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Effect of PGPR Bacterial Inoculum and Amino Acids on the Growth and Oil Quality in Three Varieties of Shrub Rose Rosa Damascene

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Abstract

The experiment was conducted in the winter season of 2023, to evaluate the effect of bacterial inoculum with three types of growth-promoting bacteria PGPR (Bacillus subtilis, Pseudomonas fluorescens, Azotobacter chroococcum) and spraying with commercial amino acids (0, 1.5, 3, or 4.5 g/L) on plant growth and quality indicators of the resulting oil in three varieties of Rosa damascena (red, pink and white). The experiment was split into three parts, and units were distributed as a randomized complete block design (RCBD) of three replications and 576 total experimental units. Experiment measurements were the plant's height, number of leaves, flowers per plant, and petals per flower. The plant's productivity was also estimated based on the flower content of oil. Findings showed that the pink variety was the most vigor with the largest number of leaves. All the PGPR bacteria did not differ in several leaves, but the P. fluorescens treatment resulted in the highest plant height. However, amino acid treatments had a negative effect on plant height compared to the untreated ones. The best results of plant height and number of leaves were obtained from the interaction treatment of the pink or red variety treated with 1.5 or 3 mg L-1 amino acids in the presence of B. subtilis. The most flowers and leaves indicators were on the pink and red varieties that interacted with B. subtilis and P. fluorescens as biofertilization. Increasing the amino acid level to 4.5 mg L-1 resulted in the highest number of rose flowers, but not the number of petals per flower. The pink variety had more oil and a higher oil content, 546.2% and a specific gravity of 18.98 g when interacted with P. fluorescens and 1.5 mg L-1 of amino acid, which did not differ much from the red variety in the same interaction.

Keywords:

Biofertilizer, plant hormones, ornamentals, rose water.

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Introduction

Rose (Rosa sp.) is one of the most famous plants for cutting (Debener & Linde, 2009, Blakeslee et al., 2005). The rose bush, or Rosa, is a very popular and pretty plant (Yuvaraj, 2017). The first record of this flower was in 4000 BC ((Al-Samarrai,2000). It is also one of the oldest in the world. Rosa trees look lovely, and they're often used as cut flowers (Al-Shaikhli, 2010). Rose oil is one of the many good chemicals that they have (Hafez, 2008). The flowers are unique because they smell great and have lots of pretty colors. There is either one flower, a walnut-shaped flower, or a half-walnut-shaped flower. There are short ones and ones that grow straight up or up and over like trees. Their flowers last a long time after being picked (Al-Sahhaf & Hammoud, 2001; Al-Abdali, 2002). The flower of a rose determines its worth and beauty, especially the shape and structure of the petals when it opens. The rose petals and flower form are also looked at, as they are very important (van Doorn & Kamdee, 2014; Berg et al., 2016; Sulborska et al., 2012). did research in 2007 and 2012 on the pattern of uneven tissue development around the apical meristem. That trend is at the heart of this difference. Cells in the rose flower are not in the same place as those in the main branch (Kaplan, 2001). For roses and their flowers, the weather is very important. Roses also change how fast or slow they grow based on how much light they get (Shaheen, 2014). According to (Mukhtar & Singh, 2006), this is usually found in safe farming methods that let you manage the air quality and weather. The best thing you can do for plants is to add fertilizer to the soil. An article from Keuskamp et al. (2010) says that these have basic parts that help plants grow, make more energy, and make more and better food. One of the most important things in farming is using bacteria and their biological activity in the soil as a safe way to give plants the nutrients they need. Chemical fertilizers leave behind toxins that are bad for plants and the earth. Organic fertilizers are used to grow many plants (Fakhrian et al., 2022). These fertilizers protect the plants and make them stronger against biological and natural stresses. Al-Khafajy et al. (2020) say that they not only cut costs and raised output, but they also didn't leave any dangerous trash behind. Living fertilizers are being thought about as a way to improve the soil. These are green ideas that are good for you and the earth, say Moussa et al., (2002). They have a lot of good things for you in them, like calcium, magnesium, iron, cobalt, copper, manganese, zinc, and potassium. Another area that has been looked into a lot is amino acids and plant nutrients, mostly lysine. This amino acid is important for plants in many ways. It's the only known polar transport hormone, and the stems and leaves are made of it (Blakeslee, 2005; Naramoto, 2017; Braybrook & Kuhlemeier, 2010). The test's purpose was to find out what happens when three kinds of bacterial bio fertilizers are added and which kind helps different kinds of bush rose plants grow better in terms of leaves, flowers, and essential oils. Before this, different amounts of industrial amino acids were put on the plants' leaves to find the most effective percentage for improving the plants' growth, flowering, and production of essential oils (Far, 2017; Al-Maliki et al., 2021; VSN International, 2009).

