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# THE EFFECT OF NANO NITROGEN AND BIO-FERTILIZER TYPES ON NPK CONCENTRATION IN SOIL AND OKRA PLANT

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## Abstract

An experiment for the summer agricultural season 2019 was carried out in one of the farmers' fields at Al-Muthanna Governorate, Al-Khidr District, to study the effect of pollination with *Azotobacter chrococcum* and *Bacillus subtilis* and spraying with nitrogenous manure and their interaction in NPK concentration in soil and okra plant (*Abelmoschus esculentus* L.), Al-Hasnawi variety, the experiment was designed according to the Completely Random Block Design RCBD with three replicates. The experiment included two factors, the first factor is biofertilizer with four levels ($A_0$, $A_1$, $A_2$ and $A_3$), the second factor is nitrogenous fertilizer with four levels ($N_0$, $N_1$, $N_2$, and $N_3$). The nanoprotective recommendation was 1 liter N Nanoparticles per 400 liters of water, the addition included two stages in the flowering phase and two weeks after the first addition. Statistically analyzed data, means were compared between coefficients averages by LSD at 0.05 probability level. The results showed that pollination with $A_3$ bacteria led to a significant increase in the readiness of nitrogen, phosphorous and potassium elements in the soil, with an increase of 30.38, 169.3 and 42.40%, respectively, compared to the comparison treatment, The interaction of bacterial inoculation had a significant effect on the ratio of nitrogen, phosphorous and potassium elements in the plant fruit with an increase of 5.71%, 15.24% and 82.75%, respectively. The effect of nano nitrogen spraying with treatments $N_1$, $N_2$ and $N_3$ had a significant effect on potassium readiness in the soil only, level N2 gave the highest concentration, with an increase of 19.67% compared to the comparison treatment, likewise, the effect of spraying with nano nitrogen with the treatments ($N_1$, $N_2$ and $N_3$) had a significant effect on increasing the ratio of nitrogen and phosphorous elements in the fruit, level $N_3$ achieved the highest concentration, with an increase of 15.25 and 18.18%, respectively, compared to the comparison treatment. The interaction was high between bio-fertilization and nanoparticle fertilization for $N_3A_1$ and $N_2A_2$, and $N_1A_2$, nitrogen-phosphorous and Available potassium parameters in the soil sequentially, with an increase of 38.03%, 283.16% and 126.96%, respectively, compared to the comparison treatment. Interaction between bio-fertilization and nanoparticle was the best value, the treatment $N_3A_3$ outperformed the nitrogen and phosphorous concentration in okra, with an increase of 26.9% and 46.23%, respectively, compared to the comparison treatment, as for the potassium concentration in okra, the treatments $N_1A_3$ and $N_3A_1$ did not differ and gave the same concentration with an increase of 83.33% compared to the comparison factor.
***Keywords*:** Nano nitrogen, bio-fertilizer. NPK concentration, soil, okra plant.

## Introduction

Okra (*Abelmoschus esculentus* L.) is a tropical crop member of the Malvaceae family, it is one of the summer vegetables grown in different parts of Iraq because of its green fruits, cultivated in most tropical and sub-tropical regions of the world (Benchasri, 2012). Widely used in many countries of Asia and Africa, also in the southern part of the continent of Europe and America, planted for fruit, which can be used in green or dry (Kumar et al., 2010). The cultivated area of the okra crop in 2014 in Iraq was about 16.75 thousand hectares, productivity is 7403 kg / hectare and the total production was 124 thousand tons (Issa and Jabbar, 2019). The world has directed in recent years to use modern technologies in the field of fertilizer production, known as (Nanotechnology), which has proven to be very effective in improving productivity and providing safety and security factors, agricultural nanostructures are based on some targeted field operations that include the use of nanoparticles with distinct characteristics in increasing productivity and reducing diseases (Scott and Chen, 2012). As well as the use of biological fertilizers in different regions of the world, including the Arab countries, microorganisms fix nitrogen, estimated that the quantity per year is about 60% of the amount of nitrogen that reaches the soil, established by microorganisms that are distinguished by the presence of the nitrogenase enzyme (Berg *et al*., 2002). Biofertilizers can be identified as commercial preparations, contain microorganisms or latent cells from the effective strains of microorganisms, helps plants absorb various nutrients by interacting in the roots of the earth (Khanna *et al*., 2019). Nanomaterials are used to cover traditional fertilizers, to improve its absorption and improve its efficiency, easily enter cells, in addition to contributing to the transportation of vehicles to leaves, roots or fruits, and metabolic processes by increasing photosynthesis activity (Lin *et al*., 2014). The present study aimed to study the effect of inoculation with *Azotobacter chrococcum* and *Bacillus subtilis* bacteria, spraying with nitrogenous manure and the interaction on the concentration of NPK in the soil and the okra plant (Al-Hasnawi).

