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# Effects of nitrogen fertilizer levels and $N_2$-fixing rhizosphere bacteria supplements, *Enterobacter asburiae*, on soil fertility, N uptake, growth and yield of sesame (*Sesamum indicum* L.) cultivated on alluvial soil in dyke

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**Abstract** The result showed the fertilization 90.0 kg N $ha^{-1}$ had total nitrogen uptake, plant height, leaves number, concentrations of chlorophyll a, b and a+b, and sesame grain yield higher than that fertilization at 0.0 kg N $ha^{-1}$, with the corresponding values of, 110.0 mg $pot^{-1}$, 75.5 cm, 17.6 leaves $plant^{-1}$, 10.9 µg $mL^{-1}$, 2.95 µg $mL^{-1}$, 13.9 µg $mL^{-1}$ and 6.34 g $pot^{-1}$, compared to 23.4 mg $pot^{-1}$, 26.6 cm, 12.3 leaves $plant^{-1}$, 8.11 µg $mL^{-1}$, 1.94 µg $mL^{-1}$, 10.1 µg $mL^{-1}$ and 2.17 g $pot^{-1}$, respectively. Supplementation of only *Enterobacter asburiae* ASD-07, only *E. asburiae* ASD-28 or both *E. asburiae* ASD-07 and *E. asburiae* ASD-28 raised the available nitrogen content in soil, the total nitrogen uptake, plant height, capsules number per plant and sesame grain yield, with the values of 4.20-6.40 mg $NH_4^+$ $kg^{-1}$, 32.4-45.0 mg N $pot^{-1}$, 1.7-4.2 cm, 3.75-5.15 capsules $plant^{-1}$ and 1.51-1.62 g $pot^{-1}$, respectively. The addition of NFRB strains either individual or dual ASD-07 and ASD-28 combined with 45.0 or 67.5 kg N $ha^{-1}$ of nitrogen fertilizer resulted in a higher sesame yield, in comparison to fertilization 90.0 kg N $ha^{-1}$ as a recommended fertilizer formula with no bacteria.

**Keywords:** Alluvial soil, In-dyke, Nitrogen-fixing, Rhizopheric bacteria, Sesame

## Introduction

Sesame is a popular annual crop for oil in Ethiopia, India and China (Gebregergis and Amare, 2019). Sesame seeds contain an amount of este of unsaturated fatty acids, including oleic acid and linolenic acid, and natural antioxidants, such as sesamines, sesamin, episesamin, sesaminol and sesamolin

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which are responsible for preventing oxidization of unsaturated fatty acids (Zenawi and Mizan, 2019; Gebregergis and Amare, 2019). Additionally, sesame seeds are used as daily diet with essentially nutritional values, due to the fact that sesame seeds have sesamin which is effective in treating obesity and in reducing the risks of cardiovascular diseases (Dalibalta *et al*., 2020). Moreover, frequent use of sesame seeds enhances the capability of accumulating oil soluble vitamins (Hernández-Pérez *et al*., 2021). According to Okpara *et al*. (2007), sesame yield in countries, including Saudi Arabia, Nigeria and Venezuela are 1,083, 300 and 1,295 kg $ha^{-1}$, respectively. For nitrogen, fertilizing 75 kg N $ha^{-1}$ on sesame results in 650 kg $ha^{-1}$ of sesame yield (Shehu, 2014), and fertilizing 80 kg $ha^{-1}$ urea led to 2,500 kg $ha^{-1}$ of sesame yield (Babajide and Oyeleke, 2014). When the fertilization reaches 90 kg N $ha^{-1}$, the sesame yield is 520 kg $ha^{-1}$ (Ibrahim *et al*., 2016) and at 92 kg $ha^{-1}$ urea, the yield is 918 kg $ha^{-1}$ (Amare *et al*., 2019). This indicates that the important role of nitrogen to and its demand for sesame is enormous and highly fluctuated at different cultivation sites. Thus, the amount of nitrogen fertilizer used is great in order to obtain the highest yield, but the potential loss of nitrogen in the forms of NO, $NO_2$ and $NH_3$ is high as well, from 12 to 60% of the total nitrogen fertilized (Zhang *et al*., 2012; Mohamed *et al*., 2019). Furthermore, the alluvial soil in dyke possesses low concentrations of total nitrogen and available nitrogen (Park *et al*., 2020). Nevertheless, the function of using rhizopheric bacteria for a provision of nitrogen for cultivars and a reduction of chemical fertilizers used for plants has been studied and widely applied (Khuong *et al*., 2018; 2020). Soil microbes play an important role in agriculture. To be more specific, it is estimated that there are about 160 million tons of nitrogen fixed by microorganisms worldwide (Li *et al*., 2019). Among the microbes, rhizopheric bacteria are able to fix nitrogen, leading to enhancement in soil fertility, growth, yield and nutrients uptake of plants (Hammad *et al*., 2015; Etesami *et al*., 2014; Pham *et al*., 2017; Godínez *et al*., 2019; Cortivo *et al*., 2020; Hala, 2020). Therefore, the isolated bacteria from soil are applied to soil in the form of suspension and microbial products, so as to improve plant growth (Hala, 2020; Khuong *et al*., 2020). Especially, plant yield can maintain in the case of reducing the amount of inorganic fertilizer by 15-40% and combining with rhizopheric bacteria (Mącik *et al*., 2020). In this study, the efficacy of nitrogen-fixing rhizosphere bacteria (NFRB), *Enterobacter asburiae*, must be evaluated. Therefore, the study was carried out in order to (i) determine the effect of nitrogen fertilizer levels and NFRB supplements on soil properties, nitrogen uptake, growth and yield of sesame, and (ii) measure the amount of nitrogen fertilizer altered by the NFRB addition but still keeping up the yield of sesame cultivated on alluvial soil in dyke.

## Materials and methods

### *Materials*

Time: A pot experiment took place from September 2019 to March 2021 at Research and Experiment Station, College of Agriculture, Can Tho University.

Soil: The alluvial soil in dyke was collected at Research and Experiment Station, College of Agriculture, Can Tho University for experiment.

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

Bacterial source: Two NFRB strains *E. asburiae* ASD-07 and *E. asburiae* ASD-28 were isolated from the rhizosphere of cultivars at An Phu district, An Giang province (our prelimitary work).

Fertilizers: The used fertilizers consisted of Urea (46% N), super phosphate (16% $P_2O_5$) and KCl fertilizer (60% $K_2O$).

### *Methods*

The two-factor experiment was followed a completely randomized block design, with five replicates, each of which was a pot of dry soil. In detail, a first factor was nitrogen fertilizer levels (0.0, 45.0, 67.5 and 90.0 kg N $ha^{-1}$) and the other one was supplementation of NFRB (no NFRB, individual supplementation of ASD-07, ASD-28 and a combined supplementation of ASD-07 and ASD-28). The nitrogen fertilizer levels were designed to decrease by 25, 50, and 100% in comparison to the recommended fertilizer formula for sesame in Mekong Delta, Vietnam.

Soil preparation: Soil was collected at the depth of 0-30 cm, eliminated from plant residues and naturally dried. Three kg of soil was scaled and put into each pot.

