Evaluation of The Biological Response of Neem (Azadirachta Indica) Spraying With Proline, and Addition of Mycorrhizal Fungi

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Abstract. This study was conducted using plastic pots of 15 kg pot capacity for the 2019 agricultural season in a private nursery in Ramadi city / Al-Anbar governorate, with the aim of studying the effect of nano-organic fertilizer and proline acid on some vegetative growth characteristics of Neem trees (Azadirachta indica). Field experiment included three proline concentrations were (0, 50 and 100) mg.L⁻¹ and three concentrations levels of Mycorrhizae were 0, 2 and 4 g per pot contains 15 kg of soil. Data averages were compared by using the Lowest Significant Difference (L.S.D) at a probability level of 0.05. The overlap between the study factors led to a clear increase in all the studied traits and the trees that were treated with the study factors outperformed them.

1. Introduction:
A medicinal plant is defined as a plant that contains in one or more parts of a chemical substance, one or more concentrations of less or more that can treat one or more specific diseases, or use to reduce the symptoms of infection if relying on this plant part in its natural form, or by the active substance extracted from it[1]. Historically medicinal plants have been shown to be an important source of molecules for therapeutic uses , and which remain an important and major group for identifying pharmaceuticals [2]. They are easy to handle and somewhat safe, so all eyes are turning towards their use at the moment. Common neem, Indian neem, Indian azdrecht, or neem tree (scientific name: Azadirachta indica) is a tree (a species) that follows the genus Neem of the family Azadrecht. In India it is called (the village pharmacy) [3], as it is a tree known there for a very long time. It is characterized by its ability to purify the soil from salts. It is a perennial tree whose age reaches 180 years, and its life in the forest may extend to 200 years. Neem is a fast-growing, dense, evergreen tree that grows heavily and represents a major greenery in the forest [4]. Its height reaches 16 meters and sometimes 25 meters, and its total vegetative diameter reaches ten meters. It is one of the woody trees that is distinguished by its large root size, as its roots extend to large areas transversely and not in the depth; Therefore, when planting neem trees, distances must be left between each tree and tree of at least 3 meters. The tree has a stiff, dark brown trunk, with a diameter of between 75 and 150 cm, and has a cracked brown rind [5]. The leaves are gathered at the ends of the twigs, the length of the leaf reaches 30 cm. Its leaves are composed of up to seventeen opposite leaflets. The inflorescence side is multifloral, reaching a length of
20 cm. The flower is white fragrant, and the fruit is elongated, up to one centimeter long, and it is green and turns yellow upon ripening. One-seed and sweet pulp is eaten [6].

Many studies have indicated the positive role of proline acid in improving the growth and yield characteristics of the plant [7]. A study has shown that foliar spraying with proline acid depends on the type and variety of the plant, the time of addition and the appropriate concentration [8], as it has a role in improving the growth and yield characteristics. Also, a study showed that proline acid had a significant effect on vegetative growth characteristics, as it increased plant height, leaf area and dry weight of the vegetative growth when spraying with different concentrations, especially at a concentration of 20 mg per liter under different levels of stress [9]. Based on the above, this research was carried out with the aim of studying the effect of the interaction of different concentrations of Proline with different levels of Mycorrhizal fungi and the effect of that on the growth of the neem tree, knowing the best concentration of proline, adding Mycorrhizal fungi and studying its effect on the growth and development of the neem tree.