## Materials and Methods

### Experiment location

The experiment was conducted in Al-Khidr District, Al-Muthanna Governorate, during the 2019-20 agricultural season in loam soil, to study the effect of *Azotobacter chrococum* and *Bacillus subtilis* and spraying with Nano-Nitrogen fertilizer on NPK concentration in okra plant soil. Random samples were taken from the soil before planting and thoroughly mixed, a composite sample was prepared for laboratory tests, the results of the tests were as shown in Table 1.

**Table 1 :** Some chemical and physical characteristics of the soil experiment field.

| Traits | | Value | Unit |
|---|---|---|---|
| Ece | | 4.39 | ds $m^{-1}$ |
| pH | | 7.45 | - |
| Organic mater | | 0.84 | g. $kg^{-1}$ |
| $CaCo_3$ | | 27.24 | |
| Dissolved calcium $Ca^{+2}$ | | 30.51 | meq L |
| Dissolved magnesium $Mg^{+2}$ | | 27.32 | |
| Dissolved sodium $N^{+}$ | | 0.81 | |
| Dissolved bicarbonate $HCO_3$ | | 1.57 | |
| Dissolved chlorine Cl | | 0.84 | |
| Dissolved potassium K | | 0.45 | |
| Sulfate $SO_4$ | | 2.74 | |
| Available elements | Nitrogen | 18.35 | Mg $kg^{-1}$ |
| | Phosphorous | 11.25 | |
| | Potassium | 162.32 | |
| Total bacteria count | | $5.6*10^{6}$ | CFU $g^{-1}$ dry soil |
| Azotobacter count | | $2.6*10^{5}$ | |
| Bacillus count | | $2.1*10^{5}$ | |
| Soil texture | | loam | |
| Sand | | 316 | g. $kg^{-1}$ |
| Loam | | 440 | |
| Clay | | 244 | |

**Study factors:**

A factorial experiment was designed with three field factors, to study the effect of adding two local isolates *Azotobacter Chrococum* and *Bacillus subtilis*, and the effect of nano nitrogen fertilizer and interaction, according to RCBD Randomized Compltely Block Design with two factors, The first factor was biofertilizer at four levels:

**$A_0$**=Control without adding, **$A_1$**=*Azotobacter chrococum* vaccine, **$A_2$**=*Bacillus subtilis* vaccine, **$A_3$**=*Azotobacter chrococum*+ *Bacillus subtilis* vaccine.

The second factor is nitrogen Nano fertilization at four levels:

**$N_0$**= Without adding, **$N_1$**= at 1 l. $h^{-1}$ concentration, **$N_2$**= at 2 l. $ha^{-1}$ concentration, **$N_3$**= at a concentration of 3 l. $ha^{-1}$.

The number of experimental units = 4 × 4 × 3 = 48

Use of one treatment with three replicates of the full Recommendation 80 kg N. $Ha^{-1}$, urea fertilizer (46%) and 80 kg P. $ha^{-1}$, triple superphosphate (P 21%) and 80 kg K. $ha^{-1}$, a 42% K sulfate fertilizer (Ali et al. 2014), the nitrogen fertilizer was added in two batches, a week after germination appears, a month after the first addition, Phosphorous fertilizer added at once before planting, Potassium fertilizer added with batch planting.