Bacterial preparation: The initial bacterial density in suspension was 1 x $10^{10}$ CFU $mL^{-1}$. Bacterial inoculation into sesame: at each pot, 3 mL of bacteria was inoculated evenly to plant ground at day 0, 7, 14, 21, 28, 35, 42 and 49 days after planting (DAP).

Seeds sowing: Sesame seeds were sowed in strays (two seeds in one hole) after 7 days. Then, planting in pots was conducted, each pot had two plants.

Inorganic fertilizer formula: The amount of NPK fertilizer for 1 hectare was 90 kg N - 60 kg $P_2O_5$ - 30 kg $K_2O$.

Soil sampling at harvest: Soil probe with 20 cm in length were used to collect 3 sites in each pot. The soil collected was put into plastic bags and let dry at room temperature. After that, the dry soil was smashed through a sieve of 2.0 mm and 0.5 mm for soil analysis.

**Soil analysis**

All of the analytical methods in this study were composed by Sparks *et al*. (1996), briefly summarized as follows:

$pH_{H2O}$ or $pH_{KCl}$ was extracted from a ratio of soil: water (1:2.5) or soil: KCl 1 M (1:2.5), and measured by a pH meter. The total nitrogen content was turned into inorganic nitrogen by a mixture of saturated $H_2SO_4$-$CuSO_4$-Se with ratio of 100-10-1 and determined by Kjeldahl and titrated by $H_2SO_4$ 0.01 N. The available nitrogen in the form of $NH_4^+$ was extracted by KCl 2 M and indicated in colors by a mixture of sodium nitroprusside, sodium salicylate, sodium citrate, sodium tartrate, sodium hydroxide and sodium hypochlorite, and detected at a wavelength of 650 nm. While the $NO_3^-$ nitrogen form was extracted by KCl 2 M, indicated in colors by a mixture of HCl 0.5 M, vanadium (III) chloride, sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride, and detected at 540 nm wavelength. Soluble phosphate was determined by Bray II method, extracted from soil by a mixture of 0.1 N HCl and 0.03 N $NH_4F$ at ratio soil: extractant (1:7). The extracted solution was determined in colors by ascorbic acid and measured at 880 nm wavelength. The organic matter content was determined by Walkley Black method: using $K_2Cr_2O_7$ to oxidize organic matter in saturated $H_2SO_4$ solution, and the excessive content of $K_2Cr_2O_7$ was titrated by $FeSO_4$ 0.5 N. The initial soil properties for cultivation were shown in Table 1.

Bacterial density in soil: According to Harada (2001), the bacterial population in soil at harvest was determined by most population density method.

**Chlorophyll concentration**

The chlorophyll a, b and total values were detected by a method of Moran (1982). Leaves were sampled at the third position from the top of apical meristem at 44 DAP, and then cleaned. Next, holes with 1 $cm^2$ in size of a pure tip were put on sesame leaves which were then transferred into tubes with 3 mL of N,N – Dimethyllformamide. After that, the tubes with leaves were covered in dark for 24 h at 20 $^oC$. Finally, the liquid in tubes was measured at wavelengths, 664 and 667 nm.

Formula for calculating the a, b and total chlorophyll:

$A_{664} = 83.9\ C_a + 10.8\ C_b$

$A_{647} = 20.2\ C_a + 45.6\ C_b$

$C_a = 12.64\ A_{664} – 2.99\ A_{647}$

$C_b = - 5.6\ A_{664} + 23.26\ A_{647}$

$$C_t = 7.04\ A_{664} + 20.27\ A_{647}$$

While:

$A_{664}$: absorbance value at wavelength of 664 nm.
$A_{667}$: absorbance value at wavelength 647 nm.
$C_a$: chlorophyll a content (µg $mL^{-1}$).
$C_b$: chlorophyll b content (µg $mL^{-1}$).
$C_{a+b}$: chlorophyll a+b content (µg $mL^{-1}$).

**Table 1.** Initial soil properties for sesame cultivation

| Property | Value | Unit |
|---|---|---|
| $pH_{H2O}$ (1:2.5) | 5.50 ±0.071 | - |
| $pH_{KCl}$ (1:2.5) | 4.01 ±0.057 | - |
| $N_{total}$ | 0.100 ±0.028 | % |
| $P_{total}$ | 0.110 ±0.007 | % |
| $NH_4^+$ | 10.2 ±1.00 | mg $kg^{-1}$ |
| $NO_3^-$ | 16.5 ±1.41 | mg $kg^{-1}$ |
| $P_{available}$ (Bray II) | 45.2 ±1.13 | mg $kg^{-1}$ |
| Organic matter | 1.00 ±0.035 | %C |

Values are mean of three replications; ±standard deviation

**Analysis of nitrogen and phosphate in seeds, stem, leaves and capsule peel**

A mixture of 100 mL of $H_2SO_4$ saturated, 18 mL of water and 6 g of salicylic acid was used to break seeds, stem, leaves and capsule peel samples. The samples were heated with an addition of $H_2O_2$ until they were completely oxidized. Then, the inorganic solution was used to detect the concentration of nitrogen and phosphate. In detail, nitrogen was determined by Kjeldahl method and phosphate was measured by colorimetric method at wavelength of 880 nm.

**Growth parameters**

All of sesame growth were determined on both plants at 51 DAP.

Plant height (cm): measurement took place from the ground to the peak of the plant. Number of leaves (leaves $plant^{-1}$): all green leaves in a plant were counted. Leaf length (cm): measurement took place from stem to tip at the fifth leaves pair of the two opposite leaves on the plant. Leaf width (cm): measurement took place at the middle of the fifth leaves pair of the 2 opposite leaves.

**Dry biomass**

Dry seed weight: seeds were dried up at 70 ℃ in 72 h, reaching the unchangeable weight to scale for dry biomass.

Dry stem, leaves and capsule peel weight: Stem, leaves and capsule peel were dried up at 70 ℃ in 72 h for unchangeable weight to scale for dry weight.

**Nutrients uptake**

Nitrogen uptake in seeds was the nitrogen content in seeds x dry seeds biomass. Nitrogen uptake in stem, leaves and capsule peel was nitrogen content in stem, leaves and capsule peel x their dry biomass. The total nitrogen uptake was the sum of nitrogen uptake in seeds, stem, leaves and capsule peel.

The similar calculation was applied for phosphate uptake in seeds, stem, leaves and capsule peel, and total phosphate.

**Yield components and yield**

Number of capsules per pot (capsule): capsules of both plants in a pot were counted.

The other criteria were determined randomly on 10 capsules. Number rows per capsule (rows): the rows number of a capsule were counted. Number of seeds per capsule (seeds): the seeds number of a capsule were counted. Number of seeds per row (seeds): the seeds on each row of a capsule were counted. Capsule length (cm): measurement took place from the bottom to the top of a capsule. Capsule diameter (cm): measurement took place at the middle of the capsule where widest diameter was. 1,000-seed weight (g): the weight of 1,000 sesame seeds was scaled. Sesame grain yield (g $pot^{-1}$): the weight and humidity of fresh seeds was measured at harvest. Then, the grain yield was converted into humidity of 8.0%.

