IAA production by *Pseudomonas putida* associated with rhizosphere of some medicine plants.

Amal Abdul S. AL-Habib

1College of Energy and Environment Science, Al-Karkh University of Sciences, Baghdad, Iraq.
Email: dr.amal.a.salam@kus.edu.iq

Abstract

The present study was carried out in the greenhouse / department of Environmental science / college of Energy and Environment Science, Al-Karkh University of Science, Baghdad. During 2019-2020 under natural environmental conditions in greenhouse.

The aim of this study was to evaluate the activity of the rhizosphere soil of two medicine plants including, Watercress (*Eruca sativa*) and parsley (*Petroselinum crispum*) plants, to evaluate the effectiveness of the soil surrounding the roots of the tested plants in stimulating rooting growth. Red hibiscus, Alternanthera purple, chrysanthemum, Rosemary and garden mint were planted in rhizosphere soil of the two medicine plants. The results indicated that parsley rhizosphere soil gave the highest values in rooting and seeds germination for the test plants than the Watercress, the results indicated the presence of pseudomonas putida and IAA hormone in the roots rhizosphere soil of the tested plants.

Key word: rhizosphere soil, Watercress, parsley, pseudomonas putida, IAA.

1. Introduction

Plants is the primary producers, in synthesize amounts of organic compounds conditions. Some of the synthesized compounds are released into the rhizosphere, (Soil refers to the part of earth on which plant grow), which surrounding the roots [1,2]. These compounds is beneficial to the stimulation of biological activity in the rhizosphere [3], including stimulation of rhizosphere bacteria [4], which provide the plant with increased nutrient solubility, fixed nitrogen, and/or competitive suppression of pathogens [5], as well as plant growth promoting molecules [6,7]. Root exuded compounds can further change the properties of the surrounding soil and are important for obtaining nutrients, mediating biological interactions, or decreasing the toxicity of pollutants [8,9]. Plant exudates and decomposing litter contain secondary plant metabolites (SPMEs) among other compounds. Beyond their role in mediating plant–microbe interactions, it is hypothesized that SPMEs can stimulate microbial metabolism of pollutants present in the environment, which is termed the “secondary compound hypothesis [10-12]. Root exudates is a key factor and acting as attractants factors to stimulant plant growth [13], in increasing microbial abundance and activity in the rhizosphere compared with soil [14,15]. The exudation were varies substantially between different plant species, as do their microbial communities and their effects [16]. These exudate contains different compounds such as (sugars, vitamins, amino acids, nucleosides, and organic acids) and signals that attract microbial community [17]. Such compounds can serve as chemo attractants for rhizosphere bacteria at very low concentrations, one of the most important production (indole-3-acetic acid) (IAA) which important for plant growth promotion, root initiation and elongation [18].

The present study was undertaken to screen the plant growth promoter microorganisms PGPMs to investigate their effect on rooting of different types of stem cuttings and seeds germination. As alternative method in rooting. Instead of the higher costs and pollution chemical promoters.

2. Materials and methods

The experiments were conducted in the greenhouse / department of Environmental science / college of Energy and Environment Science, Al-Karkh University, Baghdad. During 2019-2020 under natural environmental conditions in greenhouse.

2.1. Planting seeds the medicinal plants
Seeds of Medicinal plants including Watercress (Eruca sativa) and parsley (Petroselinum crispum) plants, planted in 15 cm pots contain loamy sand soil with nine replications for each plant. After two months of planting, the plants were uprooted with rhizosphere soil, Part of this soil was tested for the presence of IAA.

2.2. planting the stem cuttings

The second parts were shifted to 15 cm pots to be planted with stem cuttings of tested plants (Red hibiscus, Alternanthera purple, chrysanthemum, Rosemary and garden mint). After one month of planting for these cuttings, the data of rooting parameters were taken, the parameters were the (rooting percentage, numbers and lengths of roots).

2.3. planting the seeds of tested plants

The third part of the rhizosphere soils, it was tested for its effect on seeds germination. Parsley and Barley seeds were selected for testing, by cultivation 20 seeds of each type in 9 cm pots contain rhizosphere soil of Watercress and parsley. The control treatment was loamy sand uncultivated soil, with three replications for each treatment.