**Field operations**

The land was plowed at 5/19/2019 orthogonally, subject to the leveling and smoothing process at 20/3/2019, the land was divided into experimental units, the experimental unit was with dimensions (3 × 2) m, at three distance lines, between one line and another 75 cm, the distance between one hole and another is 40 cm, agriculture was on one plot, between the cultivation line and another 80 cm, the distance between one block and another is 2 m, left a distance of 60 cm between the experimental units, to ensure that the vaccine and nanoferte are not transmitted. The okra plant variety has been selected as the Al-Hasnawi variety in the region, the seeds were placed in the biological vaccine solutions for two hours, each type of vaccine in a separate container, stirring the seeds well, to ensure that the seeds are completely and accurately covered by the vital vaccines, put it in a dry place away from the sun for half an hour, transferred to the field, distributed on 29/3/2019, 3-4 seeds were placed in the hole, after the plants appeared, the removal were made, so that each neighborhood contains one plant. Fertilizers were added as fertilizer recommendation as follow; Nitrogen fertilizer was added at 20 kg ha of urea fertilizer (46% N) as a booster after two weeks of cultivation, 80 kg ha of P tertiary superphosphate fertilizer (21% p) in one batch before planting and 80 kg hectares of potassium sulfate fertilizer (42% k) in one batch with agriculture (Hussein, 2005).

**Preparing the vital vaccine:**

**Prepare the *Azotobacter chrococum* vaccine:**

Isolation of *Azotobacter chrococum* bacteria from Al-Qadisiyah University College of Agriculture from the Department of Soil and Water Resources, for polluting okra seeds, to fix more nitrogen compared to other isolates**,** as these bacteria were active on the nutrient broth, by putting 25 g of this medium and adding it to a conical flask containing 1000 ml of distilled water, pollen from the liquid bacteria farm using a vector, incubated in the incubator at a temperature of 28 °C. for a week, to preparing a sufficient amount of vaccine for the purpose of use in experiments.

**Prepare the *Bacillus subtilis* vaccine:**

Isolate *of Bacillus subtilis* from Al-Muthanna University, Faculty of Agriculture, from the Microbiology Laboratory, as these bacteria were active on the nutrient broth, by putting 25 g of this medium and adding it to a conical flask containing 1000 ml of distilled water, pollen from the liquid bacteria farm using a vector, incubated in the incubator at a temperature of 28 °C. for a week, to preparing a sufficient amount of vaccine for the purpose of use in experiments.

**Nitrogen fertilizer nanoscales**

The fertilizer was obtained from the Iranian company Sepehr Parmis, containing 20% nitrogen as an emulsion, guidelines for the recommendations for nanoferte used 1 (Nl nano liter per 400 liters of water), nano nitrogen fertilizer was used as a spray on the plant with a concentration of 20% in two batches, according to the levels used in the experiment when flowering and after two weeks from the first spray. Manure is sprayed early in the morning and use a bright cleaning solution as a spreader to increase the absorption efficiency by reducing the surface tension of the water, as for the comparison treatment, it was only sprayed with water.

**Estimate the major elements (N, P and K)%:**

The amount of elements (N, P and K) was estimated in soil and in okra fruits, after collecting a suitable sample for each experimental unit, it was dried and ground, the amount of items was estimated at the Graduate Studies Laboratory at the College of Agriculture, University of Baghdad.

**Statistical analysis**

Statistical analysis of all traits was carried out according to the design used in the medium of the statistical program Genstat version 12.1, the means were compared using the Least Significant Difference (L.S. D) at a 0.05 level (Al-Sahuki and Waheeb 1990).