***Statistical analysis***

Microsoft Excel software version 2013 was applied for data processing. Variance analysis ANOVA was applied for comparing differences among means of treatments via Duncan test at 5% by SPSS software version 16.0.

## Results

***The influence of nitrogen fertilizer levels and NFRB supplements on fertility of soil used for sesame cultivation in dyke***

The values of $pH_{H2O}$, $pH_{KCl}$, contents of total nitrogen, $NH_4^+$, $NO_3^-$ and soluble P varied insignificantly among levels of nitrogen fertilization, ranging respectively around 5.37-5.42, 3.90-4.00, 0.148-0.156%, 16.7-18.8 mg $NH_4^+$ $kg^{-1}$, 12.8-13.3 mg $NO_3^-$ $kg^{-1}$ and 108.0-111.7 mg P $kg^{-1}$. However, bacterial density showed significant differences at 5% among fertilizer nitrogen levels, including 0.0, 45.0, 67.5 and 90.0 kg N $ha^{-1}$. In detail, the density of bacteria in levels 0.0, 45.0 and 67.5 kg N $ha^{-1}$ was statistically similar, ranging from 17.2 to 19.3 x $10^5$ CFU g $soil^{-1}$, and lower than the another fertilizer level, whose value was 22.0 x $10^5$ CFU g $soil^{-1}$ (Table 2).

**Table 2.** Effect of nitrogen fertilizer levels and $N_2$-fixing rhizosphere bacteria supplements on improvement of alluvial soil fertility in dyke cultivated sesame at harvest

| **Factors** | | **$pH_{H2O}$** | **$pH_{KCl}$** | **$N_{total}$** | **$NH_4^+$** | **$NO_3^-$** | **$P_{available}$** | **Bacterial density** |
|---|---|---|---|---|---|---|---|---|
| | | 1:2.5 | 1:2.5 | % | mg $kg^{-1}$ | mg $kg^{-1}$ | mg $kg^{-1}$ | x $10^5$ CFU $g^1$ |
| Nitrogen fertilizer levels (A) ($kg\ ha^{-1}$) | 0.0 | 5.42 | 4.00 | 0.148 | 17.7 | 13.3 | 111.7 | $17.2^b$ |
| | 45.0 | 5.38 | 3.90 | 0.152 | 16.7 | 13.3 | 108.0 | $19.3^b$ |
| | 67.5 | 5.39 | 3.97 | 0.154 | 18.1 | 13.2 | 108.5 | $18.3^b$ |
| | 90.0 | 5.37 | 4.00 | 0.156 | 18.8 | 12.8 | 110.1 | $22.0^a$ |
| NFRB inoculants (B) (8 x $10^7$ CFU $g^{-1}$ soil) | No-RB | $5.23^b$ | 3.93 | 0.154 | $13.7^b$ | $12.2^b$ | $95.8^c$ | $0.04^c$ |
| | ASD-28 | $5.46^a$ | 3.96 | 0.151 | $17.9^a$ | $13.7^{ab}$ | $113.0^{ab}$ | $21.3^b$ |
| | ASD-07 | $5.48^a$ | 3.95 | 0.155 | $19.4^a$ | $12.3^{ab}$ | $109.8^b$ | $22.2^b$ |
| | Mixture | $5.40^a$ | 4.03 | 0.152 | $20.1^a$ | $14.4^a$ | $119.7^a$ | $33.8^a$ |
| Significant differences | F (A) | ns | ns | ns | ns | ns | ns | * |
| | F (B) | * | ns | ns | * | * | * | * |
| | F (A*B) | ns | * | ns | ns | ns | * | * |
| CV (%) | | 4.43 | 4.64 | 6.53 | 28.6 | 21.7 | 11.1 | 14.8 |

In a column, the different superscripts indicate significant differences at 5% ($P < 0.05$, *), and without letter is no significant difference according to Duncan's post-hoc test at 5% level. NFRB: $N_2$-fixing rhizosphere bacteria; No-RB: no applied rhizosphere bacteria; Mixture: both ASD-07 and ASD-28; CV: Coefficient of variation.

The values of $pH_{H2O}$, available content of $NH_4^+$ and $NO_3^-$, soluble phosphate and bacterial density were different statistically at 5% between in the treatments without bacteria and the ones with bacteria. The $pH_{H2O}$ in the treatment without bacteria was the lowest (5.23), compared to that in the treatments supplied with NFRB where the $pH_{H2O}$ value ranged from 5.40 to 5.48. Furthermore, $pH_{H2O}$ values between the treatment with individual strain ASD-07, ASD-28 and the one with the combination of two NFRB strains ASD-07 and ASD-28 were statistically equivalent. Sharing the same trend as $pH_{H2O}$, in the treatment added with a mixture of ASD-07 and ASD-28 reached the concentration of available nitrogen forms, $NH_4^+$ and $NO_3^-$, and soluble phosphate at 20.1 mg $NH_4^+$ $kg^{-1}$, 14.4 mg $NO_3^-$ $kg^{-1}$ and 119.7 mg P $kg^{-1}$, respectively. In the same order, these values were significantly higher than those in the treatment with no bacteria applied, whose values were 13.7 mg $NH_4^+$ $kg^{-1}$, 12.2 mg $NO_3^-$ $kg^{-1}$ and 95.8 mg P $kg^{-1}$. Besides, the concentrations of $NH_4^+$ and $NO_3^-$ in the all of treatments with bacteria, including individually supplied with ASD-07 and ASD-28, and used a mixture of ASD-07 and ASD-28, were statistically equal. The densities of bacteria among treatments with bacteria supplied was all higher than those in treatments without bacteria. To be more specific, bacterial density in the treatment served with bacteria from both ASD-07 and ASD-28 was the highest one, the second highest included those in the treatment supplied with ASD-07 and ASD-28 individually, and the lowest one belonged to the bacterial density in the treatment without bacteria, the density values were 33.8, 22.2, 21.3 and 0.04 x $10^5$ CFU g $soil^{-1}$, respectively (Table 2).

***The influence of nitrogen fertilizer levels and NFRB supplements on biomass, concentration and uptake of nitrogen and phosphate in sesame cultivated on alluvial soil in dyke***

**Dry biomass of sesame parts**

The dry biomass of seeds, and stem, leaves and capsule peel changed significantly at 5% owing to nitrogen fertilizer levels. To be more specific, at fertilizer levels of 0.0, 45.0, 67.5 and 90.0 kg N $ha^{-1}$, in the same order, the dry weights of seeds were 0.71 < 1.74 < 2.02 < 2.15 g $pot^{-1}$, and those of stem, leaves and capsule peel were 1.39 < 5.66 < 6.24 < 7.00 g $pot^{-1}$. Moreover, the treatments added with NFRB had dry weight of seeds and stem, leaves and capsule peel ranging from 1.72 and 1.84 g $pot^{-1}$ and from 5.10 to 5.36 g $pot^{-1}$, which were all higher than those in the treatment without NFRB in which the values were 1.33 and 4.54 g $pot^{-1}$, respectively (Table 3).