2. Materials and Methods
This experiment was conducted in the Department of Horticulture and Gardening Engineering / College of Agriculture / University of Anbar for the 2019 season. The study influenced the following factors: - Three concentrations of Proline acid 0, 50 and 100 mg.L^{-1}; and Mycorrhizal fungi at 0, 2 and 4 g per pot. Some chemical and physical properties of the soil used in the study were estimate Neem seeds were planted on 2/2/2019 for the spring season at a rate of 2-3 seeds per pot, then the plants were reduced to one plant at two weeks after Seedling emergence. Also, for each experimental unit, 46% N urea fertilizer was added at a rate of 6.3 g. a pot was added in two equal batches, the first was after emergence of fourth real leaves, the length of the leaf is 4 cm, the second addition was after one month from the first addition, and the triple superphosphate fertilizer 46% P_{2}O_{5} was added at a rate of 2.2 g. a pot and potassium sulfate fertilizer 41% K at a rate of 2 g. for potassium fertilizer was adding and mixed with soil before planting. Also, a solution Stok was prepared from proline acid at a weight of 1 g and dissolved in 1000 ml of distilled water to obtain a base solution at a concentration of 1000 ppm. Liters, then prepare the required concentrations of 50 and 100 mg. L according to the dilution law spraying was carried out early in the morning from sowing seeds evenly until complete wetness, according to the concentrations prepared in advance for 45 days. Control agents were sprayed with distilled water. The rest of the soil and crop service operations were carried out during the growing season as needed.

2.1 The vaccine used in the study: The Mycorrhizae Fungus (VAM) vaccine was used in this study which consisted of (spores + infected roots + dry soil) where spores of the fungus *Glomus mosseae* were obtained from the Agricultural Research Department in the field experiment, and the vaccine / Ministry of Science and Technology / Saffron for use as a bacterial inoculum (*Rhizobium leguminosarum*), where the rhizobium vaccine was isolated from the collection of plant roots of Maize.

The indicators of vegetative growth were measured at the flowering stage, and the data for the studied traits were recorded as follows:

1. *Plant height, cm*: Measured from the soil surface with a graduated measuring ruler up to the base of the disc for each experimental unit.
2. *Leaves number per plant*: The total leaves of each plant were calculated from the first green leaf near the soil surface to the top leaf.
3. *Leaf thickness (m)*
3. Result and Decision:

3.1 Plant high:
The results in Table 1 indicate the effect of proline spraying and the addition of the ground of mycorrhizae to an increase in the rate of rise, highest increase (88.04 cm) was recorded in treatment of high proline (100 mg) with a significant increase, while a lower rate of increase was obtained in the treatment Without adding. Adding the mycorrhizal fungus at average of 4 g per pot led to a significant increase in plant height. A significant high rate of plant height was recorded upon the interaction between the two experiment factors. Where a high and significant rate was recorded upon the interaction (100 mg proline with 4 grams of Mycorrhiza) Which recorded an increase rate was (98.50 cm). Proline is one of the most important biochemical contents affected in plants under conditions of salt or water stress. Proline is the amino acid content, which has a close relationship in the mechanism of plant resistance to stress conditions [10]. Among the important vital functions that the amino acid proline accumulates under stress conditions is several, the most important of which are. The function of proline in cells subjected to salt stress is to control osmotic pressure, a store of carbon and nitrogen needed to grow under stress and to prevent ammonia poisoning [10]. It has been proven that the Mycorrhizae fungus increases the effectiveness of fertilizer use by increasing the absorption of mineral elements from the soil, especially with little movement in the soil, so this fungus can be used as a biological fertilizer and reduces the use of fertilizers (up to 50%) [11].

| Mycorrhizae fungus (gm) | proline concentrations (mg) | Mean |
|------------------------|-----------------------------|------|
|                        | 0                           | 50   | 100  |
| 0                      | 45.30                       | 64.50| 88.04| 65.83|
| 2                      | 48.50                       | 71.40| 89.50| 69.57|
| 4                      | 55.20                       | 81.40| 98.50| 78.23|
| LSD (0.05)             |                             |      |      |
| Proline = 3.7          |                             |      |      |
| Mycorrhizae fungus = 3.7|                             |      |      |
| Proline × Mycorrhizae fungus = 6.5 |                   |      |      |