## Results and Discussion

Table 2 show that a significant differences when adding the bacterial vaccine in the available nitrogen concentration in the soil, as *A. chrococcum* and *B. subtilis* excelled at all levels in giving the highest concentration of available nitrogen in the soil, reached 55.08 and 44.92 mg kg $^{-1}$ of soil, respectively, with an increase of 32.18 and 7.79%, respectively, compared to the control treatment, that gave the lowest concentration of 41.67 mg kg$^{-1}$, in addition, *A. chrococcum* bacteria outperformed *B. subtilis* significantly, while it did not differ significantly with the interaction between them, due to the role of the bacteria Azotobacter in fixing atmospheric nitrogen, agreed with Ali et al. (2014), the available nitrogen in the soil increased significantly when bacteria interacted with some *A. chrococcum* and *B. subtilis*, the highest rate was 54.33 mg kg$^{-1}$ soil, a 30.38% increase compared to the control treatment, agreed with Abbasi and AL-Zuhairi (2019), this bacteria was attributed to the ability of *A. chrococcum* to stabilize atmospheric nitrogen, as well as the secretion of some hormones, enzymes, vitamins and growth regulators, that promotes the growth of treated plants and increases nitrogen in the soil (Abd-El-Gawad et al., 2009; Faraj, 2012). The bacteria *B. subtilis* may be due to its ability to improve nitrogen readiness in the soil due to its ability to analyze organic matter, in addition to ability to install atmospheric nitrogen, affected the nitrogen increase in the soil (Hassan, 2011). The nitrogen fertilizer, it had no significant effect on the ratio of available nitrogen in the soil. The interaction between the biological fertilizer and the nitrogenous fertilizer, it had a significant effect, the treatment $N_3A_1$ has been superior to all the factors significantly in the concentration of available nitrogen in the soil, which gave the highest percentage of 55.67 mg kg$^{-1}$ soil with an increase of 38.03% compared to the treatment of $N_0A_0$ which gave the lowest concentration of 40.33 mg, while the two factors $N_3A_2$ and $N_3A_3$ did not differ significantly in the amount of available nitrogen in the soil, the reason for the increase is due to the ability of the biota to stabilize nitrogen and supply other elements as a result of the decomposition of organic matter (Kumar *et al.*, 2014). Less dose of conventional chemical fertilizers with biological fertilizers *Bacillus subtilis*, *Azotobacter chroococum* and organic fertilizers can be more effective for promoting nutrients in the soil, increased absorption and productivity, treatment $N_3A_1$ gave an available nitrogen content in the soil estimated at 98.78% from the full fertilizer recommendation, result of the effectiveness of microorganisms in nitrogen readiness.

**Table 2 :** Available nitrogen in soil (mg.kg$^{-1}$ soil) after harvest.

| Vital vaccine | Nitrogen fertilizer nanoscales | | | | Means |
|---|---|---|---|---|---|
| | $N_0$ | $N_1$ | $N_2$ | $N_3$ | |
| $A_0$ | 40.33 | 41.00 | 42.67 | 42.67 | 41.67 |
| $A_1$ | 54.67 | 54.67 | 55.33 | 55.67 | 55.08 |
| $A_2$ | 45.00 | 44.67 | 44.67 | 45.33 | 44.92 |
| $A_3$ | 54.67 | 54.33 | 53.33 | 55.00 | 54.33 |
| NPK | | | | | 56.36 |
| Means | 48.67 | 48.67 | 49.00 | 49.67 | |
| $L.S.D_{0.05}$ | Vital Vaccine 1.19 | Nitrogen fertilizer nanoscales N.S | | Interaction 2.39 | |

Table 3 that the effect of the concentration of available phosphorous in the soil with the treatment of bacterial vaccine, the vaccine was significantly superior in *B. subtilis* and *A. chrococcum* at all levels, give the highest concentrations of available phosphorous in the soil, reached 14.92 and 6.33 mg. kg$^{-1}$ of soil, respectively, with an increase of 215.43% and 33.82, respectively, compare with the control treatment, gave 4.73 mg. kg$^{-1}$ of soil, *B. subtilis* bacteria significantly outperformed compare with *A. chrococcum*, available phosphorous in the soil also increased significantly when bacteria interfered with some *A. chrococcum* and *B. subtilis*, the highest rate gave 12.74 mg$^{-1}$ kg soil, an increase of 169.3% compared to the control treatment, agreed with Abbasi and AL-Zuhairi (2019).

The reason for the superiority of phosphorus when adding the bacterial vaccine is that *B. subtilis* has the ability to secrete basic and acid phosphorous enzymes, which were effective in dissolving phosphates and increasing their readiness in the soil (Chiu *et al.*, 2006). Bacillus bacteria can secrete organic acids such as Lactic acid, gluconic acid, Citric acid, Succinic acid, Proponic acid, and Ketogluconic acid, found that some of the bacterial species dissolving phosphate of the genus Bacillus and the bacteria fixing the atmospheric nitrogen *A. chroococum*, it has the ability to dissolve phosphorous and increase readiness for the plant, the highest amount of dissolved phosphorus was caused by Bacillus genus bacteria (Nguyen and Nhan, 1994).