**Concentration of nitrogen and phosphate**

Concentrations of nitrogen and phosphate differed statistically at 5% among treatments with nitrogen fertilizer levels varying from 0.0, 45.0, 67.5 to 90.0 kg N $ha^{-1}$. More specifically, the concentration of nitrogen and phosphate at fertilizing level of 90.0 kg N $ha^{-1}$ was 3.63 and 0.743%, but at the level of 0.0 kg N $ha^{-1}$, the number was 2.62 and 0.673%, respectively. Moreover, the concentrations of nitrogen and phosphate in stem, leaves and capsule peel showed significant differences at 5% based on different nitrogen fertilizer levels and opposite trends between nitrogen and phosphate as well. In detail, the highest nitrogen content peaked at 0.451% at the fertilizer level of 90.0 kg N $ha^{-1}$, but the highest phosphate content appeared in the treatment with 0.0 kg N $ha^{-1}$, 0.782%. For the case of supplying NFRB, nitrogen content in seeds and stem, leaves and capsule peel showed significant differences at 5% among treatments. In depth, the treatment with individual supplementation of either ASD-07 or ASD-28 and the one with the combination of ASD-07 and ASD-28 had an average value nitrogen concentration in seeds of 3.48%, significantly higher than that in the treatments without bacteria, only 2.06%. Furthermore, the nitrogen concentrations in stem, leaves and capsule peel in treatments applied with an individual NFRB strain and in the treatment without bacteria were statistically equal, and all lower than that in the treatment with both strains ASD-07 and ASD-28. Last but not least, the phosphate concentration in seeds and stem, leaves and capsule peel had a statistically equal result between the treatment with bacteria (0.713%) and the treatment without bacteria (0.404%) (Table 3).

**Table 3**. Effect of nitrogen fertilizer levels and $N_2$-fixing rhizosphere bacteria supplements on improvement of nutrients uptake in sesame at harvest in alluvial soil in dyke

| **Factors** | | **Dry biomass** | | **Nutrient content** | | | | **Nitrogen uptake** | | | **Phosphorus uptake** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Nitrogen (N) | | Phosphorus (P) | | | | | | | |
| | | Seed | Plant, leaf, capsule peel | Seed | Plant, leaf, capsule peel | Seed | Plant, leaf, capsule peel | Seed | Plant, leaf, capsule peel | Total | Seed | Plant, leaf, capsule peel | Total |
| | | (g pot$^{-1}$) | | (%) | | | | (mg pot$^{-1}$) | | | | | |
| Nitrogen | 0.0 | 0.71$^{d}$ | 1.39$^{d}$ | 2.62$^{d}$ | 0.314$^{d}$ | 0.673$^{b}$ | 0.782$^{a}$ | 19.1$^{d}$ | 4.35$^{d}$ | 23.4$^{d}$ | 4.73$^{c}$ | 10.9$^{b}$ | 15.6$^{c}$ |
| fertilizer levels | 45.0 | 1.74$^{c}$ | 5.66$^{c}$ | 2.99$^{c}$ | 0.341$^{cd}$ | 0.704$^{ab}$ | 0.296$^{b}$ | 55.0$^{c}$ | 19.3$^{c}$ | 74.3$^{c}$ | 12.3$^{b}$ | 16.7$^{a}$ | 29.1$^{b}$ |
| (A) | 67.5 | 2.02$^{b}$ | 6.24$^{b}$ | 3.27$^{b}$ | 0.380$^{b}$ | 0.732$^{a}$ | 0.285$^{b}$ | 67.6$^{b}$ | 23.9$^{b}$ | 91.6$^{b}$ | 14.8$^{a}$ | 17.7$^{a}$ | 32.5$^{a}$ |
| (kg ha$^{-1}$) | 90.0 | 2.15$^{a}$ | 7.00$^{a}$ | 3.63$^{a}$ | 0.451$^{a}$ | 0.743$^{a}$ | 0.253$^{b}$ | 78.4$^{a}$ | 31.4$^{a}$ | 110.0$^{a}$ | 16.0$^{a}$ | 17.5$^{a}$ | 33.5$^{a}$ |
| NFRB inoculants | No-RB | 1.33$^{b}$ | 4.54$^{b}$ | 2.06$^{b}$ | 0.350$^{b}$ | 0.699 | 0.425 | 30.6$^{c}$ | 16.4$^{b}$ | 47.0$^{c}$ | 9.40$^{b}$ | 15.3 | 24.7$^{b}$ |
| (B) | ASD-28 | 1.72$^{a}$ | 5.36$^{a}$ | 3.38$^{a}$ | 0.357$^{b}$ | 0.717 | 0.403 | 60.7$^{b}$ | 19.9$^{b}$ | 80.6$^{b}$ | 12.5$^{a}$ | 15.5 | 28.0$^{a}$ |
| (8 x $10^{7}$ CFU g$^{-1}$ | ASD-07 | 1.73$^{a}$ | 5.10$^{a}$ | 3.48$^{a}$ | 0.354$^{b}$ | 0.730 | 0.385 | 60.7$^{b}$ | 18.7$^{b}$ | 79.4$^{b}$ | 12.7$^{a}$ | 15.8 | 28.6$^{a}$ |
| soil) | Mixture | 1.84$^{a}$ | 5.30$^{a}$ | 3.59$^{a}$ | 0.426$^{a}$ | 0.707 | 0.403 | 68.1$^{a}$ | 23.9$^{a}$ | 92.0$^{a}$ | 13.3$^{a}$ | 16.1 | 29.3$^{a}$ |
| Significant differences | F (A) | * | * | * | * | * | * | * | * | * | * | * | * |
| | F (B) | * | * | * | * | ns | ns | * | * | * | * | ns | * |
| | F (A*B) | * | ns | * | ns | ns | ns | * | ns | * | * | ns | ns |
| CV (%) | | 13.0 | 5.14 | 10.5 | 26.9 | 14.7 | 17.5 | 2.00 | 3.14 | 1.80 | 2.54 | 2.08 | 1.62 |

In a column, the different superscripts indicate significant differences at 5% ($P < 0.05$, *), and without letter is no significant difference according to Duncan's post-hoc test at 5% level; NFRB: $N_2$-fixing rhizosphere bacteria; No-RB: no applied rhizosphere bacteria; Mixture: both ASD-07 and ASD-28; CV: Coefficient of variation

**Nutrients uptake in parts and in whole of sesame**

Nitrogen uptake in seeds, stem, leaves and capsule peel and the total nitrogen uptake had significant differences at 5% among nitrogen fertilizer levels. In detail, both nitrogen uptake in sesame parts and the total nitrogen uptake peaked and bottom at fertilizer levels of 90.0 and 0.0 kg N $ha^{-1}$, with the uptake values recorded were 78.4, 31.4, and 110.0 mg N $pot^{-1}$ for 90.0 kg N $ha^{-1}$ and 19.1, 4.35, and 23.4 mg N $pot^{-1}$ for the another level, respectively. Besides, in treatments with NFRB neither individual nor dual supplementation of ASD-07 and ASD-28, nitrogen uptake in seeds and total nitrogen uptake was 60.7-68.1 and 79.4-92.0 mg N $pot^{-1}$, respectively, significantly higher than those in the treatment without bacteria in which the uptake values were 30.6 and 47.0 mg N $pot^{-1}$, following the same order. Furthermore, the individual supplementation of ASD-07 or ASD-28 possessed a lower nitrogen uptake in stem, leaves and capsule peel, in comparison to the supplementation of both ASD-07 and ASD-28. The values were 23.9 mg N $pot^{-1}$ for combination of ASD-07 and ASD-28, 18.7 mg N $pot^{-1}$ for only ASD-07 and 19.9 mg N $pot^{-1}$ for only ASD-28 (Table 3).

