowest at 10.7 seeds row<sup>-1</sup>. In treatments supplemented with the NFEB bacteria individually or dually, the results ranged from 11.5 to 11.7 seeds row<sup>-1</sup>, higher than that in the treatment supplemented with no bacteria (10.7 seeds row<sup>-1</sup>).

The capsule length changed significantly at 5% according to the N fertilizer levels and the NFEB supplementation. In detail, the treatment fertilized with 100% N of RFF had the longest capsules (2.42 cm). The treatments fertilized with 50 or 75% N of RFF had the second-longest ones, 2.24 or 2.16 cm, respectively, while in the treatment fertilized with no N fertilizer, capsule length was the shortest, 1.91 cm. Additionally, the capsule length values were statistically equivalent among the treatments supplemented with the NFEB strain of ASD-48 or with the NFEB mixture of ASD-48 and ASD-21 and were 2.24 and 2.23 cm, respectively. However, the result in the treatment supplemented with the strain of ASD-21 (2.12 cm) was equivalent to that in the treatment supplemented with no bacteria (2.13 cm). The N fertilizer and NFEB factors gave out capsule diameters ranging from 1.22 to 1.24 cm and from 1.22 to 1.25 cm, respectively, all significantly at 5%, wider than those in the treatment supplemented with no N fertilizer and the one supplemented with no bacteria, which were 1.12 cm and 1.13 cm (Table 5).

In Table 5, the 1,000-seed weight was about 2.43–2.49 g among N fertilizer levels and 2.44–2.50 g among NFEB supplementation, insignificantly different between treatments.

*3.4.2. Sesame Yield.* The grain yield of sesame was significantly influenced at 5% by N fertilizer levels and NFEB supplementation. To be more specific, grain yields of 13.1,

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15.5, and 16.4 g pot<sup>-1</sup> corresponding to N fertilizer levels at 50, 75, and 100% N of RFF, higher than the 9.38 g pot<sup>-1</sup> at 0% N of RFF. Besides, the treatments supplemented with an individual strain of ASD-48, ASD-21, or their mixture had equivalent grain yield to each other, with the values of 8.75, 8.50, or 8.85 g pot<sup>-1</sup>, respectively. However, the lowest grain yield was in the treatment supplemented with no bacteria, with 7.37 g pot<sup>-1</sup> (Table 5).

In Figure 2, there were significant interactions at 5% between N fertilizer levels and NFEB supplementation in grain yield. The treatment fertilized with 100% N of RFF had a grain yield of  $9.74 \text{ g pot}^{-1}$ , statistically equal to those in the treatments fertilized with 50% N of RFF plus an individual strain of ASD-48, ASD-21, or their mixture, resulting in grain yield of 9.23, 9.10, or 9.70 g pot<sup>-1</sup>. Furthermore, the treatments fertilized with 75% N of RFF plus an individual strain of ASD-48 or ASD-21 possessed grain yield of 10.1 and  $9.75 \text{ g pot}^{-1}$ , respectively, equivalent to that in the treatment fertilized with 100% N of RFF. Surprisingly, in the treatment fertilized with 75% N of RFF plus the NFEB mixture, the grain yield was 10.3 g pot<sup>-1</sup>, significantly higher than the grain yield in the treatment fertilized with 100% N of RFF. Additionally, at 100% N of RFF, the treatments with an individual strain of ASD-48, ASD-21, or their mixture had equivalent grain yield values of 12.8 and 12.4 or 12.6 g  $pot^{-1}$ , higher than that in the treatment fertilized with only 100% N of RFF.

#### 4. Discussion

The increase in N fertilizer levels applied raised the amount of NO<sub>3</sub><sup>-</sup> in soil (Table 2). In addition, the supplementation of *E. cloacae* ASD-48, ASD-21, or their mixture boosted the concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in soil by 2.60–3.91 mg NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> and 1.31–6.83 mg NO<sub>3</sub><sup>-</sup> kg<sup>-1</sup> compared to the nonbacterial inoculation (Table 2). Moreover, treatments supplemented with NFEB resulted in higher bacterial density in soil than the treatment supplemented with no bacteria,  $2.12-2.58 \times 10^5$  CFU g<sup>-1</sup> DSW compared to  $0.02 \times 10^5$  CFU g<sup>-1</sup> DSW (Table 2). This result indicated that the supplementation with NFEB contributed to providing nutrients for sesame cultivated on alluvial soil in dykes. In other words, the bacteria carried out the nitrogen fixing function from the air or from the soil air, which produced available N content for plants.