2.4. Isolation of bacteria:

The rhizospher soil roots of each test plant was taken to isolate the rhizospheric bacteria. One gram from each sample was added to 9ml of D.W. and serially diluted with sterile distilled water up to 10-8 dilutions, then plated on nutrient agar (peptone 5.0g/l, sodium chloride 8.0g/l, beef extract 3.0g/l and agar 12.0g/l, pH 7.3±0.2) plates and incubated for 24 hours at 37 °C. The distinct morphologically colonies were selected then purified and stored in nutrient broth containing glycerol (15%) as described [17].

2.5. Detection of IAA by Salkowski reagent

The bacterial isolates were cultured in Luria-Bertani broth medium (2.1.8.1.b) and basal minimal salt medium (2.1.8.2) and incubated at 30°C for (4 – 7) days on rotary shaker. The cultures were centrifuged at 5000 rpm for 25 minutes and the IAA activity was measured in the supernatant.

Two ml of Salkowski reagent (2.1.10.5) was added to 1 ml of each concentration. The blank consisted of 1ml (LBT) and 2 ml Salkowski reagent [18]. All tubes were incubated in the dark at room temperature for 25 minutes. Two ml of Salkowski reagent was added to 1 ml of each concentration.

3. Results and Discussion

The results in table (1) indicated that there were significance differences between the treatments with control, in addition to that there was differences between different plants, in rooting percentage. The results showed also that the rhizosphere soil of Parsley plant gave the highest results in rooting percentage as show in table one. The lowest values gave by control rhizosphere soil.

|                  | Chrysanthemum | Red hibiscus | Alternanthera purple | Rosemary | garden mint |
|------------------| --------------|--------------|----------------------|----------|-------------|
| Control          | 26            | 0            | 40                   | 20       | 10          |
| Watercress       | 63            | 26           | 66                   | 50       | 26          |
| Parsley          | 76            | 50           | 90                   | 60       | 63          |
| L.S.D            | 3.1           | 1.18         | 3.7                  | 2.4      | 1.93        |

The results in table 2 showed that parsley treatment gave the highest results in rooting numbers than watercress treatments, with significance differences between treatments and control.

|                  | Chrysanthemum | Red hibiscus | Alternanthera purple | Rosemary | garden mint |
|------------------| --------------|--------------|----------------------|----------|-------------|
| Control          | 1.8           | 0            | 2.6                  | 1.9      | 3.0         |
| Watercress       | 3.0           | 2.6          | 4.3                  | 6.3      | 4.6         |
| Parsley          | 3.9           | 3.9          | 4.9                  | 5.6      | 5.9         |
| L.S.D            | 0.78          | 0.97         | 0.92                 | 1.32     | 1.29        |
Tables 3. The results also showed that Parsley rhizosphere gave the highest results in rooting lengths in all test plants. The lowest results obtained in control treatment.

Table 3. Effect of different plants rhizosphere soil on rooting length.

| Rooting percentage | Chrysanthemum | Red hibiscus | Alternanthera purple | Rosemary | garden mint |
|--------------------|---------------|--------------|----------------------|----------|-------------|
| Control            | 1.6           | 0            | 3.0                  | 1.3      | 3.0         |
| Watercress         | 2.3           | 1.6          | 5.3                  | 4.6      | 5.1         |
| Parsley            | 3.6           | 2.3          | 6.3                  | 5.9      | 5.6         |
| L.S.D              | 0.12          | 0.99         | 0.92                 | 1.11     | 1.03        |

The parsley aqueous extract showed the presence of IAA and two types of cytokinin (zeatin and kinetin) in addition to GA3 and ABA. In addition to antioxidant enzymes and many amino acids and phenolic compounds [19]. Plant roots exudate release a wide range of compounds which are sugars, polysaccharides, amino acids, aliphatic acids, aromatic acids, fatty acids, sterols, phenolics, enzymes, vitamins and other secondary metabolites and phytohormones such as auxin [20].

