Microbial Technique for The Production of Growth Regulator Compounds Indole Butyric Acid (IBA) and Its Role on The Rooting and Growth of Bougainvillea Spectabilis L. Stem Cuttings

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Abstract. The study included the application of microbial techniques to convert sesbanin and soybean seed powder into indole bio-compounds by using 3 bacterial isolates: Azotobacter chroococcum, Pseudomonas putida and Bacillus.megaterium obtained from the microbiology laboratories at the Center for Desert Studies. Peptone 1%, Then it was used alone, in pairs, or in combination to produce the indole butric acid (IBA) growth regulator compounds, using a medium containing locally prepared materials that included the dry powder of soybean seeds and sesbanin seeds. It also tested the ability of isolates to dissolve phosphate compounds and produce iron-chelating compounds. The results showed the ability of B. megaterium A. chroococcum isolates together to produce indole compounds at a rate of 182.3 mg L⁻¹ when using a mixture of sesbanin seed powder and soybeans. The highest product rate of bio-indole compounds was 67.9 mg L⁻¹ with the use of a mixture of soybean seed powder and sesbanin medium. While the highest product rate reached 103.33 mg L⁻¹ by using the two isolates mixture of B. megaterium and A. chroococcum together. Also, P. putida, B. megaterium, and A. chroococcum isolates were found are able to produce iron-chelating compounds (+++, ++, +++) according to the sequence. These isolates B. megaterium, P. putida and A. chroococcum were found ability to dissolve phosphate compounds with average dissolving diameter of 10.43, 29.9 and 29.6 mm, respectively. The ability of the Bio-IBA growth regulator product was tested with or without cells isolates compared to industrial IBA at concentrations of 300 and 600 mg L⁻¹ to influence some growth cutting of Bougainvillea spectabilis L. The results showed a significant effect on some growth characteristics such as germination percentage, number and area of leaves per plant, branch length and number of branches of the plant.

1. Introduction
The isolates of P. fluorescens and B. megaterium were able to produce quantities of IBA ranging between 17.7-22.7 mg l⁻¹ [1]. It was found that preparing the medium with tryptophan led to an improvement in the production of indole by P. putida by using tryptophan at a rate of 200 mg l⁻¹. The increase in tryptophan had a significant effect on increasing the production of indole by the bacteria, and the highest rate of production was 45 mg IBA⁻¹ after 48 hours of incubation[2]. The observed group of isolates from Azotobacter and Pseudomonas produced quantities of indole reaching 32.2 mg L⁻¹ when grown on a nutritious broth medium enriched with 0.4% of soybean seed powder as a source of tryptophan. The study showed that P.putida had a production of 32 mg l⁻¹ of indole the end of the logarithmic phase, but the survival of the culture cells after 72 hours led to the decomposition of more than 25% of the indole into other compounds [3]. There were significant differences in the amount of indole produced by the isolates used in their studies[4] who selected three isolates out of a total of 106 isolates obtained from soil that were indole producing [5]. The chemical composition was indicated of these compounds varies according to the microorganisms that produce IBA [6].

The isolates are showed of Pseudomonas have produced Siderophore compounds in an iron-free medium [2]. was able to select three indole producing isolates that were the most efficient out of a
The use of a growth regulator is one of the factors in stimulating the plant cutting rooting, one of the oldest methods and accelerating its rooting, one of the oldest known uses of growth regulators. Indole butyric acid IBA is also the best growth regulator for this purpose, because its degradation is relatively slow in plants, and is slow to transfer, remains great in the treatment area, non-toxic to plants and does not cause collateral damage to plants [7]. Indole biotic IBA promotes the formation, growth and development of adventitious roots principles, and the increase in the rate of roots formed [8,9]. There are many studies on the positive effect of using IBA on the propagation of plants at different concentrations, including what has been done on rubber cuttings [10], razaki (Alfol) [11], Aldamas [12] and alhana [13]. The growth regulator IAA produced by the root ganglia bacteria increased the germination of Brassica Campestris, as the germination rate reached 72-75% compared to the control treatment, in which the germination rate was 54% after 15 days after planting [14].

The growth regulators from auxins have an important role in regulating biological processes, transporting compounds and their transformations within plant cells [15]. The importance of these compounds by transporting and transforming compounds within the plant [16]. As explained by [17]; [18]; [19] the role and importance of microbes producing auxins in the root zone of the plant and the importance of these compounds in the formation and activity of plant roots. The indole compounds produced microbes in the root zone can be metabolized botanically and transformed into more useful compounds for plant transport and food conversion [20]. The present study aimed to find a microbial technique for producing IBA and to test its ability to influence some characteristics of bougainvillea plant growth.

