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RATIONALIZING THE USE OF CHEMICAL FERTILIZERS BY USING SOME MICRO-ORGANISMS AND THEIR IMPACT ON THE PRODUCTIVITY AND QUALITY OF *OENOTHERA BIENNIS* L. PLANT

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ABSTRACT: In order to examine the effects of bio-agent technology, the current study, which used a complete randomized block design, was conducted in the Farm of Cairo University's Pharmacy Department throughout the two succeeding growing seasons of 2020/2021 and 2021/2022. In order to investigate the growth, herb yield, fixed oil %, and fixed oil yield and its components, manipulation of a few plant-growth-promoting PGPs such as *Azotobacter chroococcum, Azospirillum lipoferum*, and *Bacillus polymyxa* under 50, 75, and 100% of N fertilizer on *Oenothera biennis* L. (evening primrose) was tested. Before planting, seeds were soaked for half an hour in a distillated water. Plants were sprayed with the bioagent suspension four times a month after planting during the two seasons in which these bioagents. The plant height, number of branches/plant, number of pods/plant, herb fresh and dry weights per plant (g), seed weight/plant (g), fixed oil % in seeds, and fixed oil production per plant (ml) were significantly increased after inoculation with these bio-agents. The soil's nitrogenase and dehydrogenase activity, total carbohydrates (%), and percentages of N, P, and K were all measured chemically. When plants were treated with *Azotobacter chroococcum* (Ac), *Azospirillum lipoferum* (Al) and *Bacillus polymyxa* (B) in the presence of 75% N fertilizer, the maximum rise in these parameters were recorded. The plants that received 50% of their N fertilization had the lowest mean of all the parameters in both seasons. The same process yielded the highest percentages of linoleic, oleic, palmitic, and linolenic acids in the fixed oil. Additionally, the plants receiving this treatment showed the highest percentages of total carbohydrates, N, P, and K in the soil as well as the highest levels of nitrogenase and dehydrogenase activities.

Keywords: *Oenothera biennis* L., evening primrose, plant growth promoters (PGP), *Azotobacter chroococcum*, *Azopirillum lipoferum*, *Bacillus polymyxa*, dehydrogenase activities

INTRODUCTION

One of the main techniques for enhancing the availability of soil nutrients to plants is the use of fertilizers. Plant growth rates, times for reaching maturity, the size of plant components, the phytochemical contents of the plants, and seed capacity can all be altered by fertilization. Numerous difficulties in the areas of ecology, economics, and society have been brought about by high-input methods like the extensive use of chemical fertilizers. In addition, farmers are powerless due to the rising costs of chemical inputs, which led to a decline in the quality of some crops' seeds, a decline in commodity prices, and a decrease in farm revenue (Khadem *et al.,* 2010).

Many microbial inoculants, such as *Azospirillum*, *Azotobacter*, and phosphor-bacterium, are employed as biofertilizers in the modern agricultural techniques. These inoculants have received a lot of attention since they increase crop output and plant growth when applied to fields. Through biological processes, bio-fertilizers can mobilize nutritional components from an unusable state to a useable one. P-solubilizers are a type of biofertilizers that help plants to absorb phosphorus from the soil by solubilizing it. They can raise the crop's production, growth, and yield (Galavi *et al.,* 2011).

The term "bio-fertilizer" refers to a material containing living microorganisms. They colonize the rhizosphere, or the interior of the plant, whether applied to seed, plant surfaces, or soil. This increases the host plant's supply or availability of primary nutrients, which stimulates growth. Bio-fertilizers supply soil with nutrients by utilizing the body's natural mechanisms of fixing nitrogen, phosphorus solubilization, and growth-promoting material production. It is projected that the consumption of chemical pesticides and fertilizers would decline with the use of biofertilizers. The natural nutrient cycle of the soil is restored and soil organic matter is increased by the microorganisms found in biofertilizers. It is possible to cultivate healthy plants and improve soil health and sustainability by using biofertilizers. Plant-growth-promoting rhizobacteria (PGPR) is the chosen scientific nomenclature for these helpful bacteria because of their multi-faceted activities. As a result, they are very helpful in increasing soil fertility and meeting plant nutrient needs since they provide organic nutrients through bacteria and their waste products. Therefore, there are no chemicals in biofertilizers that could harm the living soil. Eco-friendly and more economical than chemical fertilizers, biofertilizers are an organic agricultural input including *Azotobacter, Rhizobium*, and *Azospirillum* (Esitken *et al.,* 2009).

In addition to organic materials obtained from plant residues, animal fertilizers, green manure, etc., bio-fertilizers also comprise bacterial and fungal microorganisms, particularly microbacterial plant growth (also known as Plant Growth Promoting Rizobacteria, or PGPR) and materials derived from their activities, of which bio-fertilizers are the most significant. In order to promote plant growth and development, they employ one or more specific systems. Research has demonstrated that certain growth-stimulating bacteria release organics and phosphates to enhance the amount of phosphorus that is absorbable by dissolving the inorganic phosphate compounds and converting the insoluble forms of phosphorus into forms that are soluble for plants. The synthesis of plant growth regulators including auxin, cytokinin, and gibberellins is another advantageous outcome of growth-stimulating microorganisms (Soleimanzadeh *et al*., 2010).

Bio-fertilizers are materials that harbor living microorganisms, which settle in the plant's rhizosphere and augment the target crop's availability of primary nutrients and/or growth stimulants (PGPR). The bacteria Bacillus, Pseudomonas, Lactobacillus, photosynthetic bacteria, nitrogen-fixing bacteria, Trichoderma fungi, and yeasts are the microorganisms used in biofertilizers because they can convert inert soil nutrients into plant-available form through microbial processes. The term "plant growth promotion by microorganisms" refers to the chemical compounds that plants produce in response to interactions with certain microorganism metabolites. These compounds are used as nitrogen fixers, phosphate dissolvers, phytopathogen resistance agents, and environmental conditions that are not suitable for them (Ismail *et al*., 2014).

One plant in the Onagraceae family is the evening primrose (*Oenothera biennis). Oenothera* L. is a genus that contains approximately 145 species. Evening primrose is an attractive plant with therapeutic qualities. The accumulation of body fat, diabetes, premenstrual syndrome, and many illnesses such as rheumatism, cancer, multiple sclerosis, and eczema are treated with evening primrose seed oil. The polyphenols in evening primrose seeds have been shown to have antioxidant activity in recent research.