Materials and Methods

In the winter of 2023 and 2024, the test was done at the Agricultural Research Station of the University of Kufa in Najaf Governorate. The dirt and wood cover was put up with only half of the light on. The trees came from a farm in Baghdad that was allowed to sell seeds. When the plants were one year old, they were put in 15-cm pots filled with river sand one at a time. The plants were moved to plastic pots that were 29 cm across and 30 cm high on December 31, 2023. A 1:3 mix of sand and peat moss was used to fill the pots. Each pot with a plant in it now weighed 10 kg. The plants were taken care of under a wooden cover. The types of peat moss in the growth medium's dirt were also checked (Table 1).

Table 1. Soil mix (growing medium) used in the study was determined by its components and contents of some nutritional elements

Constituent	**%**
N	2.2 – 2.8
P2O5	0.1
K2O	0.1
Na	≥0.01
CL	≥0.8
O.M.	60 –70
PH	0.5-5.9
Moisture	12 – 15
C: N	**1**:14 - 1:18

Agricultural services were carried out during the experimental period, where all experimental plants were fertilized with 50 g per 20 litres of water with granular NPK compound fertilizer (0-46-18), and fertilization was repeated every 21 days four times (Ranjan & Bhagat, 2024; Sredić et al., 2024; Scholberg et al., 2001). The plants were irrigated uniformly and whenever needed before the planting medium was exposed to drought. The plants were covered during the winter with a thick polyethylene cover to protect the plants from low temperatures in winter, while the plants were covered with a light mesh cover (Saran) to protect the plants from high temperatures in summer.

Preparation of Biofertilizers

The biofertilizer was prepared from bacterial isolates (Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus subtilis). They were obtained from the accredited Al-Amin Laboratory in Najaf. The bacterial inoculum was activated and multiplied on the sterile Nutient broth liquid medium using an Autoclave, where the inoculated media plates were incubated at 28±2 °C in a shaking incubator for 7 days. After one week of growth, the bacterial treatments were added to the rose seedlings by injection on 1/25/2024, and the number of bacterial colonies was counted every three weeks (Table 2).

Estimation of Bacterial Numbers in the Inoculum

The Plate Count Technique was used to find the total number of bacterial species in dilutions 10-5 to 10-7. This was done by moving 1 ml of the plates that had been kept at 28 ± 2 °C for three days. Then, Kirchman et al., (1982) found the number of bacterial cells.

The experimental treatments were distributed in a RCBD factorial manner, where the first factor included: using three varieties of shrub roses, which are red, pink, and white. The second factor was inoculation with biofertilizers (Bacillus subtilis, Pseudomonas fluorescens, Azotobacter chroococcum).

The third factor included spraying with amino acids at four concentrations (0, 1.5, 3, 4.5) g, with three replicates. Amino acids were sprayed five times, the first after 21 days of transplanting, and four subsequent sprays with a time interval of 14 days. Spraying was applied in the early morning using a 2-litre hand sprayer. All experimental units were serviced same operations of irrigation and weeding whenever necessary.

Table 2. Number of bacterial colonies of three bacterial types in two dilutions, estimated after 20 days of inoculation DPI on three cultivars of bush roses

Rose type	No. of days post inoculation DPI	Azotobacter chroococcum		Pseudomonas fluorescens		Bacillus subtilis	
		N*10-5	N*10-7	N*10-5	N*10-7	N*10-5	N*10-7
Red	20	23	4	55	12	30	3
	40	35	12	66	20	73	68
	60	29	12	60	7	110	23
Pink	20	43	6	18	2	15	2
	40	46	23	90	72	80	69
	60	25	15	33	4	33	9
White	20	3	0	6	1	14	1
	40	40	13	59	35	90	76
	60	50	20	125	9	95	14

Studied Indicators

At the end of each season, the plant's height (cm), number of leaves (leaf plant-1), flowers (flower plant-1) (the number of flowers made on each plant from the start of blooming to the end of flowering), and petals (petal flower-1) were all recorded. Oil content and quality in flowers was also estimated: A hundred microliters of clean water weighed the same as the same amount of essential oil (Khatiri et al., 2019) (Jayapriya, 2021). This is to facilitate estimation of actual oil weight using a highly sensitive scale (200, Japanese HR) set at 25°C (Guenther, 1972).