3.2 Leaves number per plant:
Results showed in Table 2 that there was a clear increase in the number of plant leaves in the trees that were treated with Proline and Mycorrhizae. where high rates of the number of leaves were recorded when spraying plants with propylene 100 mg, with a clear difference from the rest of the other treatments, as the highest rate reached (712 leaflets), while the lowest rates were in the no-add treatments. Likewise, in the case of adding Mycorrhizae to the pots, high rates of leaflet number were recorded at the highest addition of Mycorrhiza (4 grams), as it gave the Leaves where given (675 leaflets per plant), this increase was significant compared to the rest of the other treatments. The lowest average number of leaflets was recorded in control. In the case of overlapping experiment workers, the highest average number of leaflets was recorded when the combination.

Proline accumulation is due to disturbance in the catabolism of amino acids related to protein breakdown and aging resulting from salinity [12]. Accumulation of glutamate and ammonia in plants subjected to salt stress leads to induction of proline formation by direct or indirect effect of building material / material ratio. Another reason for the accumulation of proline in plants is the lack of activity of both proline & dehydrogenase. Proline oxidase some scientists have pointed to the importance of soluble carbohydrates in inducing proline
accumulation by inhibiting them Proline catabolic enzymes leading to increased proline building and accumulation [13].

Mycorrhizae fungus helps to improve water absorption from the soil and reduce water stress problems for plants. The formation of Mycorrhizae fungi of a large network of fungal strings outside the roots in the soil helps the plant to reach a greater amount of water relatively far from the root and as a result, the water relations in the plant improve. Which leads to improving plant tolerance of environmental stress factors such as drought, heat, salinity, etc [14].

Table (2) Effect of Proline concentrations (ppm) and Mycorrhizae fungus ( g per pot)on the Leaves number per plant

| Mycorrhizae fungus (gm) | Proline concentrations (mg) | Mean |
|-------------------------|-----------------------------|------|
| 0                       | 0, 100, 200                 |      |
| 2                       | 682, 740, 712               | 732  |
| 4                       | 709, 741, 763               | 761  |

LSD (0.05) Mycorrhizae fungus = 5.3
Proline = 5.3
Mycorrhizae fungus × proline = 9.8

3.3 Leaf thickness (mm).

The results in Table 3 indicate the effect of proline spraying and the addition of the ground of mycorrhizae to an increase in the rate of rise, as a rate of increase of 0.720mm was recorded in the first treatment of high proline (100 mg) with a significant increase, while a lower rate of increase was obtained in the treatment Without adding.

Likewise, in the case of adding Mycorrhizae to the pots, high rates of the number of Leaves were recorded at the highest addition of Mycorrhiza (4 grams), as it gave the Leaf thickness per plant 0.470, this increase was significant compared to the rest of the other transactions. The lowest average Leaflets thickness was recorded in Control treatment. In the case of overlapping experiment workers, the highest average Leaflets thickness was recorded when the combination.

Like other amino acids, it forms part of proteins important to cells. Proline is the amino acid that the largest numbers of studies interested in overcoming all types of stress are focused on: salinity, dehydration, heavy metals, high and low temperatures, hypoxia, air pollution, oxidative stress, pathogenic infection or UV rays. In addition to its role in stress tolerance, its signaling activity in plant development has also been demonstrated [15].

Mycorrhizae fungus increases water efficiency improving the ability of plants to absorb water from the soil and thus increasing water use efficiency. Where research has shown that the use of the microscopic technique saves the use of water in irrigation by 10-30%. Mycorrhizae fungus helps protect plants from diseases and pests. Research results have indicated that the Mycorrhizae sometimes secrete antibiotics that kill pathogens. The formulas also act as a barrier against root diseases.
4. Conclusion
Spraying the plant with proline at the level of 100 milligrams resulted in good and promising results in most of the studied growth treatments had a good effect on the development and growth of the neem tree. This indicates that Proline and mycorrhizae have significant effects on plant growth and development.

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