The sterols sitosterol, oenotheralanosterol A, and oenotheralanosterol B are found in evening primrose roots. The root contains the following carbohydrates: arabinose, galactose, glucose, mannose, galacturonic acid, and glucuronic acid. It also contains the triterpenes maslinic acid and oleanolic acid. Additionally present are the tannins gallic acid, oenostacin, tetramethylellagic acid, and 2,7,8-trimethylellagic acid.

The oil found in evening primrose seeds contains two important fatty acids: linoleic acid (LA) and gamma-linolenic acid (GLA), which the body cannot produce on its own. It is stated that GLA and LA both help to lessen pain and inflammation. Gamma-linolenic acid (GLA), a very useful fatty acid that is uncommon in plants, is made up of unsaturated fatty acids (also known as anticoagulant compounds) and is essential to many bodily processes. It is well recognized that this fatty acid (GLA) can help avoid heart disease, multiple sclerosis, eczema, cirrhosis, rheumatoid arthritis, menopause, and arterial hardening. It has a favorable impact on responses to sex hormones, such as testosterone and estrogen. Furthermore, it is significant since it decreases cholesterol levels.

This experiment aimed to investigate the effects of exudates from nitrogen fixers (*Azotobacter chroococcum, Azospirillum lipoferum,* and *Bacillus polymyxa,* respectively) on the growth, quality, and quantity of oil produced by evening primrose plants, as well as the ability of replacing 25 and 50% of the recommended nitrogen dose with root.

MATERIALS AND METHODS

At the Expt. Farm of the Faculty of Pharmacy, Cairo University, Bain El-Srayat, Giza, Egypt, two field experiments were carried out during the two consecutive growing seasons of 2020/2021 and 2021/2022 to examine the impact of the exudates (*Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus polymyxa*, respectively) on the growth, quality, and quantity of oil of the evening primrose plant at 50 and 75% of the recommended nitrogen dose.

The seeds were planted in pots (as a seed bed) after being acquired from the Farm of the Faculty of Agriculture at Moshtohor, Benha University.

October 15th marked the start of the trial for both seasons. The germination process took 12 days to finish. Forty-five days later, seedlings measuring fifteen centimeters in height with four to six leaves per leaf were transplanted.

The experiment was designed as a randomized complete block with three replicates (blocks) and 17 treatments. They were planted in 2.0 × 3.0 m plots, with three rows per plot spaced 50 cm apart within the same row, in hills. In every season, eighteen plants were present in each plot. According to Chapman and Pratt's (1961) physical and chemical parameters of the used soil are displayed in Table (a).

Table a. Physical and chemical properties of the experimental soil.

Properties	Physical analysis				Chemical analysis						
	Clay %	Silt %	Fine sand %	Coarse sand %	pH	N ppm	P_2O_5 ppm	K_2O ppm	Zn ppm	Fe ppm	Mn ppm
2020	37.73	33.62	26.45	2.20	7.0	25.40	102	166	4.17	2.69	2.54
2021	36.13	32.30	26.10	5.47	7.2	23.24	105	175	5.32	3.88	3.12

Mineral fertilizers:

The plants were treated with NPK at the prescribed rate in both seasons. 300 kg of ammonium sulfate (20.5% N), 200 kg of calcium superphosphate (15.5% P_2O_5), and 100 kg of potassium sulfate (48% K_2O) per feddan were the NPK fertilizers utilized. Two equal doses of all the phosphorus provided during soil preparation (N and K) were applied thirty and sixty days after transplanting.

Plant growth promoters (PGP):

Bacillus polymyxa, *Azotobacter chroococcum*, and *Azospirillum lipoferum* are a few of the root-associated bacteria known as plant growth promoters (PGP), and they were kindly provided by the Microbiology Department of the Soil, Water, and Environment Research Institute (SWERI), Agriculture Research Center (ARC), Ministry of Agriculture, Giza, Egypt. For a whole day, these PGPR were cultivated at their highest density in Difco nutrient broth medium (Difco, 1984). Seedlings of marigold were watered for half an hour prior to being planted in the PGP culture. Once in transit, the plants were sprayed with these PGPs until they reached the flowering stage.

Isolation of rhizospheric bacteria:

A conical flask (250 ml) containing 10 grams of rhizosphere soil was filled with 90 ml of sterilized water, agitated well for 10 minutes, and dilution series up to 107 were created. King's Media were used to plate dilutions of every soil sample (Atlas, 2002). After one to three-days incubation period at 28 °C ± 2, individual colonies were removed from the plates, purified, and their morphological features were studied under a microscope. At 40 °C, isolates were kept on nutrient agar. Every month, the purifird isolates were subcultured.

Quantitative determination of plant growth-promoting substances in culture media:

Azotobacter chroococcum, *Azospirillum lipoferum,* and *Bacillus polymyxa* isolates were tested for their quantitative capabilities to produce plant growth-promoting substances, i.e., indole acetic acid (IAA) and gibberellins (GA), according to Rahal (2006), as shown in Table (b).

With 17 treatments and three replicates (blocks), the experiment was planned as a fully randomized block design. Five PGP treatments were given to the plants. The first time, seeds were soaked for 30 minutes prior to sowing. In the two seasons, the other times were sprayed once a month. Plants were fertilized with 100% (as a control), 75%, and 50% N received fertilization. Thus, the following 17 treatments were applied:

1. Control (100% N)
2. Control (75% N)
3. Control (50% N)
4. 75% N + *Azotobacter chroococcum* (Ac)
5. 75% N + *Azospirillum lipoferum* (Al)
6. 75% N + *Bacillus polymyxa* (B)
7. 75% N + *A. chroococcum* (Ac) + *Azospirillum lipoferum* (Al)
8. 75% N + *A. chroococcum* (Ac) + *B. polymyxa* (B)
9. 75% N + *A. lipoferum* (Al)+ *B. polymyxa* (B).
10. 75% N + *A. chroococcum* (Ac) + *A. lipoferum* (Al)+ *B. polymyxa* (B)
11. 50% N + *A. chroococcum* (Ac)
12. 50% N + *A. lipoferum* (Al)
13. 50% N + *B. polymyxa* (B)
14. 50% N + *A. chroococcum* (Ac) + *A. lipoferum* (Al)
15. 50% N + *A. chroococcum* (Ac) + *B. polymyxa* (B)
16. 50% N + *A. lipoferum* (Al) + *B. polymyxa* (B)
17. 50% N + *A. chroococcum* (Ac) + *A. lipoferum* (Al) + *B. polymyxa* (B)

Table b. Some characteristics of plant growth promotors in the study.