Statistical Analysis

Genstat 12 (VSN, 2009), a statistical analysis tool that was made from scratch, was used to make the ANOVA charts. We used a 0.05 chance level for the LSD test to see if there was a difference between the means (Al-Rawi & Mohammed, 2000, Garcia-Gomez et al., 2002, Mohammed et al., 2012).

Results and Discussion

Table 3 shows that the different types of plants are very tall in different ways. When they were young, the red plants were 46.33 cm tall and the pink plants were 39.99 cm tall. For the white type, the plants were 39.99 cm tall. The bacteria B. subtilis had the least effect on plants that were 40.59 cm tall. On the other hand, the bacteria P. fluorescens had the most effect on plants that were 47.17 cm tall. It was also seen that plants grew a lot taller when there were fewer amino acids in the soil than when there were more. In general, this was most true when the height was 30.25 cm and the amino acid amount was 4.5 mg.L-1. There were also different numbers of leaves on each type. The red type had the most leaves, with 61.80 per plant. The pink type came in second with an average of 41.57 leaves per plant, and the white type came in third with no more than 41.57 leaves per plant. If you look at the bacteria, they all changed the number of leaves in the same way. But the amino acid treatment made a big difference, especially when it was 1.5 mg/L-1. The trees usually had 57.84 leaves, while the test group only had 48 leaves.

Several flowers and leaves in a flower were affected in different ways. There were only 15.24 flowers on each white plant, but each flower had 31.73 petals. While pink plants had 16.99 flowers, red plants had 46.10 leaves per flower. Most of the time, bacterial fertilizers increase petals to 43.99 petals per flower-1. However, several flowers/plant was slightly affected by amino acid concentration, where 1.5 mg.L-1 resulted in 15.60 flowers per plant while 4.5 mg. L-1 had 16.49 flowers per plant (Table 4).

Table 3. Effect of soil application with three PGPR bacteria and foliar amino acids concentrations on plant height and number of leaves in three varieties of shrub rose.

PGPR bacterial treatments	**Amino acids**	**Plant height (cm)**			**No. of leaves. Plant-1**		
		Rose varieties					
		RED	**Pink**	**White**	**RED**	**Pink**	**White**
0	0	53.97	47.79	56.36	68.46	54.79	43.72
	1.5	19.77	23.56	62.85	49.21	31.16	53.5
	3	25.55	62.25	50.78	49.11	56.09	52.9
	4.5	46.99	39.31	15.88	53	63.77	33.75
Agrobacterium chroococcum	0	61.05	62.75	54.77	61.08	64.37	46.72
	2	26.85	86.78	20.36	55.79	77.83	35.45
	4	56.96	43.1	35.02	71.3	47.91	39.64
	6	44.5	11.09	27.84	79.33	38.44	46.07
Pseudomonas florescens	0	63.94	63.74	61.65	68.75	53.9	43.53
	2	64.04	36.92	31.03	75.74	41.13	44.12
	4	53.57	57.76	52.48	61.57	50.9	41.13
	6	13.58	35.22	32.13	48.61	54	27.17
Bacillus subtilis	0	68.03	53.77	43.6	68.46	67.26	53
	2	28.84	28.94	49.48	42.63	42.43	27.07
	4	37.82	50.58	29.64	79.48	79.33	34.25
	6	42.7	37.72	15.98	56.29	43.97	43.18
L.S.D. (P≤0.05)	Bacteria	5.305			4.904		
	Amino acids	5.305			4.904		
	Varieties	4.595			4.247		
	Interaction	18.378			16.988		

Table 4. Effect of soil application with three PGPR bacteria and foliar amino acids concentrations on number of flowers (flower plant-1) and petals (petal flower-1) in three varieties of shrub rose

PGPR bacterial treatments	**Amino acids**	**Number of flowers /plant**			**Number of petals of flower-1**		
		Rose varieties					
		RED	**Pink**	**White**	**RED**	**Pink**	**White**
0	0	12.9	21.9	12	39.5	38.7	33.2
	1.5	14.5	13.8	11.8	44.5	42.9	31.7
	3	15.2	16.9	17.6	46.3	42.1	23.7
	4.5	14.3	16.4	19.2	54	46.9	26.6
Agrobacterium chroococcum	0	14.3	15.4	15.6	48.7	41.5	22.6
	2	19.7	13.2	13.4	52.7	40.5	26.8
	4	18.6	14.6	17.1	58.8	46.9	20.5
	6	15.4	14.1	19.1	58.3	47.2	39.1
Pseudomonas florescens	0	15.1	19	13.7	52.4	44.1	67.4
	2	15.8	19.1	14.9	44.8	34.8	56.1
	4	16.9	20	12.7	40.7	34.5	39.7
	6	12.2	20.1	13.6	48.3	42	23.1
Bacillus subtilis	0	19.3	18.7	13.4	34.7	32.5	19
	2	16.8	14.9	19.3	35.6	39.6	26
	4	18.6	13.8	15.4	40.4	44.7	23.6
	6	18.7	19.8	15	37.9	58.4	28.6
L.S.D. (P≤0.05)	Bacteria	1.128			4.266		
	Amino acids	1.128			4.266		
	Varieties	0.976			3.695		
	Interaction	3.906			14.779		