The nitrogen fertilizer nanoscales had no significant effect on the percentage of available phosphorous in the soil. The interaction between bio-fertilizer and nitrogenous fertilizer had a significant effect. The treatment $N_2A_2$ outperformed all the treatments significantly in the concentration of available phosphorous in the soil, gave the highest concentration, reached 19.12 mg. $kg^{-1}$ of soil, with an increase of 283.16% compared to the measurement treatment that gave the lowest concentration, which reached 4.99 mg. $kg^{-1}$ of soil. The reason may be attributed to the role of Bacillus bacteria in reducing soil PH, to produce organic acids or produce enzymes like Phosphatase, Kumar *et al.* (2014) indicated that a lower dose of conventional chemical fertilizers with biological fertilizers *Bacillus subtilis*, *Azotobacter chroococum* and organic matrix, it can be more effective for enhancing nutrients in the soil, as well as increasing absorption and productivity, treatment $N_2A_2$ gave 82.6% increase of the phosphorous concentration in the soil at the end of the season compared to the full fertilizer recommendation.

**Table 3 :** Available Phosphorus in soil (mg.$kg^{-1}$ soil) after harvest.

| Vital vaccine | Nitrogen fertilizer nanoscales | | | | Means |
|---|---|---|---|---|---|
| | **$N_0$** | **$N_1$** | **$N_2$** | **$N_3$** | |
| $A_0$ | 4.99 | 5.73 | 4.02 | 4.19 | 4.73 |
| $A_1$ | 6.22 | 6.69 | 6.51 | 5.92 | 6.33 |
| $A_2$ | 15.27 | 11.93 | 19.12 | 13.38 | 14.92 |
| $A_3$ | 13.86 | 12.69 | 12.41 | 12.01 | 12.74 |
| NPK | | | | | 15.8 |
| Means | 10.08 | 9.26 | 10.51 | 8.87 | |
| $L.S.D_{0.05}$ | Vital Vaccine 1.40 | Nitrogen fertilizer nanoscales N.S | | Interaction 2.81 | |

Table 4 notes that the concentration of available potassium in the soil has significantly increased when adding manure, as the vaccine with *B. subtilis* and *A. chrococcum* vaccine exceeded all levels in giving the highest concentrations of available potassium in the soil, reached 266.6 and 202.4 mg. $kg^{-1}$ of soil, respectively, with an increase of 61.96 and 22.96%, respectively, compared to the measurement treatment that gave the lowest rate 164.6 mg. $kg^{-1}$ of soil, in addition, *B. subtilis* bacteria excelled significantly on *A. chrococcum* and on the interaction of bacteria with each other, also, the available potassium in the soil increased significantly when bacteria interfered with some *A. chrococcum* and *B. subtilis*, gave the highest rate of 234.4 mg. $kg^{-1}$ of soil, with an increase of 42.40% compared to the control treatment, due to the microorganisms that assist in potassium readiness and a list of fixations and losses, by secreting some enzymes and organic acids (Khan *et al.*, 2013), agreed with Shanware *et al.* (2014), mentioned that the addition of biological fertilizers leads to an increase in some nutrients, such as nitrogen, phosphorus and potassium. The concentration of available potassium in the soil increased significantly, by increasing the level of foliar spray with nano fertilizer, as $N_2$ exceeded all levels, reached 234.8 mg. $kg^{-1}$ of soil with an increase of 19.67% compared to the control treatment, which gave the lowest potassium concentration, reached 196.2 mg. $kg^{-1}$ of soil. As for the interaction between the biological fertilizer and the nitrogenous fertilizer, it had a significant effect, the treatment $N_1A_2$ outperformed all the treatments significantly in the available potassium concentration in the soil, gave the highest concentration of 279.4 mg. $kg^{-1}$ of soil, with an increase of 126.96% compared to the measurement treatment, which gave the lowest concentration as it reached 123.1 mg. $kg^{-1}$ of soil, the reason may be due to the role of microorganisms in the release of elements, including potassium (Khan et al., 2013), or may be due to the addition of potassium fertilizer potassium sulfate, it was characterized by high solubility compared to other fertilizer sources of potassium, as well as the role of this fertilizer in reducing the degree of interaction of the soil, especially the root zone, will lead to readiness, also increases the readiness of other elements such as phosphorus, iron, and zinc in the soil (Ali et al., 2014), the treatment $N_1A_2$ also gave an increase of 77.31% of the available potassium concentration in the soil by the end of the season compared to the full fertilizer recommendation.