Strain	N_2-ase µmole C_2H_4/ml/hr	Phytohormons (µg/l culture)		
		Indole acetic acid	Gibberellins	Cytokinins
Azotobacter chroococcum	286.5	69.3	100.2	146.0
Azospirillum lipoferum	60.23	4.4	80.1	297.0
Bacillus polymyxa	56.18	33.5	98.2	97.0

Data recorded:

During the two experimental seasons, the following data were recorded for each season:

1. Plant growth and herb yield:

For both seasons, data of plant height (cm), number of branches/plant, number of pods/plant, and herb fresh and dry weights (g/plant) were noted. Every week, a predetermined oil % and the amount of seed output (g/plant) were recorded after the capsules were tallied for each plant at regular intervals.

2. Essential oil determinations:

a. Fixed oil content (% of the seeds).

b. Fixed oil was extracted from seeds by using Soxhlet apparatus. The oil percentage was determined according to the methylation (change fixed oil into fatty acid). Oil yield per plant (ml) was then calculated. Also, G.L.C. analysis was recorded by G.C. mass in the National Research Center, Dokki, according to Kinsella (1966).

3. Chemical analysis:

Chemical determinations were done in harvested seed samples of the two tested seasons. Total carbohydrates percentage was determined using the method described by Herbert *et al. (*1971), total nitrogen was determined using the modified micro Kjeildahle method as described by Pregl (1945), phosphorus was determined according to King (1951), and potassium was determined using atomic absorption spectroscopy (Rawe, 1973).

4. Enzymatic activates:

Evening primrose rhizosphere soil was sampled after harvesting plants in each season to determine the soil biological activity in terms of dehydroengenase enzyme (DHA) activity (Casida *et al.,* 1964) and nitrogenase enzyme (N_2-ase) activity of the rhizosphere according to Hegazi *et al. (*1979).

Statistical Analysis

The collected data were subjected to statistical analysis according to Little and Hills (1978). Mean separation was done using the least significant difference test at the 5% level (LSD, 0.05).

RESULTS

1. Growth and herb yield:

a. Plant height:

According to Table (1) results, plants that received 50% of the recommended amount of N fertilization without the use of a bioagent in the first season grew to a significantly lower height (62.100 cm) than plants that received another N fertilization treatment. Furthermore, a continuous rise in plant height was observed when 75% of the necessary dose of N was applied with either a mixture of bacteria or simply microbes. As a result, the plants that were fertilized with 75% of the suggested dose of N using the combination of three microorganisms were the tallest (110.127 cm).

In the second season, a similar trend was shown, with the tallest plants (106.235 cm) coming from the application of the 75% recommended dose of N with the combination of the three used microbes, and the shortest plants (61.242 cm) coming from the application of the 50% recommended dose of N fertilization without the use of any bioagent.

With the exception of control, the first season was the best overall. Plant height was

Table 1. Rationalizing the use of chemical fertilizers by using some micro-organisms and their impact on vegetative qualities of the *Oenothera beinnis* L. during 2020 and 2021 seasons.

Treatments	Plant height		Number of branches/plant		Number of pods/plant	
	First season	Second season	First season	Second season	First season	Second season
Control 100% N	112.167	108.333	17.500	15.100	445.000	411.000
Control 75% N	77.200	70.700	6.800	6.300	230.667	219.133
Control 50% N	62.100	61.242	3.874	3.212	155.243	148.340
Ac +75% N	86.140	78.521	8.575	7.012	280.32	278.56
Al+75% N	83.214	74.853	8.001	6.854	274.14	265.49
B +75% N	79.433	72.067	7.467	6.467	252.000	247.533
Ac+Al+75% N	90.320	82.321	9.112	7.851	289.12	280.34
Ac+B+75% N	100.141	95.533	13.833	11.167	338.333	326.167
Al+B+75% N	96.033	89.400	10.167	9.800	305.333	301.467
Ac+Al+B+75% N	110.127	106.235	16.541	14.100	440.471	416.725
Ac +50% N	63.933	62.300	4.667	4.433	168.000	160.700
Al +50% N	63.014	62.108	4.210	4.105	165.421	155.640
B +50% N	62.147	61.542	4.008	3.865	160.172	151.024
Ac+Al+50% N	64.267	64.300	4.767	4.567	190.000	181.900
Ac+B+50% N	75.267	68.733	5.704	5.467	228.333	210.533
Al+B+50% N	69.415	65.174	5.011	4.874	217.245	205.547
Ac+Al+B+50% N	105.267	102.367	14.933	12.400	432.667	389.567
L.S.D. at 0.05	3.147	2.872	2.521	2.014	8.531	6.852

Ac: ***Azotobacter chroococcum;*** **Al:** ***Azopirillum lipoferum*****; B:** ***Bacillus polymyxa***

impacted by reducing N fertilization to 50 or 75 percent in the absence of bioagent treatments. Treatments using bioagent fertilization had a significant impact on plant height. However, the results of plant height at 75% of the recommended dose of N with the mixture of the three used microbes were almost equal to the recommended dose without any bio-agent addition, the half of the recommended dose of N fertilization with the mixture of microbes used was superior to 75% of the recommended dose of N without bio-agent addition. In the initial and subsequent growing seasons, the plant heights were 110.127 and 106.235 cm, respectively, at 75% of N indicated dose when the combination of three microorganisms was employed, and 112.167 and 108.333 cm, at 100 N recommended dose were obtained when no bioagent was added.

b. Number of branches/plants:

Due to root resistance against infections, data presented in Table (1) demonstrated a favorable response and noteworthy variations in evening primrose growth throughout the course of the two seasons in terms of the number of branches/plant. When comparing plants treated with 50% of the acceptable dose of N fertilization without the use of any bioagent to those treated with 75% or 50% of the recommended dose of N with a mixture of the three microorganisms gave significant increases in number of branches/plant were observed. With the mixture of three microorganisms utilized (16.541 and 14.100 in the first and second seasons, respectively), plants with the highest number of branches/plant during the two seasons were obtained fertilizing by 75% of the recommended dose of nitrogen, as opposed to the full dose of nitrogen.

Conversely, the plants treated with 50% of the necessary dose of N fertilization without any bio-agent had the fewest branches/plants during the two seasons (3.874 and 3.212 in the first and second seasons, respectively). The increase in the values of number of branches/plant was observed when fertilizer was applied at 50% of the recommended dose of N fertilization, together with any bio-agent.