2. Materials and Methods
The ability of 3 isolates were tested. Azotobacter chroococcum, Bacillus megaterium and Pseudomonas putida on the production of indole compounds in medium peptone water 1% [21]. Sesbanin and soybean seed powder were also selected with a good content of the amino acid Tryptophan, whose metabolism from isolates leads to the formation of indole acid. Prepare dry powder of sesban seeds and soybeans. Pass each ingredient through a sieve with a 0.2 mm hole in diameter. Soak 350 g of each powder in 1750 ml distilled at 50°C, after a 24-hour soak, the solutions were filtered by centrifugation (3000 rpm), then the filter was sterilized through a filter whose aperture diameter was 0.45 mm. The filtrate was collected up to 1650 ml of each powder. The pH was adjusted to 7.23. From each filtrate, he distributed 50 ml / vials of 100 volumes, as well as 25 + 25 ml / vial from the two filtrates. Media were inoculated from 7 inoculation: A. chroococcum, B. megaterium, P. putida, (A. chroococcum + B. megaterium), (A. chroococcum + P. putida), (B. megaterium + P. putida) and (P. putida + B. megaterium + A. chroococcum). With All experiments in this study used a complete randomized design (CRD) with three replicates of the activated isolate in the broth medium at a rate of 1 ml / 50 ml medium, they were incubated in a shaking incubator at 120 revolutions / min at a temperature of 28 ± 2°C for 72 hours, and the amount of the produced indole compounds was calculated [21]. For the purpose of knowing the role of the product in germination and growth of cutting of Bougainvillea spectabilis L.

Collection of the most productive replicate filtrates of 182 mg L⁻¹ treatment from B. megaterium and A. chroococcum combined with a mixture of sesbanin and soybean seed powder. It was divided into two halves, the first was filtered through filters with a diameter of less than 420 micrometers to get rid of the isolates cells, and the other half the cells were left for isolates in it. He also attended a concentration of 300 and 600 micrograms l-1 industrial IBA in addition to the control treatment to be 5 treatments (control, IBA300, IBA600, BioIBA¹ and Bio IBA²). It was placed in liter bakers and dipped in each treatment 10 cuttings (20 cm length of cuttings) for an hour to a depth of 10 cm. The cuttings were prepared with 150 cuttings from Bougainvillea spectabilis L. on 20/12/2018. cutting contains 6 nodes and is 1 cm in diameter, used plastic boxes and put a plastic cover, and filled sandy...
soil with a depth of 20 cm, 15 planting boxes in each box of 10 cuttings to a depth of 12 cm and watered, then covered with a transparent plastic cover and placed in a place in the wooden canopy and monitored. Service operations were conducted for it and the germination percentage, number of leaves, leaf area, number of branches, average length of branches and flowering were calculated according to [22].

It also examined the ability of isolates used in the production of iron-related compounds Sidrophores and followed the method described by [23] with Chrome Azurel Sulfonate (CAS). The ability of isolates to dissolve phosphate compounds was also examined using pico-viscaya medium [24].

3. Results and Discussion

3.1. The role of inoculation isolates, sesbanin seed powder and soybeans in the production of indole compounds:

The test results of the used isolates showed their ability to produce indole compounds in 1% peptone water medium. The results showed that the medium prepared from a mixture of sesbanin seed powder and soybeans gave the highest yield in it, reaching 67.9 mg L\(^{-1}\), while the lowest yield was 30.88 mg L\(^{-1}\) from the medium of sesbanin seed powder. It was also found that the use of the inoculation consisting of a mixture of \(B.\ megaterium\) and \(A.\ Chroococcum\) together gave the highest production rate of 103.33 mg L\(^{-1}\), while the lowest production rate of bioindole compounds from inoculation use was 17.93 mg L\(^{-1}\). While the interaction factors achieved the highest yield of 182 mg L\(^{-1}\) from the treatment consisting of a mixture of \(B.\ megaterium\) and \(A.\ chroococcum\) together with a mixture of sesbanin and soybean seed powder, the lowest quantity produced was 12.8 mg L\(^{-1}\) from the treatment consisting of inoculation of \(P.\ putida\) with sesbanin seed powder medium.

Table 1. The role of inoculation of isolates, sesbanin seed powder and soybean in the production of indole compounds

| Isolates | Seeding powder | rate |
| --- | --- | --- |
| | Sesbania | Soyabean | Mixed |
| A. chroococcum | 32.8 | 54.6 | 78.5 | 55.30 |
| B. megaterium | 48.5 | 56.2 | 64.6 | 54.42 |
| P. putida | 12.8 | 18.6 | 22.4 | 17.93 |
| A. chroococcum; B. megaterium | 56.4 | 71.3 | 182.3 | 103.33 |
| A. chroococcum, P. putida | 16.6 | 20.8 | 25.4 | 20.93 |
| B. megaterium; P. putida | 20.5 | 30.2 | 33.6 | 28.10 |
| A. chroococcum, B. megaterium; P. putida | 28.6 | 41.4 | 68.5 | 46.16 |
| Rate | 30.88 | 41.86 | 67.9 |
| LSD p ≥ 0.05 | 1l = 6.54, S = 8.92, 1sS = 11.18 |

This confirms the importance of the substances associated with the amino acid tryptophan in these materials and the ability of bacteria to use them. Carbon sources enabled the used microorganisms to produce enzymes for metabolizing the amino acid Tryptophan. Tryptophan is an essential amino acid in the production of indole compounds, this amino acid is found in many seeds of leguminous plants [25], the use of such substances in the medium improves the production of indole compounds by bacteria, this is confirmed by [2] who found that the production of Azotobacter and Pseudomonas reached 32.2 mg L\(^{-1}\) when using broth medium enriched with powder from the seeds of the soybean plant.