**Table 4 :** Available Potassium in soil (mg.$kg^{-1}$ soil) after harvest.

| Vital vaccine | Nitrogen fertilizer nanoscales | | | | Means |
|---|---|---|---|---|---|
| | **$N_0$** | **$N_1$** | **$N_2$** | **$N_3$** | |
| $A_0$ | 123.1 | 171.2 | 174.5 | 189.7 | 164.6 |
| $A_1$ | 208.5 | 183.0 | 249.1 | 169.1 | 202.4 |
| $A_2$ | 246.6 | 279.4 | 267.2 | 273.3 | 266.6 |
| $A_3$ | 206.6 | 238.2 | 248.5 | 244.5 | 234.4 |
| NPK | | | | | 216 |
| Means | 196.2 | 217.9 | 234.8 | 219.1 | |
| $L.S.D_{0.05}$ | Vital Vaccine 13.21 | Nitrogen fertilizer nanoscales 13.21 | | Interaction 26.42 | |

Table 5 showed that the nitrogen ratio in the plant was significantly superior when adding fertilizer, especially the vaccine in *A. chrococcum*, gave the highest concentration, reaching 2.60%, the percentage increase 6.12% compared to the measurement treatment, gave less concentrate reached 2.45%, likewise, the vaccine with *B. subtilis*, which gave less nitrogen in okra than the *A. chrococcum*, reached 2.50%, with an increase 2.04% compared to the control treatment, gave the lowest concentration, as it reached 2.45%, in addition, *A. chrococcum* bacteria outperformed *B. subtilis* significantly, while did not differ significantly with the interaction between them in the ratio of nitrogen in the fruits, the percentage of nitrogen in the fruit also increased significantly when it interacted with some *B. subtilis* and *A. chrococcum*, reached 2.59%, or 5.71%, compared to the control treatment, agreed with Kahil *et al.* (2017), they found that N, P and K concentrations were increased, this increase boosted the growth and productivity of hibiscus plants, may be because microorganisms also produce substances that aid growth, resulting in a more efficient absorption of nutrients, the reason for the increase in nitrogen was attributed to the fruit, this may be because microorganisms also produce substances that aid growth, led to a more efficient absorption of nutrients (Umesha *et al.*, 2018). As for the manure, the manure was significantly superior to that of the compost and the comparison treatment, gave the highest level at the fourth level ($N_3$) reached 2.72% with an increase of 15.25% compared to the measurement treatment, which gave the lowest rate of nitrogen in okra reached 2.36%, other nanoscale levels $N_1$ and $N_2$ did not differ significantly with control treatment, the spraying with nitrogen fertilizer manufactured according to nanotechnology at different levels, instrumental in increasing growth and increasing absorption of nutrients, the increase in the vegetable content of nitrogen as a result of spraying with manure, due to the fact that fertilizers provide more surface area for the different metabolism reactions in the plant, which increases the rate of metabolism, it targets the cell wall, which facilitates the penetration of nutrients into the vegetative system of the plant, contributes to increasing cell divisions and encouraging branch formation, provides a constant demand for nutrients that the plant pulls or absorbs from the soil (Prasad *et al.*, 2014; Naresh *et al.*, 2019), agreed with Hegab *et al.* (2018) found that nitrogenous fertilizers increased the efficiency of their absorption in plants when using nanofertures. The interaction between the biological fertilizer and the nitrogenous fertilizer, it had a significant effect on increasing the nitrogen ratio in the fruit of the okra plant, treatment $N_3A_3$ surpassed compare with all treatments, gave the highest concentration of 2.83% and an increase of 26.9% compared to the treatment of $N_0A_0$, gave the lowest concentration of 2.23%, whereas the $N_3A_1$ and $N_2A_3$ treatments differed in the nitrogen ratio of okra fruit significantly between them, but it significantly outperformed the $N_0A_0$, note that the interference treatment $N_3A_3$ did not differ significantly with many other interaction treatments, due to the role of the nitrogenous fertilizer because of its unique properties due to its small surface area with high absorption, therefore it enables to penetrate the leaf tissue of the plant, easily absorb and act, increase enzymatic activity and speed of reactions (Venkatachalam *et al.*, 2017), this contributes to increasing cell divisions, encourage branching, provides a constant demand for nutrients that the plant pulls or absorbs from the soil (Naresh *et al.*, 2019), agreed with Al-Fahdawi and Allawi (2019) in the eggplant plant, which showed that the biological and nano fertilizers gave the highest nitrogen concentrations in the plant, the same treatment $N_3A_3$ gave a nitrogen ratio in the plant estimated at 93.4% of the complete fertilizer recommendation.