The total N uptake in sesame in treatments supplemented with the bacteria mixture, with an individual strain of ASD-48 or ASD-21, and with no bacteria was sequentially  $112.3 > 102.0 - 102.9 > 86.0 \text{ mg N} \text{ pot}^{-1}$  (Table 3). Furthermore, with N fertilizer levels at 0, 50, 75, and 100% of RFF, the total Ν uptake rose correspondingly:  $30.3 < 95.5 < 120.7 < 156.7 \text{ mg N pot}^{-1}$ . Simultaneously, the supplementation of NFEB and the N fertilizer levels interacted with each other significantly at 5%. Thus, N content fixed by the bacteria positively impacted N uptake in sesame seeds, stems, leaves, and capsule peel compared to the treatment supplemented with no bacteria (Table 3).

The supplementation of NFEB strains of *E. cloacae* ASD-48 and ASD-21 individually or together enhanced the

amount of available N content in soil and N uptake in plants (Tables 2 and 3). Syaputra and Hidayati [18] have conducted a study on sugarcane which shows the highest N uptake in the treatment fertilized with 100% of inorganic fertilizers plus biofertilizers containing nitrogen fixing bacteria. The result was 62.56% higher than that in the treatment with only 100% of inorganic fertilizers. In consequence, in treatments

pius biolertilizers containing introgen fixing bacteria. The result was 62.56% higher than that in the treatment with only 100% of inorganic fertilizers. In consequence, in treatments with NFEB supplementation, the growth of sesame was stimulated in its height, number of leaves per plant, leaf size, and concentrations of chlorophyll a and b and their total (Table 4). In a study by Din et al. [19], biofertilizers containing the nitrogen fixing bacterial strain *Azotobacter* SR4 improved the plant height of gourds and okra compared to no biofertilizers applied. In addition, the growth parameters of sesame, including the number of capsules per plant, the capsule length, the number of rows per capsule, and the number of seeds per row, were improved by the application of NFEB (Table 5).

The application of NFEB improved sesame yield components, leading to better grain yield. To be more specific, adding a mixture of E. cloacae ASD-48 and ASD-21 or applying them individually led to higher grain yield values than the treatment supplemented with no bacteria, 8.50-8.85 g pot<sup>-1</sup> compared to 7.37 g pot<sup>-1</sup>. The result was in accordance with that in a study by Shakeri et al. [20], where in 2010, sesame supplied with a mixture of nitrogen fixing strains of Azospirillum and Azotobacter had 121.0 capsules plant<sup>-1</sup>, 74.8 seeds capsule<sup>-1</sup>, and a yield of 1218.3 kg ha<sup>-1</sup>, which were increased by 23.6, 15.5, and 27.6% compared to no bacteria applied. According to Shakeri et al. [20], with N fertilizer levels at 25 and 50 kg ha<sup>-1</sup>, the number of capsules per plant, the number of seeds per capsule, and the grain yield were higher than those in the case of no N fertilizer, 117.9 and 145.3 capsules plant<sup>-1</sup>, 73.9 and 78.6 seeds capsule<sup>-1</sup>, and 1149.3 and  $1309.7 \text{ kg ha}^{-1}$  compared to  $65.1 \text{ capsules plant}^{-1}$ , 56.8seeds capsule<sup>-1</sup>, and 800.4 kg ha<sup>-1</sup>. Furthermore, there were interactions at 5% among N fertilizer levels and NFEB supplementation in sesame yield. In detail, when applying any strains of E. cloacae ASD-48 and ASD-21 individually or together plus N fertilizer levels at 50 and 75% of RFF, the grain yield obtained was not lower than that in the treatment fertilized with only 100% N of RFF (Table 5). This result was consistent with a study by Shakeri et al. [20], where at 25 and 50 kg N ha<sup>-1</sup> of N fertilizer plus supplementation with Azospirillum and Azotobacter combination, the grain yield dominated that in the treatment supplemented with no bacteria. In detail, in 2010, at 25 or 50 kg N ha<sup>-1</sup>, the sesame Darab-14 and GL-13 had higher grain yields (1341.7 or 1340.7 kg ha<sup>-1</sup> and 1520.8 or  $1500.0 \text{ kg ha}^{-1}$ ) than the treatments supplemented with no bacteria (1054.17 or 1091.67 kg ha<sup>-1</sup> and 1204.7 or 1195.8 kg  $ha^{-1}$ ). The lowest grain yield was in the treatment with neither N fertilizer nor bacteria, ranging around 675.1-1006.3 kg ha<sup>-1</sup>. Moreover, the grain yield in the treatment with 100% N was not significant difference with 75% N in case of no bacteria. However, yield is continuously increasing for the NFEB supplementation at these levels. This showed that potential yield can be higher in the