In this regard, the first season was the best overall, while at 75% of the recommended dose of N with the mixture of three microbes used, the results were almost equal to the recommended dose without any bioagent addition, while the half of the recommended dose of N fertilization with the mixture of microbes used was superior to 75% of the recommended dose of N without bioagent addition. In comparison to 17.500 and 15.100 at the 100 N recommended dose without any bioagent addition, respectively, number of branches/plant in the first and second seasons were 16.541 and 14.100 at 75% of the N recommended dose with the mixture of utilized microorganisms.

c. Number of pods/plant:

According to Table (1) results, plants that received 50% of the necessary dose of N fertilization without the use of a bioagent in the first season had values that were significantly higher (155.243) than plants that received another N fertilization treatment. Moreover, a continuous rise in the number of pods/plant was observed when 75% of the necessary amount of N was applied with either a mixture of microbes or soleln microbes. As a result, the samples that were fertilized with 75% of the suggested amount of N using the combination of three microorganisms recorded the highest results (440.471).

During the second season, a similar trend was observed: plants receiving 50% of the recommended dose of N fertilization without the use of bioagent (148.340) gave the lowest numbers of pods/plant, whereas plants receiving the 75% recommended dose of N with a mixture of three microbes applied gave the highest values (416.725).

With the exception of control, the first season was the best overall. Number of pods per plant was impacted when N fertilization was reduced to 50 or 75% without the use of bioagent treatments. The quantity of pods per plant was significantly influenced by the bio-agent fertilization treatments, while number of pods/plant at 75% of the recommended dose of N with the mixture of three microbes used was almost equal to the recommended dose without any bio-agent addition, the half of the recommended dose of N fertilization with the mixture of microbes used was superior to 75% of the recommended dose of N without bio-agent addition. In comparison to 445.000 and 411.000 at 100 N recommended dose without any bioagent addition, number of pods/plant in the first and second seasons were 440.471 and 416.725 at 75% of the N recommended dose with the mixture of three utilized microorganisms.

d. Herb fresh and dry weights/plant (g):

When compared to plants fed with a full dose of nitrogen, plants fertilized with 75% of the necessary dose of nitrogen and utilizing the combination of the three microorganisms there were substantial improvements in both fresh and dry weights per plant (Table, 2). Both the fresh and dry weights of the herb increased steadily when 75% of the necessary dose of N was employed, regardless of the bioagent or combination of microorganisms used. In contrast, to the full or 75% of the required dose of N fertilization with or without any bio, 50% of the recommended dose of N fertilization with or without any bio-agent caused a considerable reduction in the majority of cases. These outcomes persisted for the duration of the two seasons.

When compared to plants fed with a full dose of nitrogen, plants fertilized with 75% of the necessary dose of nitrogen and utilizing the combination of three microorganisms substantial improvements in both fresh and

Table 2. Rationalizing the use of chemical fertilizers by using some micro-organisms and their impact on vegetative qualities of the *Oenothera beinnis* L. during 2020 and 2021 seasons.

Treatments	Herb fresh weight/plant (g)		Herb dry weight/plant (g)		Seeds weight/plant (g)	
	First season	Second season	First season	Second season	First season	Second season
Control 100% N	412.547	421.326	105.451	108.334	35.542	44.104
Control 75% N	348.723	354.262	64.530	68.789	18.112	20.127
Control 50% N	233.412	240.745	42.200	45.167	10.800	17.200
Ac +75% N	359.203	366.521	75.451	78.853	20.405	24.356
Al+75% N	355.423	362.704	71.456	75.362	19.202	22.470
B +75% N	351.112	358.362	68.254	71.523	18.452	20.778
Ac+Al+75% N	367.221	376.415	78.347	80.214	21.100	27.243
Ac+B+75% N	380.733	389.000	86.633	90.633	22.100	31.967
Al+B+75% N	372.342	380.662	84.326	85.961	21.500	30.252
Ac+Al+B+75% N	409.667	418.767	101.667	103.785	30.600	38.000
Ac +50% N	275.367	281.633	56.321	60.167	15.100	18.767
Al +50% N	243.700	258.367	51.333	55.312	14.900	18.700
B +50% N	238.067	249.900	46.200	49.167	13.800	18.200
Ac+Al+50% N	280.133	286.603	57.600	61.365	15.367	19.600
Ac+B+50% N	318.267	326.667	62.733	66.733	17.600	20.032
Al+B+50% N	282.367	296.633	62.167	63.412	16.300	19.767
Ac+Al+B+50% N	384.214	395.145	92.241	97.354	26.745	34.851
L.S.D. at 0.05	7.215	8.365	5.304	6.417	3.742	4.321

Ac: ***Azotobacter chroococcum;*** **Al:** ***Azopirillum lipoferum*****; B:** ***Bacillus polymyxa***

dry weights per plant were gained (Table, 2). Both the fresh and dry weights of the herb increased steadily when 75% of the necessary dose of N was employed, regardless of the bioagent or combination of the used microorganisms. In contrast to the full or 75% of the required dose of N fertilization with or without any bio, 50% of the recommended dose of N fertilization with or without any bio-agent caused a considerable reduction in the majority of cases. These outcomes persisted for the duration of the two seasons.

However, when 75% of the necessary quantity of N was applied with a combination of three microorganisms, the greatest increases in fresh and dry weights/plants of herbs were obtained. In the first and second seasons, the values for the 75% N effect were 409.667 and 418.767 g/plant for herb fresh weight and 101.667 and 103.785 g/plant for herb dry weight, respectively.

Using any bio-agent or combination of microorganisms 50% of the suggested amount of N was applied to the various fertilizer treatments. Application of 50% N with any bio-agent was generally most beneficial when combined with a mixture of microorganisms (50% N+Ac+Al+B). In the first and second seasons, these values were 384.214 and 395.145 g fresh weight/plant and 92.241 and 97.354 g dry weight/plant, respectively.