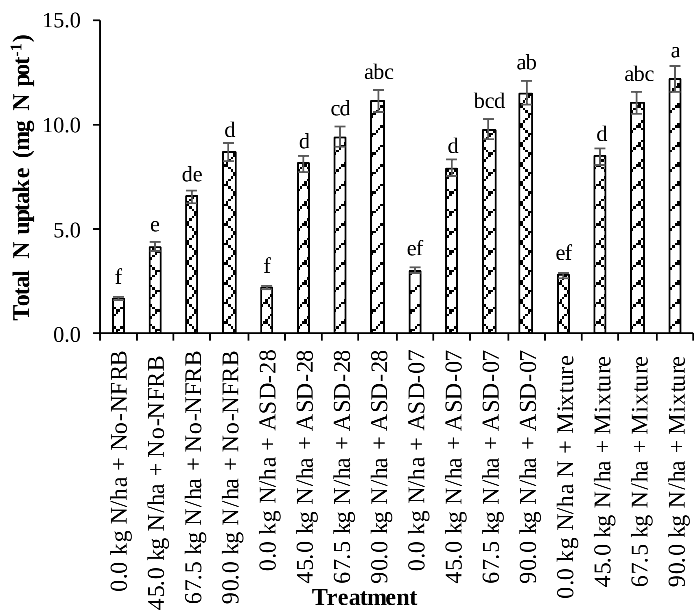


**Figure 1.** Effect of nitrogen fertilizer levels and $N_2$-fixing rhizosphere bacteria supplements on improvement of N uptake in sesame at harvest in alluvial soil in dyke

Mean of four replications and its standard deviation are presented; while different letters above the bars reveal significant differences at $P < 0.05$; No-NFRB: no applied $N_2$-fixing rhizosphere bacteria; Mixture: both ASD-07 and ASD-28

There was an interaction in total nitrogen content between the two factors. The total nitrogen uptake rose following the increase of nitrogen fertilizer levels from 0.0 to 90.0 kg N ha$^{-1}$. The treatment with addition of NFRB strains ASD-07, ASD-28 and their mixture plus 67.5 kg N ha$^{-1}$ of nitrogen fertilizer level had a nitrogen uptake of 10.6 mg N pot$^{-1}$, significantly higher than that in treatment fertilized with 90.0 kg N ha$^{-1}$ but no bacteria. Moreover, the total nitrogen uptake recorded in the treatment with 90.0 kg N ha$^{-1}$ but no supplementation of bacteria was equivalent to that in the treatment with 45.0 kg N ha$^{-1}$ plus bacteria neither individual nor dual supplementation of ASD-07 and ASD-28 (Figure 1).

**Phosphate uptake in parts and in whole of sesame plants**

Phosphate uptake in plant parts and in whole showed statically differences at 5% among nitrogen fertilizer levels. In detail, phosphate uptake in seeds and stem, leaves and capsule peel, and the total nitrogen uptake at nitrogen fertilizer levels of 90.0 and 67.5 kg N ha$^{-1}$ was statistically similar, ranging around 14.8-16.0, 17.5-17.7 and 32.5-33.5 mg P pot$^{-1}$, and all higher than those in the treatment with a nitrogen level of 0.0 kg N ha$^{-1}$, in which the values were 4.73, 10.9 and 15.6 mg P pot$^{-1}$, respectively. In addition, phosphate uptake in seeds and total phosphate uptake among bacterial treatments were equivalent, with a mean of 12.8 and 28.6 mg P pot$^{-1}$, respectively, and all higher than those in the treatment without bacteria (9.40 and 24.7 mg P pot$^{-1}$, respectively) (Table 3).

**Table 4.** Effect of nitrogen fertilizer levels and $N_2$-fixing rhizosphere bacteria supplements on growth of sesame cultivated in alluvial soil in dyke at harvest

| Factors | | Plant height (cm) | Number of leaves per plant (leaf) | Leaf length (cm) | Leaf width (cm) | Chl a (µg mL$^{-1}$) | Chl b (µg mL$^{-1}$) | Chl a+b (µg mL$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Nitrogen | 0.0 | 26.6$^{d}$ | 12.3$^{d}$ | 3.89$^{c}$ | 1.98$^{d}$ | 8.11$^{d}$ | 1.94$^{d}$ | 10.1$^{d}$ |
| fertilizer levels | 45.0 | 42.9$^{c}$ | 15.6$^{c}$ | 6.76$^{b}$ | 3.64$^{c}$ | 9.16$^{c}$ | 2.24$^{c}$ | 11.4$^{c}$ |
| (A) | 67.5 | 55.2$^{b}$ | 16.9$^{b}$ | 7.51$^{a}$ | 4.06$^{b}$ | 9.96$^{b}$ | 2.52$^{b}$ | 12.5$^{b}$ |
| (kg ha$^{-1}$) | 90.0 | 75.5$^{a}$ | 17.6$^{a}$ | 7.76$^{a}$ | 4.23$^{a}$ | 10.9$^{a}$ | 2.95$^{a}$ | 13.9$^{a}$ |
| NFRB | No-RB | 40.3$^{c}$ | 14.2$^{c}$ | 6.11$^{b}$ | 3.05$^{c}$ | 9.08$^{b}$ | 2.30 | 11.4$^{b}$ |
| inoculants (B) | ASD-28 | 42.0$^{b}$ | 15.7$^{b}$ | 6.69$^{a}$ | 3.54$^{b}$ | 9.83$^{a}$ | 2.54 | 12.4$^{a}$ |
| (8 x 10$^{7}$ CFU g$^{-}$ | ASD-07 | 42.4$^{b}$ | 16.4$^{a}$ | 6.34$^{b}$ | 3.45$^{b}$ | 9.65$^{a}$ | 2.41 | 12.1$^{a}$ |
| $^{1}$ soil) | Mixture | 44.5$^{a}$ | 16.2$^{a}$ | 6.80$^{a}$ | 3.86$^{a}$ | 9.58$^{a}$ | 2.42 | 12.0$^{a}$ |
| Significant differences | F (A) | * | * | * | * | * | * | * |
| | F (B) | * | * | * | * | * | ns | * |
| | F (A*B) | * | * | * | * | ns | * | ns |
| CV (%) | | 3.41 | 3.64 | 8.05 | 13.3 | 6.23 | 11.9 | 6.14 |

In a column, the different superscripts indicate significant differences at 5% (P < 0.05, *), and without letter is no significant difference according to Duncan's post-hoc test at 5% level; NFRB: $N_2$-fixing rhizosphere bacteria; No-RB: no applied rhizosphere bacteria; Mixture: both ASD-07 and ASD-28; CV: Coefficient of variation.