treatment of NFEB application at higher chemical N fertilizer.

In summary, the supplementation of NFEB strains of *E. cloacae* ASD-48 and ASD-21 was efficient in nitrogen fixation, with the concentrations of  $NH_4^+$  and  $NO_3^-$  in the range of 14.0–15.3 mg  $NH_4^+$  kg<sup>-1</sup> and 17.0–22.5 mg  $NO_3^-$  kg<sup>-1</sup>, compared to 11.4 mg  $NH_4^+$  kg<sup>-1</sup> and 15.7 mg  $NO_3^-$  kg<sup>-1</sup> in the treatment supplemented with no bacteria. Thus, the total N uptake in sesame went up by 16.0, 16.9, or 26.3 mg N pot<sup>-1</sup> correspondingly in the treatment supplemented with an individual strain of ASD-48 or ASD-21 or their mixture compared to the treatment supplemented with no bacteria. The increase in available N content and total N uptake led to a 20.0% higher grain yield compared to no bacteria. To sum up, the highest efficacy in total N uptake and sesame yield was recorded in the treatment supplemented with the mixture of two NFEB strains.

#### 5. Conclusions

Plant height, number of capsules per plant, leaf size, the concentrations of chlorophyll a and b and their total, total N uptake, and sesame yield rose in the treatment fertilized with 100% N of RFF compared to those in the treatment with no N fertilizer.

Supplementation with two strains of *Enterobacter clo-acae*, ASD-48 and ASD-21, enhanced the amount of  $NH_4^+$  and  $NO_3^-$ , the total N uptake, and the grain yield in comparison to the treatment supplemented with no bacteria: 15.0 mg  $NH_4^+$  kg<sup>-1</sup>, 22.5 mg  $NO_3^-$  kg<sup>-1</sup>, 112.3 mg N pot<sup>-1</sup>, and 8.85 g pot<sup>-1</sup> versus 11.4 mg  $NH_4^+$  kg<sup>-1</sup>, 15.7 mg  $NO_3^-$  kg<sup>-1</sup>, 86.0 mg N pot<sup>-1</sup>, and 7.37 g pot<sup>-1</sup>, respectively.

Sesame plants treated with 75% N of RFF plus an individual strain of ASD-48, ASD-21, or their mixture had N uptake equivalent to that in the treatment fertilized with only 100% N of RFF:  $120.8-125.5 \text{ mg N pot}^{-1}$  compared to  $124.4 \text{ mg N pot}^{-1}$ .

The sesame yield in the treatments fertilized with 50-75% N of RFF plus an individual strain of ASD-48, ASD-21, or their mixture was equivalent to or even higher than the results in the treatment fertilized with only 100% of RFF, 9.23-10.3 g pot<sup>-1</sup> compared to 9.47 g pot<sup>-1</sup>.

#### **Data Availability**

The data presented in this article are available from the corresponding author upon request.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

#### **Authors' Contributions**

Le Vinh Thuc, Tran Ngoc Huu, Trinh Minh Ngoc, Nguyen Hong Hue, Do Thi Xuan, Tran Chi Nhan, Ly Ngoc Thanh Xuan, and Le Thi My Thu collected, analyzed, and interpreted the data. Le Vinh Thuc and Le Thanh Quang drafted the paper. Isao Akag, Jun-Ichi Sakagami, and Nguyen Quoc Khuong revised the paper. All authors read and approved the final manuscript.

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