Table 5 shows the different ways that the quality indicators, such as the amount of oil in the flowers and the exact weight of the oil that was made, changed. The pink flower had the most oil, at 209.8%. The red flower, which came in second, had a lot more oil than the white flower, which had only 183.2%. There was more oil when germs were around. P. florecens made 220.2% of the oil, resulting from all the bacterial treatments. It was much more than that of the control group, which resulted in only 155.9% oil. Even so, only the 1.5 mg L-1 of amino acids resulted in a higher oil increase of 223.9%, when 3 mg L-1 had only 169.3%. When flowers were touched, they usually got the most oil, as the oil of each variety had a different pressure. The pink rose showed an oil specific gravity of 2.49 g, but the red variety only had an oil specific gravity of 0.52 g. P. florecens always resulted in higher oil specific gravity, showing significant differences from other bacteria, especially when interacting with amino acid at 1.5 mg L-1 (Table 5).

Table 5. Effect of soil application with three PGPR bacteria and foliar amino acids concentrations on oil yield (%) and oil specific gravity in three varieties of shrub rose

PGPR bacterial treatments	**Amino acids mg L-1**	**Flower content of oil% %**			**Oil Specific gravity**		
		Rose varieties					
		RED	**Pink**	**White**	**RED**	**Pink**	**White**
0	0	141.2	173.6	208.9	0.26	0.43	0.48
	1.5	140.4	233.9	106.9	0.74	4.62	0.35
	3	140.8	84	176.1	1.33	4.02	0.43
	4.5	116.1	209.6	109.3	0.17	4.17	0.36
Agrobacterium chroococcum	0	133.7	206.8	204.5	0.42	0.23	0.29
	1.5	209.3	209.2	208.8	0.00	4.43	0.23
	3	205.1	113.4	185.9	0.06	0.35	0.05
	4.5	185.9	184	230.7	0.02	0.57	0.5
Pseudomonas florescens	0	103.6	274.7	186.8	0.08	0.6	0.07
	1.5	213.2	185.7	213.5	0.05	4.18	0.14
	3	248.7	215.8	178.7	0.47	0.05	0.3
	4.5	184.8	214.7	222.3	0.52	0.2	4.72
Bacillus subtilis	0	203.7	202.2	148.1	0.3	0.3	0.29
	1.5	546.2	212.7	207.2	0.57	0.21	0.33
	3	109.6	230.1	143.6	0.47	0.45	0.25
	4.5	202.3	205.7	199.6	2.77	0.2	0.39
L.S.D. ($P \leq 0.05$)	Bacteria	24.53			1.028		
	Amino acids	24.53			1.026		
	Varieties	12.25			0.889		
	Interaction	84.98			3.554		

The results of the study showed that the treatments made the plants taller and gave them more leaves. More studies have shown that peat moss and amino acids added to the growing medium make plants taller and have more leaves. This means they show more signs of vegetative growth (Gome, 2002; Schoberg et al., 2001; George et al., 2008; Nowak & Strojy, 2003). When amino acids were sprayed on plants, they grew a lot faster. Their 2013 study found that the best way to grow Spathiphyllum was with a 3:1 mix of perlite, sand, and amino acids. The plants got more leaves because of this. Rajvanshi & Dwivedi, (2014), Kauffman et al., (2005) say that adding bacterial bio fertilizer to dirt that already had Z. elegans plants in it made the plants bigger and gave them more leaves. When 40% bio fertilizer was put on dirt that already had Calendula officinalis L. in it, a lot more leaves grew. The same thing happened when Hasan et al., (2014) used a growth medium mix of (3 mixtures: 1 peat moss). There were a lot more good changes to the green growth traits.