In general, fresh and dry weights/plant of herb yielded the highest results during the second season. According to the results, when 50% and 75% of the N suggested dose were administered with the combination of bio-agents, the fresh herb in the second season produced the greatest values when compared to the first, one recording increasing by 2.8% and 2.22%, respectively. Furthermore, in the second season, the mean fresh weight of the herb per plant at 421.326 g/plant at the N recommended dose was compared to 418.767 g/plant at 75% of the complete dose of N fertilization with the mixture of bio-agents. Following the same fresh weight trend, the dry weight at 75% of the required N dose was 103.785 g/plant, whereas at the full N dose was 108.334 g/plant.

e. Seeds weight/plant (g)

When rooting against diseases across two seasons, data presented in Table (2) demonstrated a good response and substantial changes in growth in terms of seed weight/plant. Comparing plants treated with 50% of the required dose of N fertilization without any bioagent to those treated with 50 or 75% of the recommended dose of N with the mixture of three utilized microorganisms, significant increases in the number of seeds and weight per plant were observed. With the mixture of three utilized microorganisms (30.600 and 38.000 g/plant in the first and second seasons, respectively), plants with the highest seed weight per plant during the two seasons were fertilized by 75% of the necessary dose of nitrogen, as opposed to the full dose of nitrogen.

However, the plants treated with 50% of the recommended dose of N fertilization without any bio-agent recorded the lowest number of seeds per plant during the two seasons (10.800 and 17.200 g/plant in the first and second seasons, respectively). The increasing of the values of the seed weight/plant when compared to 50% of the recommended dose of N fertilization alone was noted when fertilizer was applied at 50% of the recommended dose of N with any bio-agent.

In terms of all outcomes, the second season was the best, while at 75% of the recommended dose of N with the mixture of three used microbes, the results were almost equal to the recommended dose without any bioagent addition, the half of the recommended dose of N fertilization with the mixture of microbes used was superior to 75% of the recommended dose of N without bioagent addition. In comparison to 35.542 and 44.104 g/plant at 100 N recommended dose without any bioagent addition, the seeds weight per plant in the first and second seasons were 30.600 and 38.000 g/plant at 75% of the N recommended dose with the mixture of the utilized microorganisms.

2. Fixed oil determinations:

Recorded data representing fixed oil determinations are in Tables (3) and (4).

Effect of 50% of the recommended dose of N:

According to Table (3) results, during the two seasons, 50%N+Ac+Al+B considerably raised the proportion of fixed oil in seeds as compared to 50% N that was left untreated.

The fixed oil percentage was 17.1% overall, down from 17.32% in the first season of untreated 50% N plants and 17.58% in the second season of untreated 50% N plants.

Data listed in Table (3) demonstrated that, when compared to 50% N fertilization alone, all tested 50% N fertilization with the microbial treatments greatly enhanced fixed oil yield/plant (ml). The oil yield/plant of 50% N+B was significantly lower than that of 50% N+Ac+Al+B, despite the fact that gradual increases were typically reported as 50% N+Ac+Al+B. Both seasons had the same consequences.

The primary constituents of the resulting fixed oil were affected by 50% N fertilization and the microbial treatments, according to data registered in Table (4). It is evident that when 50% N + Ac + Al + B was applied to plants, the percentages of the primary fixed oil constituents were higher than when 75% N was applied alone or when the plants were treated with alternative treatments applying the same amount of N. Under 50% N+Ac+Al+B, however, the resultant fixed oil had 10.663% linolenic, 52.412% linoleic, 24.741% oleic, and 11.998% palmitic acid.

Table 3. Rationalizing the use of chemical fertilizers by using some micro-organisms and their impact on fixed oil determinations of the *Oenothera beinnis* L. during 2020 and 2021 seasons.

Treatments	Oil (%)		Oil yield/plant (ml)	
	First season	Second season	First season	Second season
Control 100% N	17.321	18.212	6.156	8.032
Control 75% N	13.223	13.723	2.395	2.762
Control 50% N	10.074	10.835	1.088	1.864
Ac +75% N	14.325	14.865	2.923	3.621
Al+75% N	14.182	14.415	2.723	3.239
B +75% N	13.652	14.005	2.519	2.909
Ac+Al+75% N	15.111	15.253	3.188	4.155
Ac+B+75% N	16.424	16.874	3.629	5.394
Al+B+75% N	15.742	16.103	3.385	4.871
Ac+Al+B+75% N	17.130	18.041	5.242	6.856
Ac +50% N	11.986	12.121	1.809	2.275
Al +50% N	11.342	11.732	1.689	2.194
B +50% N	10.578	11.255	1.459	2.048
Ac+Al+50% N	12.362	12.475	1.899	2.445
Ac+B+50% N	13.000	13.301	2.288	2.664
Al+B+50% N	12.652	12.986	2.062	2.566
Ac+Al+B+50% N	17.100	17.580	4.573	6.127
L.S.D. at 0.05	1.006	1.241	3.252	4.435

Ac: ***Azotobacter chroococcum;*** **Al:** ***Azopirillum lipoferum*****; B:** ***Bacillus polymyxa***

Effect of 75% of the recommended dose of N:

During both seasons, there were no discernible differences in fixed oil percentage between the 75% N tested treatments and the control group (Table, 3) aside from 75% N+Ac+Al+B

The results indicated that applying 75% N fertilizer with any microbe generally had no significant effect on the resultant oil production per plant (ml) compared to control throughout the two seasons. This is especially true for fixed oil yield per plant (Table, 3), which was affected by 75% N fertilization with the microbe treatments. In contrast to the control, treated plants with 75% N+Ac+Al+B only produced the highest meaningful values for fixed oil yield per plant. These outcomes corroborated each other during the two seasons. In the first and second seasons, the overall mean was 5.242 and 6.856 ml/plant, respectively.

The largest percentages of linoleic, oleic, palmitic, and linolenic acids (52.862%, 25.087%, 12.357%, and 10.852%, respectively) were found in fixed oil extracted from plants treated with 75% N+Ac+Al+B compared to 75% of the recommended dose of N fertilization without any bio-agent. These data represent the main components of the resultant fixed oil as affected with 75% N application treatments (Table, 4).

While the yield of oil in the recommended amount of nitrogen was 8.032 ml/plant, the fixed oil yield/plant in the second season with 75% N+Ac+Al+B was 6.856 ml/plant, as opposed to 2.762 ml/plant without any bio-agent addition.

Table 4. Rationalizing the use of chemical fertilizers by using some micro-organisms and their impact on oil main components (%) of the *Oenothera beinnis* L. during 2020 and 2021 seasons.