**Table 5 :** Available Nitrogen in plant (%).

| Vital vaccine | Nitrogen fertilizer nanoscales | | | | Means |
|---|---|---|---|---|---|
| | $N_0$ | $N_1$ | $N_2$ | $N_3$ | |
| $A_0$ | 2.23 | 2.40 | 2.50 | 2.66 | 2.45 |
| $A_1$ | 2.50 | 2.56 | 2.60 | 2.76 | 2.60 |
| $A_2$ | 2.36 | 2.50 | 2.50 | 2.63 | 2.50 |
| $A_3$ | 2.36 | 2.50 | 2.70 | 2.83 | 2.59 |
| NPK | | | | | 3.03 |
| Means | 2.36 | 2.49 | 2.57 | 2.72 | |
| $L.S.D_{0.05}$ | Vital Vaccine 0.06 | Nitrogen fertilizer nanoscales 0.06 | | Interaction 0.13 | |

Table 6 shows that the percentage of phosphorus in the plant significantly increased when adding the *B. subtilis* vaccine, gave the highest rate, reached 2.51%, outperform 12.55% lead over the comparison treatment, then treatment of the bacterial vaccine, *A. chrococcum*, was 2.34% (with an increase of 4.93%) compared to the control treatment, which gave the lowest concentration 2.23%, the phosphorous ratio increased significantly with the bilateral vaccine between *A. chrococcum* and *B. subtilis*, gave the highest rate, reached 2.57%, with an increase of 15.24% compared to the measurement treatment. Additionally, *B. subtilis* bacteria significantly outperformed *A. chrococcum*, the addition of the vaccine can be overlapped, with some being superior to adding it individually, due to the microbiology, *Aspergillus* spp., *Penicillium* spp. and *Bacillus* spp., works to form complex materials with calcium, $H^+$ ion was produced, which leads to a decrease in pH, increases the solubility of phosphorous in the soil in the rhizosphere, microorganisms can reduce pH by forming $CO_2$ when analyzing organic matter in the soil, carbonic acid was formed, leads to a relative decrease in soil pH, which increases the solubility of insoluble phosphate compounds (Kumari *et al.*, 2008; Nehwani *et al.*, 2010), agreed with Kahil *et al.* (2017) they found that N, P and K concentrations were increased, resulted in enhancing the growth and productivity of hibiscus plants, may be due to the fact that microorganisms also produce growth-enhancing substances leading to a more efficient absorption of nutrients (Umesha *et al.*, 2018). As for nanoferture, may significantly outperform bio-fertilizer and comparison treatment, gave the highest level at the fourth level ($N_3$), reached 2.60% and an increase of 18.18% compared to the measurement treatment, which gave the lowest rate of phosphorous in okra, reached 2.20%, other nanoscale levels $N_1$ and $N_2$ did not differ significantly with control treatment, due to the increased nitrogen concentration, stimulates and encourages the absorption of phosphorous plant roots, by increasing their growth, then increasing the top of the plant and their production (Ali *et al*., 2014). I agreed with Hegab *et al.* (2018), showed that the efficiency of the use of phosphorous (UPE) increased with the increase of nano nitrogen. As for the interaction between the biological fertilizer and the nitrogenous fertilizer, it had a significant effect in increasing the percentage of phosphorus in the fruit of okra, treatment $N_3A_3$ surpassed all treatments, gave the highest concentration of phosphorus in okra, reached 2.72% with an increase of 46.23% compared to the measured treatment ($N_0A_0$), gave the lowest concentration of 1.86%, agreed with Al-Fahdawi and Allawi (2019) on the eggplant plant, showed that the biological and nano fertilizers gave the highest concentrations of phosphorous in the plant, the treatment ($N_3A_3$) also gave a phosphorous percentage in the plant estimated to be 94.14% of the full fertilizer recommendation.