***The influence of nitrogen fertilizer levels and NFRB supplementation on the growth, yield components and yield of sesame cultivated on alluvial soil in dyke***

**Sesame growth**

Plant height, leaves number and leaf size had significant differences at 5% according to nitrogen fertilizer levels. Plant height rose gradually from 26.6, 42.9, 55.2 to 75.5 cm when the amount of nitrogen fertilizer applied went up from 0.0, 45.0, 67.5 to 90.0 kg N $ha^{-1}$, respectively. In the same line, the leaves number and the leaf width valued 12.3, 15.6, 16.9 and 17.6 leaves $plant^{-1}$; and 1.98, 3.64, 4.06 and 4.23 cm, following the order of nitrogen fertilizer levels. Furthermore, leaf length recorded at fertilizer levels of 67.5 and 90.0 kg N $ha^{-1}$ did not show significant difference, with 7.76 and 7.51 cm in values and significantly higher than that of the other nitrogen fertilizer levels. In addition, the amount of chlorophyll a, b and chlorophyll a+b at 90.0 kg N $ha^{-1}$ was 10.9, 2.95 and 13.9 µg $mL^{-1}$, respectively; at 67.5 kg, 9.96, 2.52 and 12.5 µg $mL^{-1}$; at 45.0 kg N $ha^{-1}$, 9.16, 2.24 and 11.4 µg $mL^{-1}$; and at 0.0 kg N $ha^{-1}$, 8.11, 1.94 and 10.1 µg $mL^{-1}$ (Table 4). This indicated an influence of nitrogen fertilizer on sesame growth.

For bacterial factor, the plant height, leaves number, concentrations of chlorophyll a and a+b, leaf size changed significantly at 5%. Treatments with bacteria had dominant values of plant height, leaves number, leaf width, and chlorophyll a and a+b contents whose numbers fluctuated around 42.0-44.5 cm, 15.7-16.4 leaves $plant^{-1}$, 3.45-3.86 cm, 9.58-9.83 µg $mL^{-1}$ and 12.0-12.4 µg $mL^{-1}$, respectively, significantly higher than the control treatments with the results of 40.3 cm, 14.2 leaves $plant^{-1}$, 3.05 cm, 9.08 µg $mL^{-1}$ and 11.4 µg $mL^{-1}$, in the same order. The supplementation with NFRB strains of ASD-07 and ASD-28 either individually or dually enhanced plant height, leaves number and chlorophyll a and a+b contents. Nevertheless, the leaf length increase happened only in the treatment with only ASD-28 and the one with the combination of ASD-07 and ASD-28 and the improvement in leaf width took place only in the treatment with bacterial mixture of ASD-07 and ASD-28, in comparison the treatment without bacteria (Table 4).

**Yield components**

The number of capsules per plant and seeds per capsule varied significantly at 5% under the influences of nitrogen fertilizer levels and bacterial supplementation. In brief, the lowest number of capsules per plant was 2.77 capsules $plant^{-1}$ at 0.0 kg N $ha^{-1}$ of nitrogen fertilizer; the following was 10.1 and 10.9 capsules $plant^{-1}$ at 45.0 and 67.5 kg N $ha^{-1}$ of nitrogen fertilizer, respectively; and the highest number of capsules was 12.3 capsules plant$^{-1}$ at 90.0 kg N ha$^{-1}$ of fertilizer. In the same line, with nitrogen fertilizer levels at 0.0, 45.0, 67.5 and 90.0 kg N ha$^{-1}$, the number of seeds per capsules was correspondently 65.1, 87.2, 92.3 and 103.0 seeds capsule$^{-1}$. Moreover, every treatment supplied with NFRB resulted in higher capsules per plant and seeds per plant than treatments without bacteria, 9.40-10.8 capsules plant$^{-1}$ and 88.0-89.7 seeds capsule$^{-1}$ compared to 5.65 capsules plant$^{-1}$ and 81.6 seeds capsule$^{-1}$ (Table 5).

Capsule size was affected significantly at 5% by nitrogen fertilizer levels. In detail, capsule length values at nitrogen fertilizer levels of 45.0, 67.5 and 90.0 kg N ha$^{-1}$ were similar, ranging from 2.29 to 2.37, significantly higher than that at 0.0 kg N ha$^{-1}$ of nitrogen fertilizer level whose capsule was 1.90 cm long. Furthermore, capsule diameter at a nitrogen fertilizer level of 0.0 kg N ha$^{-1}$ was 1.20 cm, remarkably smaller than that at 45.0 and 90.0 kg N ha$^{-1}$ nitrogen fertilizer levels (1.40 and 1.32 cm, respectively). Besides, the capsule length was statistically similar among treatments with NFRB (2.20-2.25 cm), but there were significant differences at 5% among them. To be more specific, the treatment with only strain ASD-07 had a capsule diameter of 1.41 cm, remarkably higher than the other treatments with NFRB, including individual supplement of strain ASD-28 and a mixture of both strains ASD-07 and ASD-28, and the non-bacterial treatment (Table 5).

The number of rows per capsule and seeds per row showed a significant impact of nitrogen fertilizer levels at 5%. The rows per capsule and seeds per row numbers at the nitrogen fertilizer levels of 67.5 and 90.0 kg N ha$^{-1}$ were equivalent, with values of 7.58, 7.53 rows capsule$^{-1}$ and 13.6, 12.4 seeds row$^{-1}$. These were significantly higher than those at 0.0 kg N ha$^{-1}$ of fertilizer, 6.98 rows capsule$^{-1}$ and 9.44 seeds row$^{-1}$, respectively. In contrast, the numbers of rows per capsule and seeds per row did not receive any significant effect from NFRB. The means were 7.38 rows capsule$^{-1}$ and 11.9 seeds row$^{-1}$ (Table 5).

The 1,000-seed weight was averagely 2.62 g and remained statistically unchanged under the influence of both nitrogen fertilizer levels and NFRB supplements (Table 5).

The grain yield changed remarkably at 5% under the impacts from levels of nitrogen fertilization (0.0, 45.0, 67.5, 90.0 kg N ha$^{-1}$) and an addition of nitrogen-fixing rhizosphere bacteria. For further evaluation, grain yield peaked at 6.34 g pot$^{-1}$ (90.0 kg N ha$^{-1}$), the second highest was 5.94 g pot$^{-1}$ (67.5 kg N ha$^{-1}$), the third highest was 5.36 g pot$^{-1}$ (45.0 kg N ha$^{-1}$) and the least was 2.17 g pot$^{-1}$ (0.0 kg N ha$^{-1}$). For the bacterial factor, treatments with NFRB had grain yield ranging from 5.28 to 5.39 g pot$^{-1}$, significantly higher than that in the treatment without bacteria, 3.78 g pot$^{-1}$ (Table 5).