Treatments	Caprylic	Arachidic	Lauric	Palmitic	Oleic	Linoleic	Linolenic
Control 100% N	2.521	1.874	1.088	12.635	25.220	53.412	11.122
Control 75% N	0.920	1.357	0.738	10.542	22.174	50.522	9.058
Control 50% N	0.545	0.624	0.352	9.554	19.745	49.575	7.828
Ac +75% N	1.320	1.448	0.905	11.184	23.201	51.145	9.863
Al+75% N	1.101	1.403	0.832	11.021	22.850	51.031	9.652
B +75% N	0.952	1.392	0.774	10.760	22.462	50.865	9.347
Ac+Al+75% N	1.603	1.576	0.941	11.342	23.652	51.303	10.035
Ac+B+75% N	2.008	1.632	1.022	11.747	24.350	52.011	10.542
Al+B+75% N	1.854	1.602	1.005	11.532	24.056	51.623	10.236
Ac+Al+B+75% N	2.324	1.742	1.073	12.357	25.087	52.862	10.852
Ac +50% N	0.712	1.014	0.513	10.147	20.856	49.962	8.336
Al +50% N	0.647	0.955	0.462	10.085	20.441	49.823	8.117
B +50% N	0.600	0.810	0.404	9.852	20.103	49.672	8.009
Ac+Al+50% N	0.745	1.244	0.550	10.352	21.263	50.075	8.523
Ac+B+50% N	0.863	1.304	0.685	10.441	21.854	50.330	8.954
Al+B+50% N	0.821	1.298	0.612	10.201	21.456	50.198	8.742
Ac+Al+B+50% N	2.105	1.686	1.056	11.998	24.741	52.412	10.663

Ac: ***Azotobacter chroococcum;*** **Al:** ***Azopirillum lipoferum*****; B:** ***Bacillus polymyxa***

3. Chemical analysis:

a. Total carbohydrates (%) in seeds

The effects of bioagents on the carbohydrate % in seeds were determined over the course of two consecutive testing seasons, as shown in (Fig., 1). Comparing plants treated with 50% of the acceptable dose of N fertilization without the use of any bioagent to those treated with 50 or 75% of the necessary dose of N with the mixture of three microorganisms, significant increases in total carbohydrates (%) were obtained in the seeds.

With the combination of three utilized microorganisms (19.13% and 20.041% in the first and second seasons, respectively), plants with the highest total carbohydrates (%) in seeds during the two seasons were those fertilized by 75% of the recommended dose of nitrogen, as opposed to the full dose of nitrogen.

Conversely, the plants treated with 50% of the recommended amount of N fertilization without any bio-agent recorded the lowest total carbohydrates (%) in seeds in the two seasons (12.074% and 12.835% in the first and second seasons, respectively). The values of total carbohydrates (%) in fruits were found to increase when fertilizer was applied at 50% of the recommended dose of N fertilization with any bio-agent, as opposed to 50% of the recommended dose of N fertilization alone.

In terms of all outcomes, the second season was the best, while at 75% of the recommended dose of N with the mixture of three used microbes, the results were almost equal to the recommended dose without any bioagent addition, the half of the recommended dose of N fertilization with the mixture of microbes used was superior to 75% of the recommended dose of N without bioagent addition. In the first and second

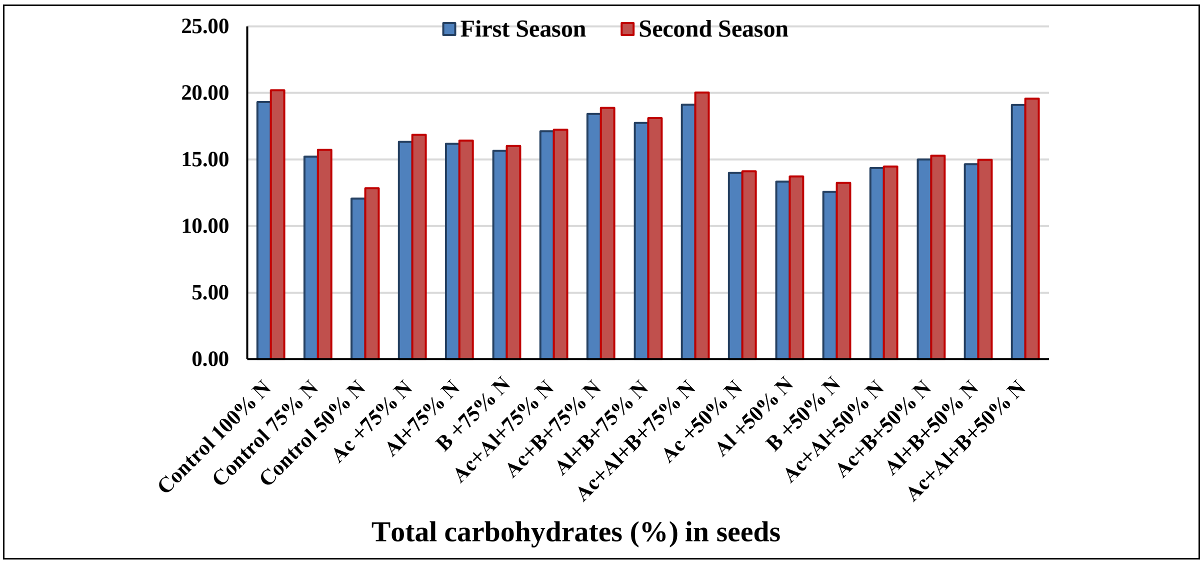

Fig. 1. Rationalizing the use of chemical fertilizers by using some micro-organisms and their impact on total carbohydrates (%) in seeds of the *Oenothera beinnis* L. during 2020 and 2021 seasons.

seasons, the total carbohydrates (%) in the seeds were, at 75% of the N indicated dose, 19.13% and 20.041% with the mixture of utilized microbes, and, at 100% of the N recommended dose, 19.321% and 20.212%) without any addition of bioagents.

b. Seed content of elements

Through two consecutive testing seasons, the effects of bio-agents on the percentages of elements (nitrogen, phosphorous, and potassium) in seeds were obtained, as shown in (Fig., 2).

Comparing plants treated with 50% of the acceptable dose of N fertilization without the use of any bioagent to those treated with 50 or 75% of the recommended dose of N with the mixture of three microorganisms, significant increases in the percentage of elements in the seeds were observed. With the combination of three utilized microorganisms, plants fertilized with 75% of the recommended dose of nitrogen (as opposed to the full dose), recorded the largest percentage of elements in seeds across the two seasons.

Conversely, the plants treated with 50% of the necessary dose of N fertilization without any bio-agent had the lowest percentage of elements in their seeds over the course of the two seasons. The percentage of elements in seeds increased when fertilizer was applied at 50% of the recommended dose of N, along with any bio-agent. However, when fertilizer was applied at 50% of the recommended dose of N alone, the values did not change.