**Table 6 :** Available Phosphorus in plant (%).

| Vital vaccine | Nitrogen fertilizer nanoscales | | | | Means |
|---|---|---|---|---|---|
| | $N_0$ | $N_1$ | $N_2$ | $N_3$ | |
| $A_0$ | 1.86 | 2.23 | 2.36 | 2.46 | 2.23 |
| $A_1$ | 2.10 | 2.26 | 2.40 | 2.60 | 2.34 |
| $A_2$ | 2.43 | 2.46 | 2.56 | 2.60 | 2.51 |
| $A_3$ | 2.43 | 2.50 | 2.63 | 2.73 | 2.57 |
| NPK | | | | | 2.9 |
| Means | 2.20 | 2.36 | 2.49 | 2.60 | |
| $L.S.D_{0.05}$ | Vital Vaccine 0.03 | Nitrogen fertilizer nanoscales 0.03 | | Interaction 0.07 | |

Table 7 shows that the percentage of potassium in the fruit of the okra plant has significantly increased when adding the fertilizer, in particular, the bacterial vaccine *A. chrococcum*, the highest rate was 0.51%, with an increase of 75.86% compared to the measurement, the treatment of *B. subtilis*, amounted to 0.45%, an increase of 55.17% compared to the measurement treatment, which gave the lowest concentration 0.29%, in addition, *A. chrococcum* bacteria outperformed *B. subtilis* significantly, while it did not differ significantly with the interaction between them, also, the potassium level increased significantly in the bilateral vaccine between *A. chrococcum* and *B. subtilis* bacteria, gave the highest rate of 0.53%, which is 82.75% higher compared to the measurement treatment, agreed with Kahil *et al*. (2017), they found that N, P and K concentrations were increased, resulted in enhancing the growth and productivity of hibiscus plants, may be because microorganisms also produce substances that aid growth, resulting in a more efficient absorption of nutrients (Umesha *et al*., 2018). As for the nitrogen fertilizer, it had no significant effect on the potassium level in the fruit of okra. As for the interaction between the biological fertilizer and the nitrogenous fertilizer, it had a significant effect in increasing the proportion of potassium in the fruit of the okra plant, the treatments $N_1A_3$ and $N_3A_1$, outperformed all other treatments, the concentration was 0.55%, outperformed 83.33% higher in comparison with the measurement treatment, gave the lowest concentration of 0.30%, agreed with Al-Fahdawi and Allawi (2019) on the eggplant plant, they showed that the biological and nano fertilizers gave the highest concentrations of potassium in the plant, the same treatment also gave potassium in the plant an estimated 98.21% of the full fertilizer recommendation.

**Table 7 :** Available Potassium in plant (%).

| Vital vaccine | Nitrogen fertilizer nanoscales | | | | Means |
|---|---|---|---|---|---|
| | $N_0$ | $N_1$ | $N_2$ | $N_3$ | |
| $A_0$ | 0.30 | 0.28 | 0.31 | 0.28 | 0.29 |
| $A_1$ | 0.48 | 0.52 | 0.51 | 0.55 | 0.51 |
| $A_2$ | 0.46 | 0.41 | 0.45 | 0.50 | 0.45 |
| $A_3$ | 0.51 | 0.55 | 0.53 | 0.52 | 0.53 |
| NPK | | | | | 0.56 |
| Means | 0.44 | 0.44 | 0.45 | 0.46 | |
| $L.S.D_{0.05}$ | Vital Vaccine 0.03 | Nitrogen fertilizer nanoscales N.S | | Interaction 0.07 | |

## Conclusions

Biological fertilization represented by *A. chrococcum* and *B. subtilis* vaccine in NPK increased in soil and concentrated in the plant, nano-nitrogen fertilization spraying increased the nitrogen and phosphorous concentration in the fruit, the increase was significant with an increase in the added concentrate, the interaction between nanoparticle and fertilization achieved increased concentrations of nitrogen and phosphorous elements in the fruits, therefore, this reflected positively on the increase in growth and yield, and the improvement of production and quality.

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