**Table 5.** Effect of nitrogen fertilizer levels and $N_2$-fixing rhizosphere bacteria supplements on yield components and grain yield of sesame cultivated in alluvial soil in dyke at harvest

| Factors | | Yield components | | | | | | | Grain yield |
|---|---|---|---|---|---|---|---|---|---|
| | | Number of capsule per plant | Capsule length | Capsule diameter | Number of rows per capsule | Number of seeds per capsule | Number of seeds per row | Weight 1,000 seeds | |
| | | capsule | cm | cm | rows | seed | seed | g | g pot$^{-1}$ |
| Nitrogen fertilizer levels (A) (kg ha$^{-1}$) | 0.0 | $2.77^{c}$ | $1.90^{b}$ | $1.20^{c}$ | $6.98^{b}$ | $65.1^{d}$ | $9.44^{b}$ | 2.62 | $2.17^{d}$ |
| | 45.0 | $10.1^{b}$ | $2.34^{a}$ | $1.40^{a}$ | $7.32^{ab}$ | $87.2^{c}$ | $12.0^{a}$ | 2.61 | $5.36^{c}$ |
| | 67.5 | $10.9^{b}$ | $2.29^{a}$ | $1.30^{bc}$ | $7.53^{a}$ | $92.3^{b}$ | $12.4^{a}$ | 2.63 | $5.94^{b}$ |
| | 90.0 | $12.3^{a}$ | $2.37^{a}$ | $1.32^{ab}$ | $7.58^{a}$ | $103.0^{a}$ | $13.6^{a}$ | 2.64 | $6.34^{a}$ |
| NFRB inoculants (B) (8 x $10^{7}$ CFU g$^{-1}$ soil) | No-RB | $5.65^{b}$ | 2.20 | $1.29^{b}$ | 7.23 | $81.6^{b}$ | 11.3 | 2.61 | $3.77^{b}$ |
| | ASD-28 | $10.8^{a}$ | 2.25 | $1.27^{b}$ | 7.38 | $88.0^{a}$ | 11.9 | 2.62 | $5.28^{a}$ |
| | ASD-07 | $9.40^{a}$ | 2.25 | $1.41^{a}$ | 7.45 | $88.8^{a}$ | 12.0 | 2.63 | $5.36^{a}$ |
| | Mixture | $10.1^{a}$ | 2.20 | $1.24^{b}$ | 7.45 | $89.7^{a}$ | 12.3 | 2.63 | $5.39^{a}$ |
| Significant differences | F (A) | * | * | * | * | * | * | ns | * |
| | F (B) | * | ns | * | ns | * | ns | ns | * |
| | F (A*B) | * | ns | * | ns | * | * | ns | * |
| CV (%) | | 12.6 | 8.16 | 11.4 | 8.27 | 6.48 | 8.20 | 2.09 | 4.52 |

In a column, the different superscripts indicate significant differences at 5% ($P < 0.05$, *), and without letter is no significant difference according to Duncan's post-hoc test at 5% level; NFRB: $N_2$-fixing rhizosphere bacteria; No-RB: no applied rhizosphere bacteria; Mixture: both ASD-07 and ASD-28; CV: Coefficient of variation.

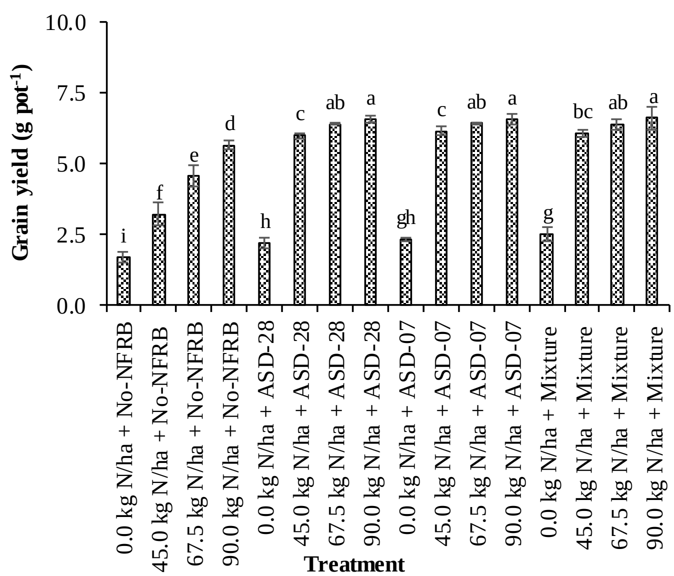


**Figure 2.** Effect of nitrogen fertilizer levels and $N_2$-fixing rhizosphere bacteria supplements on improvement of sesame yield cultivated in alluvial soil in dyke

Mean of four replications and its standard deviation are presented; while different letters above the bars reveal significant differences at $P < 0.05$; No-NFRB: no applied $N_2$-fixing rhizosphere bacteria; Mixture: both ASD-07 and ASD-28.

**Sesame grain yield**

Both factors interacted with each other and changed sesame yield values, which made significant differences at 5% among the results. When combining with NFRB, all of the treatments with a reduction by 50% of nitrogen fertilizer level gained a better yield than fertilizing with 100% of recommended fertilizer formula, 6.00-6.55 (combined with strain ASD-07), 6.15-6.56 (combined with strain ASD-28) and 6.07-7.20 (combined with a mixture of both strains ASD-07 and ASD-28) compared to 6.09 g pot$^{-1}$ (100% of RFF) (Figure 2).

## Discussion

The nitrogen of either available or total content at 90.0 kg N ha$^{-1}$ as RFF was similar to that at 0.0 kg N ha$^{-1}$ of RFF. This result was in accordance with a study of Noor *et al*. (2020), treatments with nitrogen fertilizer applied or not had insignificantly different nitrogen concentration in soil. However, in comparison of no application of bacteria, supplement of NFRB raised in soil properties, including $pH_{H2O}$ (5.40-5.48 compared to 5.23), available nitrogen of $NH_4^+$ (17.9-20.1 compared to 13.7 mg $NH_4^+$ kg$^{-1}$) and soluble phosphate (109.8-119.2 compared to 95.8 mg P kg$^{-1}$). This result was consistent with a research of supplying a bioproduct of NFRB *Azobacteria* to increase soil pH due to the fact that *Azobacteria* strains are strongly active in tropical and neutral pH conditions (Kakhki *et al*., 2020). According to Artyszak and Gozdowski (2020), a bioproduct of a mixture *Azotobacter chroococcum, A. brasilense* and *Bacillus megaterium* with 30% reduction in RFF outputs a higher amount of $NO_3^-$ and $NH_4^+$ in soil compared to the negative control using only chemical fertilizers. Bacterial strains of *A. lipoferum* and *B. megaterium* are capable of providing nutrients via their nitrogen-fixing and $Ca_3(PO_4)_2$-solubilizng processes from which the concentration of available nitrogen and soluble phosphate rises (El-Komy, 2005). Moreover, the bacterial density in treatments with bacteria was higher than that in the treatment without them. Simultaneously, NFRB strains *E. asburiae* ASD-07 and ASD-28 fixed nitrogen from the atmosphere, leading to the higher $NH_4^+$ content in soil.