In terms of all outcomes, the second season was the best, while the percentage of elements in seeds was almost equal to the recommended dose without any bioagent addition at 75% of the recommended dose of N with the mixture of three microbes used, the half of the recommended dose of N fertilization with the mixture of microbes used was superior to 75% of the recommended dose of N without bioagent addition.

c. Soil properties:

Through two consecutive testing seasons, the results illustrated in (Fig., 3) show the impacts of bio-agents on soil properties such as the rhizosphere's dehydrogenase enzyme (DHA) and nitrogenase enzyme (N_2-ase) activities. In comparison to plants treated with 50% of the recommended dose of N fertilization without the use of any bioagent, there were notable increases in the soil properties such as dehydroengenase enzyme (DHA) and nitrogenase enzyme (N_2-ase) in 50 or 75% of the recommended dose of N with the combination of three microbes.

Fig. 2. Rationalizing the use of chemical fertilizers by using some micro-organisms and their impact on percentage of nitrogen, phosphorous and potassium in seeds of the *Oenothera beinnis* L. during 2020 and 2021 seasons.

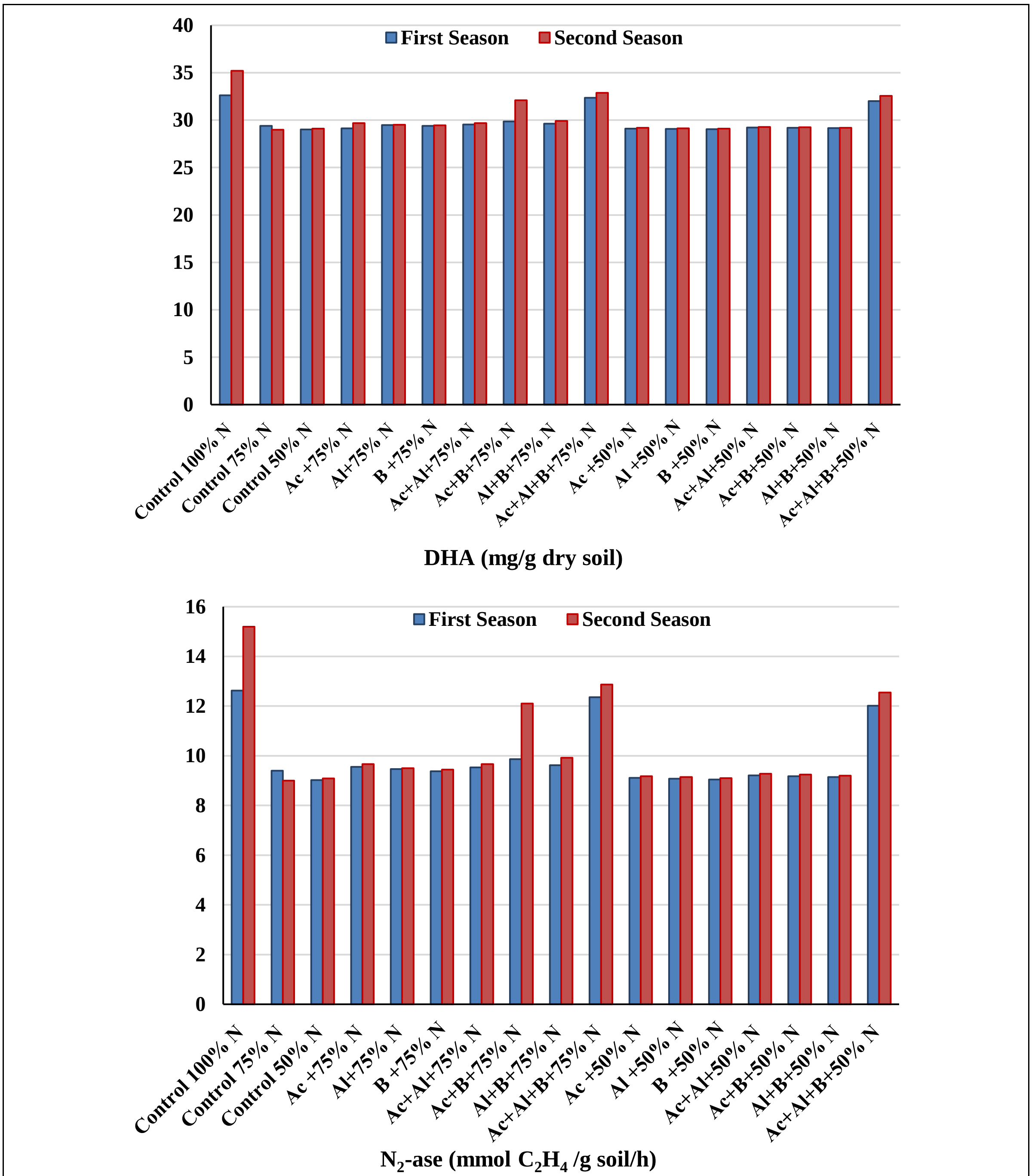

Fig. 3. Rationalizing the use of chemical fertilizers by using some micro-organisms and their impact on dehydroengenase enzyme (DHA) and nitrogenase enzyme (N_2-ase) activities of the *Oenothera beinnis* L. during 2020 and 2021 seasons.

When the combination of three microorganisms was utilized instead of the entire amount of nitrogen, plants fertilized by 75% of the recommended dose of N gave the highest levels of the enzymes Dehydroengenase (DHA) and Nitrogenase (N_2-ase) during the two seasons.

Conversely, plants treated with 50% of the recommended amount of N fertilization without any bio-agent had the lowest values of the enzymes dehydroengenase (DHA) and nitrogenase (N_2-ase) across the two seasons. The values of the enzymes dehydrogenase (DHA) and nitrogenase (N_2-ase) rose when fertilizer was applied at 50% of the recommended dose of nitrogen fertilization alone, regardless of the used bio-agent.

In terms of all outcomes, the second season was the best. Dehydroengenase (DHA) and nitrogenase (N_2-ase) values at 75% of the recommended dose of N with the mixture of three microbes used were nearly equal to the recommended dose without any bioagent addition, while the half of the recommended dose of N fertilization with the mixture of microbes used was superior to 75% of the recommended dose of N without bioagent addition.

DISCUSSION

Chemical contamination, whether from pesticides or fertilizers, resulted in the emergence of numerous diseases in recent times.