Adding acids called amino acids to some enzy mes can change how they work and how they look. They also let water and heavy metals out of the plant, control the flow of ions, and keep the osmotic pressure steady (Rai, 2002; Kauffmanll et al., 2005, Polo et al., (2006). Claussen (2004) and Nur (2006) did tests that showed amino acids with a lot of nitrogen helped plants get bigger and make more shoots. Mohammad (2012) and Bergougnoux et al., 2007 says that nitrogen helps the plant make auxins, which help cells divide and make the plant grow taller. In this case, proteins in cells get bigger.

To help plants grow, fungicides are the most important thing that can be added to the soil. They make plants grow and stay healthy. They can also help the plant make more and better food (Keuskamp et al., 2010). The study found that flower growth markers went up when peat moss and bacterial fertilizers were added to the soil. Bio fertilizers bring more and different bugs to the soil. It helps the ground keep its temperature steady and is more alive when it has living things in it, like peat moss. Hormones or enzymes help the plant grow faster, which helps it get bigger. If you change how the elements in the earth or those that are added to it get ready, you can also make more and better things (Taha, 2007, Kakoei & Salehi, 2013). As Abd El-Samad et al., (2010) say, amino acids can help plants' biological processes in any way you look at them. Herons need amino acids before they can be made by plants. The first step in making ethylene is methionine, and the first step in making auxin is tryptophan (Davies, 2010). These are some examples. Al-Sheikhly agreed in 2010 that the best way for flowers to grow, have more flowers, and bloom more is to mix peat moss medium with soft dirt. Bio fertilizers that were mixed into the growing medium were also named as one of the most important things that made different breeding methods work. The plants' growth and change will show you this. It depends on the material you pick, how the roots grow and branch out. This helps farms grow as many plants and seeds as they can. According to Berg et al. (2016), free amino acids are a key way for plants to get nitrogen, which they need to make enzymes and energy that help their stems and leaves grow. Not only Jackson et al., (2004), but also Omer et al., (2006) and Ballaa and Abd-Aziz (2007) did find it. If you spray arginine and proline on the plant, you can change when the stomata open and close. This makes photosynthesis work better for the plant, which helps it grow faster and get more leaves and flowers (Saddon & Zuraini, 2016; Ibrahim et al., (2010) say that amino acids help make many different chemicals, such as enzymes, vitamins, alkaloids, terpenoids, amines, and other groups of chemicals. Amino acids help plants grow and make more food, both good food and more food. The plant needs different amino acids at different stages of its growth. Through the stomata in the leaves, they can get in. The temperature of the air around the plant affects this process (Stino et al., 2010). Bio fertilizers are a group of bugs that do a lot of work. They are also called bacterial inoculants. They help plants grow better and keep the ground healthy for these living things. Organic acids, amino acids, and sugars are just a few of the good things that build up in the root zone. In this, germs find a lot of food and fuel (Smith et al., 2015a, Ishibashi et al., 2013).

It was also clear that the biological factors and amino acid amounts had different effects on the different types of rose stems. Rosies come in over 200 different types, but only a few are used to make essential oils. The Damask rose, Rosa damascene Mill, is one of these. You can get Rosa gallica L. x Rosa moschata Herrm. Rose oil can be made from it. Rose water, pure oil, and oil mix are what the business makes most of. Baydar et al. (2016) say that these are made by taking liquids out of water and boiling them. Taif got up. Rosa damascena-trigintipetala is a Damask rose, but it's not clear what kind it is. The strong, deep smell of this rose makes it one of the best for making rose oil. This 30-flower rose with a lot of oil has been grown in Taif for 300 years (Bahaffi, 2005, Abdel-Aziz & Balbaa, 2007).

Conclusion

Findings of this study showed that the rose varieties differed in their growth and quality indicators and their responses to biofertilization and amino acids foliar spray. The highest growth and rose qualitative indicators were recorded in the pink variety, followed by the red one then the white rose. It was also found that the three PGPR bacteria had a similar positive effect on several leaves, but P. fluorescens resulted in the highest plant height. However, plant height was negatively affected by higher foliar amino acid concentrations. The best growth measurements were obtained in the interaction treatment of the pink or red rose sprayed with 1.5 or 3 mg L-1 amino acids in the presence of B. subtilis. The flowering indicators were at their highest values in the pink and red varieties fertilized with B. subtilis or P. fluorescens. Increasing amino acid concentration increased the number of rose flowers, but not the number of petals per flower. The best results of rose flower content of oil and oil specific gravity were recorded in the pink variety fertilised with P. fluorescens and 1.5 mg L-1 of amino acid.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

All authors' contributions are equal for the preparation of research in the manuscript

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