The amount of nitrogen in sesame seeds were recorded to be 2.06-3.59%. In the same line, that value is 3.30-4.84% for sesame cultivated in Uranda and Iran (Anguria *et al.*, 2017; Kakhki *et al*., 2020). The lower nitrogen concentration in seeds and stem, leaves is 3.01 and 1.08% in the treatment without nitrogen fertilization, whereas, at 60 kg N ha$^{-1}$ of nitrogen fertilization, the number peaks at 3.45 and 1.31%, respectively (Bijarnia *et al*., 2019). The average phosphate content in seeds among treatments in this study was 0.713%, higher than 0.265-0.660% in previous studies (Anguria *et al*., 2017; Kakhki *et* *al*., 2020). However, the differences might be due to features of a sesame variety, and soil properties and nutrition status. The total nitrogen uptake at 90.0 kg N $ha^{-1}$ of fertilizer reached 110.0 mg N $pot^{-1}$, and without nitrogen fertilization resulted in 23.4 mg N $pot^{-1}$. According Bijarnia *et al.* (2019), on sandy loam soil in Rajasthan, the total nitrogen uptake is 73 kg N $ha^{-1}$ in the treatment with 60 kg N $ha^{-1}$ of fertilizer, while in the control treatment without nitrogen fertilizer, the value is 38.8 kg N $ha^{-1}$. An increase in nitrogen fertilizer levels from 0.0 to 90.0 kg N $ha^{-1}$ raised an amount of phosphate uptake from 15.6 to 33.5 mg P $pot^{-1}$, which is in accordance with a study of Bijarnia *et al*. (2019), total phosphate uptake is 12.8 kg P $ha^{-1}$ at 40 kg N $ha^{-1}$, higher than 7.6 kg P $ha^{-1}$ at 0.0 kg N $ha^{-1}$. The reason for this is that nitrogen and phosphate are synergistic nutrients, nitrogen fertilization enhances plant growth and root development, leading to better nitrogen and phosphate uptake (Bijarnia *et al.*, 2019).

The sesame height is 111.9 cm in the case of 60 kg N $ha^{-1}$ of RFF (Shamsuzzoha *et al*., 2019), at 92 kg N $ha^{-1}$ of fertilizer, it is 104 cm in Ethiopia (Amare *et al*., 2019), and at 100 kg N $ha^{-1}$, the height is 116 cm (Haruna, 2011). In Vietnam, combining 30 kg N and 30 ton of manure per hectare, plant height reaches 106.4 cm for V6 sesame variety (Binh and Lieu, 2016). In this research, the plant height for the low-height sesame ADB1 was 75.5 cm, when being fertilized with 90.0 kg N $ha^{-1}$. Addition of *Azotobacter* and *Azospirillum* potential in nitrogen-fixation makes the sesame 11-19 cm higher than those in the control treatment without bacteria supplied (Kakhki *et al.*, 2020). For V6 sesame variety, leaves number is 41.7 leaves $plant^{-1}$ at 30 tons $ha^{-1}$ of manure (Binh and Lieu, 2016). Fertilization with nitrogen and NFRB supplementation increases chlorophyll content in sesame leaves (Tulukcu and Baba, 2019; Shamsuzzoha *et al*., 2019). This is resulted from that an increasing amount of nitrogen leads to activation of $CO_2$-fixing enzyme, faster photosynthesis rate and better dry biomass accumulation. Thus, addition of bacteria raised chlorophyll a and a+b content, compared to the control treatment, i.e more efficient photosynthesis, leading to better growth and yield.

The number of capsules per plant was 10.1-12.3 capsules $plant^{-1}$ in treatments with nitrogen fertilizer level and 2.77 capsules $plant^{-1}$ in the treatment without nitrogen fertilizer. Although this result was lower, the trend was similar to a study of Shamsuzzoha *et al.* (2019), number of capsules per plant peaks at 65.2 capsules $plant^{-1}$ in the treatment fertilized with 60 kg N $ha^{-1}$ and the result is 57.8 capsule $plant^{-1}$ in the treatment without nitrogen fertilizer. This indicates the essential role of nitrogen in increasing capsules number in sesame. On the other hand, treatments with NFRB possessed a number of capsules range of 9.40-10.8 capsule $plant^{-1}$, higher than that in the treatment without bacteria with 5.65 capsule plant$^{-1}$, and the seeds number per capsule range of 88.0-89.7 seeds capsule$^{-1}$, compared to 81.6 seeds capsule$^{-1}$, respectively. Supplementation of NFRB strains of *Azotobacter* sp., *Azospirillum* sp., *Bacillus* sp. and *Pseudomonas* sp. improves number of capsules per plant to 59.4 capsules plant$^{-1}$, compared to no bacteria, with 43.6 capsules plant$^{-1}$ (Kakhki *et al.*, 2020). The maximum seeds number per capsule is 57.1 seeds capsule$^{-1}$ at 60 kg N ha$^{-1}$ of nitrogen fertilizer, while at the control treatment with no nitrogen fertilizer, the number is 50.0 seeds capsule$^{-1}$ (Shamsuzzoha *et al*., 2019). The 1,000-seed weight of sesame BARI Til-3 is 2.52 g in the treatment with 60 kg N ha$^{-1}$ of fertilizer (Shamsuzzoha *et al*., 2019), while the 1,000-seed weight of sesame ADB1 at 90.0 kg N ha$^{-1}$ of fertilizer was 2.81 g. The sesame yield increased by 4.17 g pot$^{-1}$, approximately 192% increase when comparing the fertilizing level of 0.0 kg N ha$^{-1}$ with the one of 90.0 kg N ha$^{-1}$. According to Shehu, (2014), sesame yield grows by 56.1% in the treatment with 75 kg N ha$^{-1}$, compared to the no nitrogen fertilization. Apart from sesame, as reported by Artyszak and Gozdowski (2020), the yield of beetroot remains while it is being applied by a bioproduct of *Azotobacter chroococcum*, *A. brasilense* and *B. megaterium* plus 70% chemical fertilizer of RFF.

The study results illustrated the response of growth and yield to nitrogen fertilizer levels. This mean that nitrogen fertilization promotes to increase plant height and grain yield of sesame, compared to no nitrogen fertilization. While, bacterial strains *E. asburiae* ASD-07 and ASD-28 were able to fix nitrogen, contributing to enhancing the amount of available nitrogen, $NH_4^+$ and $NO_3^-$, in soil. This also stated that by bacterial density, the value could reach 3.38 x $10^5$ CFU g$^{-1}$ in treatment with bacteria, while in the case of no bacteria applied, the density was 0.040 x $10^5$ CFU g$^{-1}$. Therefore, treatments with NFRB had higher total nitrogen uptake than the treatment without bacteria, the uptake values were 79.4-92.0 and 47.0 mg N pot$^{-1}$, respectively. Treatments with an individual supplementation of *E. asburiae* ASD-07 and *E. asburiae* ASD-28, and the treatment with a mixture of *E. asburiae* ASD-07 and ASD-28 were responsible for improving sesame growth, including sesame plant height and yield components, including capsules number per plant and seeds number per capsule, leading to higher sesame yield. The interaction analysis showed that every increase in nitrogen fertilizer levels always led to an increase in sesame grain yield, even in the case of with and without NFRB strains *E. asburiae* ASD-07 and *E. asburiae* ASD-28. The interaction between nitrogen fertilizer levels illustrated that reducing 50% of nitrogen fertilizer as RFF combined with either individual *E. asburiae* ASD-07 or individual *E. asburiae* ASD-28 or *E.*

*asburiae* ASD-07 and ASD-28 mixture always gave out higher sesame yield than fertilization according to 100% N of RFF.

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