However, one of the biggest obstacles to the development of agricultural commodities, including aromatic and medicinal plants, is the high cost of chemical fertilizers and the difficulty in obtaining them. Finding alternatives to chemical fertilizers or minimizing their use has become necessary in order to preserve the yield and caliber of agricultural produce.

Plant growth-promoting bacteria (PGP) bio-amelioration using microorganisms like bacteria is a potential technique for the remediation of calcareous sodic and saline-sodic soil because of its high efficiency, environmentally benign features, and low cost. Plant symbionts may benefit indirectly from PGPR through the induction of systemic resistance, augmentation of antibiotic or antifungal impact, modification of quorum sensing, and/or elevation of plant cellular metabolites (Compant *et al.,* 2019).

Still, the most potent PGPR strains probably work through a combination of ways. Both organic and inorganic forms of phosphorus (P), an important macronutrient for biological growth and development, are widely available in soils. Despite having a big reservoir, soil contains insoluble, non-utilizable P, which means that plant availability of P is typically poor. P can be found in soil in both organic and inorganic forms, such as inositol phosphate, phosphomonoesters, and phosphotriesters. Inorganic forms are primarily found in insoluble mineral complexes like apatite. By secreting organic acids or extracellular hydrolytic enzymes (acid phosphatase), a number of PGPRs have been shown to have the ability to mineralize and solubilize both organic and inorganic insoluble complex forms of phosphorus, improving the accessibility of nutrients to plants (Sharma *et al.,* 2016).

Auxins are produced by several PGPR and have been shown to have particularly powerful impacts on root growth and architecture (Jha and Saraf, 2015; Gupta *et al.,* 2015). The auxin that is produced by PGPR that has been investigated, the most was indole-3-acetic acid (IAA). It plays a role in the interactions between plants and microbes (for example, the function of exogenous IAA depends on the amounts of endogenous IAA in plants). Application of bacterial IAA may have neutral, favorable, or unfavorable effects on plant growth at optimal IAA concentrations in plants. It has been demonstrated that auxin-producing PGPR induces transcriptional changes in genes related to hormones, defense, and cell walls; additionally, it increases root biomass, lengthens roots, and reduces the size and density of stomata; lastly, it activates auxin response genes that promote plant growth (Llorente *et al.,* 2016; Ruzzi and Aroca, 2015). Following its identification as a plant hormone, a great deal of research has been done on IAA because it has been shown to be crucial for the growth and development of plants. It has been discovered that a variety of bacteria, fungi, and algae may produce quantities of IAA that are physiologically active.

Of all the genera, *Bacillus* species are thought to be the safest microbes, possessing exceptional capabilities to synthesize a wide range of advantageous compounds (Stein, 2005). Many horticultural, agricultural, and medicinal crops have produced *Bacillus* spp. with strong plant growth-promoting traits like IAA production, phosphate solubilization, nitrogen fixation, and biocontrol attributes like production of HCN, siderophores, hydrolytic enzymes, and antibiotics (Sharma *et al.,* 2016). The biocontrol properties of *Bacillus* against numerous common phytopathogens have also been discovered by a number of other researchers (Gajbhiye *et al.,* 2010).

CONCLUSION

While maintaining the quality and productivity of the *Oenothera biennis* L. (evening primrose) plant, it may be suggested that the use of some plant-growth-promoting [*Azotobacter chroococcum, Azopirillum lipoferum,* and *Bacillus polymyxa*] can help lower the usage of chemical fertilizers.

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ترشيد إستخدام الأسمدة الكيماوية بإستخدام بعض الكائنات الحية الدقيقة وأثرها على إنتاجية و جودة نبات الأونيثرا

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أُجريت هذه الدراسة بتصميم قطاعات كاملة العشوائية خلال موسمي النمو المتعاقبين ٢٠٢٠/٢٠٢١ و٢٠٢١/٢٠٢٢ في مزرعة كلية الصيدلة بجامعة القاهرة، لدراسة تأثير تكنولوجيا العوامل الحيوية. تم اختبار تأثير بعض محفزات نمو النباتات مثل *Azotobacter chroococcum* و*Azospirillum lipoferum* و*Bacillus polymyxa* مع تركيزات ٥٠٪ و٧٥٪ و١٠٠٪ من سماد النيتروجين على نبات الأونيثرا (*Oenothera biennis* L.) لدراسة النمو، ومحصول العشب، ونسبة الزيت الثابت، ومحصول الزيت الثابت ومكوناته. تم نقع بذور الأونيثرا لمدة ٣٠ دقيقة قبل الزراعة. في الموسمين اللذين تم استخدام هذه العوامل الحيوية فيهما، تم رش النباتات بمعلق العوامل الحيوية أربع مرات شهرياً بعد

الزراعة. أشارت النتائج إلى أن التسميد بهذه العوامل الحيوية أدى إلى زيادة ملحوظة في ارتفاع النبات، وعدد الفروع لكل نبات، وعدد القرون لكل نبات، ووزن العشب الطازج والجاف لكل نبات (جم)، ووزن البذور لكل نبات (جم)، ونسبة الزيت الثابت في البذور، وإنتاج الزيت الثابت لكل نبات (مل)، التحليل الكيميائي بالإضافة إلى نسبة الكربوهيدرات الكلية (%)، ونسبة النيتروجين، والفوسفور، والبوتاسيوم في البذور، والنشاط لإنزيمى النيتروجينيز والديهدروجينيز في التربة. وكانت أعلى زيادة في هذه القياسات تم الحصول عليها عندما تم معالجة النباتات بـ *Azotobacter chroococcum* (Ac) و *Azospirillum lipoferum* (Al) و *Bacillus polymyxa* (B) في وجود ٧٥٪ من سماد النيتروجين. وأقل متوسط لجميع القياسات في كلا الموسمين تم الحصول عليه في النباتات التي تم معاملتها بنسبة ٥٠٪ من النيتروجين. تم تسجيل أعلى النسب من حمض اللينوليك، وحمض الأوليك، وحمض البالمتيك، وحمض اللينولينيك في الزيت الثابت مع نفس المعاملة. بالإضافة إلى ذلك، تم تسجيل أعلى نسبة من الكربوهيدرات الكلية، النيتروجين، الفوسفور و البوتاسيوم في البذور، وكذلك أنشطة النيتروجينيز والديهدروجينيز في التربة، في النباتات التي تلقت هذه المعاملة.