Auxin is the primary endogenous hormone which regulator the adventitious root induction, the formation of roots from non-root tissues [21]. Endogenous IAA levels increase temporarily during the induction phase , in the other hand simultaneously of the plant tissue increased sensitivity to auxin signals. IAA content has a direct correlation with rooting, appearing as the adventitious roots were breaking through the epidermis in cuttings. this can explain the better performance for rooting and roots growth of testing plants in rhizosphere of parsley plant. The tables 4, 5 and 6 indicated the effects of rhizosphere soil of parsley and watercress on speeding germination, seed germination percentage and root growth, the tables showed that parsley treatment was the highest In all required data (speeding germination, seeds germination percentage and root growth.

Table 4. Effect of different plants rhizosphere soil on speeding time germination in days.

| Treatments | Parsley | Barley |
|------------|---------|--------|
| Control    | 12      | 10     |
| Watercress | 7       | 8      |
| Parsley    | 5       | 6      |
| L.S.D      | 0.61    | 0.69   |

Table 5. Effect of different plants rhizosphere soil on seeds germination percentage.

| Treatments | Parsley | Barley |
|------------|---------|--------|
| Control    | 0       | 20     |
| Watercress | 40      | 50     |
| Parsley    | 55      | 60     |
| L.S.D      | 1.28    | 1.39   |

Table 6. Effect of different plants rhizosphere soil on roots length.

| Treatments | Parsley | Barley |
|------------|---------|--------|
| Control    | 0       | 0.8    |
| Watercress | 1.0     | 1.3    |
| Parsley    | 1.6     | 2.1    |
| L.S.D      | 0.26    | 0.34   |
4. Identification of bacteria.

The results indicated the presence of *Pseudomonas putida* bacteria in the rhizospheres soil of parsley and watercress. These bacteria are distinguished from a group of (PGPMs) plant growth promoting bacteria [22], which enhance plant rooting and growth [23]. The PGPMs, have many different mechanisms, such as biofertilizer which promote and increase the supply of nutrients, such as nitrogen and phosphate. The second function have ability to produce plant growth regulators such as auxins, gibberellins and cytokinins [24]. The *Pseudomonas* genus has a medical and biotechnological (biocontrol and biofertilizer) importance its aerobic genus. *Pseudomonas* genus is widely spread in the environment such as soil or water [25]. *Pseudomonas putida* which had Antifungal activity, and phosphate solubilization, in addition to produce IAA, the bacterial IAA increases root surface area and length, so provides the plant with soil nutrients. These bacteria loosen plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria [26].

Indole-3-acetic acid (IAA) is the most abundant endogenous auxin which has roles in stem elongation and root growth. The auxin level is usually higher in the rhizosphere, where high percentage of rhizosphere bacteria is likely to synthesize auxin as secondary metabolites because of the rich supplies of root exudates. The production of auxin (IAA) stimulate lateral roots that increase nutrient absorbing surfaces and results in better assimilation of water and nutrients from the soil. As a result the plant will increased the shoot and root length [27].

5- Detection of IAA in rhizosphere of the test plants

The detection of IAA, indicated the presence of IAA in the two broth rhizosphere, (Parsley and Watercress), because of the formation of characteristic pink to red or bulbous color in the medium, this is because of the formation of chromatic complex between IAA produced by bacteria and Salkowski reagent as the IAA is a carboxylic acid in which the carbonyl group bounded with methyl group by C-3 in the indole ring [26]. But in parsley The color intensity was more than watercress, that mean the IAA concentration in parsley rhizosphere is more than in the rhizosphere of watercress.

![Figure 1.](image1.png)

*Figure 1.* represents the colony of *P.putida* in Parsley and watercress rhizosphere.

The superiority in percentage of seed germination in the rhizosphere soil of parsley plant may be due to the presence of compounds stimulating the seeds germination in the root exudate of the parsley plant, also may be to the presence of some hormones like (GAs) gibberellic acid that stimulate the germination of seeds, in rhizosphere of parsley plant.

Conclusion

From this study it is clear that rhizosphere soil of some medicine plants can provide a source of IAA producing bacteria such as parsley plant. It is concluded that presence of such growth promoting in the field also cheap and prevent environmental pollution by avoiding excessive applications of industrially chemical rooting and growth promoter to cultivated fields.
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