3.2. The role of inoculation isolates by producing Siderophage compounds and dissolving phosphate compounds:

It was found that the selected isolates used in the production of indole compounds have the ability to produce iron-chelating compounds (Table 2) \(B.\ megaterium\). and \(A.\ chroococcum\), this confirms that it is able to produce iron-chelating compounds (+++, ++, ++), and the production of these compounds is important to increase the availability of some nutrients [26] whose unavailability is poor under conditions of neutral pH in neutral and alkaline soils. It shows the ability of isolates used to dissolve tri-calcium phosphate (TCaP) in the medium, the solubility of isolates varied according respectively B.
megaterium, P.putida and A. chroococcum with rate dissolution diameter of 10.43, 29.9 and 29.6 mm, respectively.

The apparent variation of isolates in adiameter soluble zone is due to their ability to produce organic acids, and the nature and type of the organic acid produced [27], and the presence of such organisms in the soil and increasing their effectiveness is an important matter due to their ability to dissolve phosphate compounds and make them ready for the plant.

Table 2. inoculation of isolates by producing Sidrofur and dissolving phosphate compounds

| Dissolution diameter m | A. chroococcum | B. megaterium | P. putida |
|------------------------|----------------|---------------|-----------|
| Siderophore compounds  | ++             | ++            | +++       |

3.3. Effect of bioindole compounds and industrial IBA on some germination and growth traits: cutting of Bougainvillea spectabilis L.

The results are shown in Table (3) and Pictures (1), there was a significant effect in the percentage of cutting, the highest rate was reached at 100% in the two treatments IBA600 and BioIBA-2, they were followed by BioIBA-1 treatment with the cutting ratio, it reached 92.6%, while it was 76.5% and 53.2% for each of the IBA300 and control transactions, respectively. As shown in Table 3, there is also a significant effect of the paper area rate, the three transactions, BioIBA1, BioIBA2, and IBA600 achieved the highest average paper surface averages of 47.74 45.75 and 45.83 Decm². The average area leaf decreased to 20.23 and 16.45 dm² for the IBA300 and control treatments, respectively.

Table (3) Effect of bioindole compounds and industrial IBA on some germination and growth traits cutting of Bougainvillea spectabilis L.

| BiBA × IBA | Germination rate (%) | area of leaves (dec²) | Number leaves | Branch length cm | Branch No/plant | Flower percentage (%) |
|------------|----------------------|-----------------------|---------------|------------------|-----------------|----------------------|
| 0 × 0      | 53.20                | 16.45                 | 11.60         | 12.57            | 1.267           | 0.0                  |
| IBA 300 mgL⁻¹ | 76.52                | 20.23                 | 15.47         | 18.80            | 2.212           | 0.0                  |
| IBA 600 mgL⁻¹ | 100                  | 45.83                 | 24.70         | 44.67            | 2.899           | 0.0                  |
| Bio-IBA2   | 92.64                | 45.75                 | 23.40         | 40.60            | 3.467           | 0.0                  |
| Bio-IBA1   | 100                  | 47.74                 | 28.37         | 49.83            | 3.800           | 30.23                |
| LSD p ≥ 0.05 | 6.64                | 3.844                 | 2.874         | 2.880            | 0.508           | 0.0                  |

Mean while, Bio-IBA1 achieved the highest significant average for the number of leaves with 28.37 leaves, followed by the IBA600 and BioIBA2 treatments with the average number of leaves reaching 24.70 and 23.40 sheets respectively. As for the number of branches of the plant, the two treatments were achieved by Bio-IBA1 and BioIBA2, the highest was 3,800 and 3,467 branches of plants, respectively, followed by the IBA600 and IBA300 treatments, averaging 2,899 and 2,212 plant branches, while the lowest mean was for the branches of 1.267 branches with the control treatment. Flower formation was seen before seedling transplantation in Bio-IBA1 treatment at a rate of 30.23% and no flowering was seen in other treatments.

The growth regulator IAA produced by the root ganglia bacteria increased the germination of Brassica Campestris, as the germination rate reached 72-75% compared to the control treatment, in which the germination rate was 54% after 15 days after planting [14]. Several References confirmed the importance of auxins compounds in increasing plant growth and activity and vehicle transformations and their transport within plant cells [15]; [16]; [20].
4. Conclusions
The possibility of using sesbanin and soybean seed powder to produce indole acetic acid through the biology role of bacterial isolates such as Azotobacter chroococcum, Pseudomonas putida and Bacillus megaterium. It was significant of the role for this compound was found in regulating plant growth by studying some of the growth characteristics studied.

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