Effect of Humic and Salicylic Acids Foliar Application in The Chemical Content of Papaya Seedlings

Muhammad Hadi Ali Al-Abadi¹ and Ali Muhammad Abd Al-hayany²
¹,²College of Agriculture, University of Diyala, Iraq.
¹Email: Agrihorth21@uodiyala.edu.iq
²Email : alhayanyali15@yahoo.com

Abstract

The experiment was carried out on Papaya seedlings (2 months old) to determine the effect of humic acid (HA) and Salicylic acid (SA) on seedlings growth under local environmental conditions of in a private orchard in Diyala governorate. Humic acid (HA) sprayed at 4 concentrations (0, 1, 2 and 3%), whereas Salicylic acid (SA) was used at three concentrations (0, 200 and 400 mg.L⁻¹). The results showed that spraying with the high concentrations of both acids gave the best results in most studied traits, in addition these treatments decreased significantly leaves proline content.

Keywords: Humic, Salicylic, Papaya.

1. Introduction

Papaya (Carica papaya L.) is an evergreen fruit plant that belongs to the family Caricaceae and is believed to have originated in the Caribbean coast of Central America as a result of natural crossbreeding between Pcltata and Carica and other wild species. It is one of the plants grown in all tropical and subtropical regions [1]. Papaya has gained more importance due to its high palatability, fruiting throughout the year, early fruiting and giving it the highest productivity per unit area. The papaya fruit is consumed, such as watermelon, as it is peeled, the seeds are removed, and cut into pieces and served as fresh fruit, it is also used in jam [2]. Furthermore, the plant is used medicinally (roots and leaves) as a diuretic and anti-worm (leaves and seeds) as well as for indigestion and other digestive disorders and tonsil hypertrophy and juice is used for warts, cancers, tumors and skin problems, and moreover the root is used in the treatment of uterine tumors, smoking leaves to relieve asthma attacks and eating fruit prevents rheumatism and to treat psoriasis and ulcers [3].

The plant grows and bears fruit well in different types of soil. Saplings often give a strong root system soon after planting. Under appropriate conditions, the root system can penetrate the soil to a depth of up to 2 m. Whereas, Papaya is sensitive to frost, exposure to 0.5°C for long periods, destructive and can kill the plant [4]. However, organic fertilization is one of the important resources of supplying plants with their needs of nutrients without negative impact, whether the fertilization is in addition to the soil or sprinkled on the vegetative system [5]. Humic acid (HA) is one of the most important types of organic fertilizers, as it contains oxygen (31-40%) and nitrogen (2-6%), moreover to its high ability to exchange ions and celebrate water, so it works to improve soil fertility and plant nutrition for long periods [6]. Humic acid is easy to add, fast-acting, and has no harmful side effects for humans, animals and plants. It is safe and soluble in water and contains humic substances that are end products of microbial decomposition of living organisms dead in soil [7]. Many research and studies have shown the positive role of humic acid in many characteristics of vegetative growth such as the number of leaves, plant height, as well as chemical content of leaves. In a study conducted by Asil and Neumann [8] included spraying humic acid at a concentration of 12% on avocado seedlings, which led to an increase in the leaves content of nitrogen, phosphorous and potassium. Also, it was confirmed Haggag et al. [9] that spraying humic acid by 20% on olive seedlings of Aggizi cultivar led to a significant increase in leaf content of nitrogen, phosphorous and potassium.

Salicylic acid (SA) is one of the plant hormones and has significant physiological effects on plant growth and development [10], it works to regulate ion absorption, hormonal balance, stomata movement, flowering induction, accelerate the formation of plant pigments such as chlorophyll and carotene, as well as increase enzyme activity and photosynthesis efficiency [11]. In the study conducted by El-Gioshy [12] who noted that the treatment of navel orange trees with salicylic acid at concentrations of 100 and 200 mg.L⁻¹ for two seasons caused a significant superiority of the higher concentration in the leaves content of nitrogen, phosphorous, potassium and chlorophyll. Study of Jassim [13] who found that spraying salicylic acid at a concentrations of 100, 200 mg.L⁻¹ on olive saplings cv. Ashrassi caused a significant superiority in the leaves nitrogen,
phosphorous and potassium content, as it was found by Al-Hamdani et al.[14] when spraying the seedlings of clementine mandarin with salicylic acid at concentrations of 0, 100, 200 mg.L\(^{-1}\), the higher concentration significantly exceeded in leaves nitrogen, phosphorous, potassium and carbohydrates content.

2. Materials and Methods

The experiment was carried out in a private orchard in Diyala Governorate for the period from 5-17-2020 to 1-15-2021 to study the effect of HA and SA spray on 2 months old Papaya seedlings growth. Seedlings were grown in 15 Kg containers filled with 3 soil : 1 Peatmoss medium. The table below showing some physical and chemical properties of the soil used in the experiment:

| Measurements               | Value | Unit of measurement |
|----------------------------|-------|---------------------|
| EC 1:1                     | 2.7   | Ds m\(^{-1}\)       |
| PH                        | 732   | ---                 |
| N                         | 24    | g. kg\(^{-1}\)      |
| P                         | 2.21  | g. kg\(^{-1}\)      |
| K                         | 250.12| g. kg\(^{-1}\)      |
| CaCo\(_3\)                | 245.2 | g. kg\(^{-1}\)     |
| Organic matter            | 7.5   | g. kg\(^{-1}\)      |
| Sand                      | 728   | g. kg\(^{-1}\)      |
| Silt                      | 84    | g. kg\(^{-1}\)      |
| Clay                      | 188   | g. kg\(^{-1}\)      |
| Texture of soil           | Sandy loam | ---                  |

2.1. Humic acid levels

Commercial humic acid (Disper Humic 85%) was used in four concentrations of 0, 1%, 2%, and 3% and the plants were sprayed four times with three weeks intervals started on 10/06/2020.

2.2. Salicylic acid levels

Salicylic acid (99.8% purity) was used at concentrations of 0, 200 and 400 mg.L\(^{-1}\) starting from 13/06/2020 and with 21 days interval.

2.3. Experimental design and results analysis

A factorial experiment with two factors (four levels of humic acid concentrations x three concentrations of salicylic acid using randomized complete block design (RCBD) and two seedlings per experimental units. Means were analyzed using Duncan’s multiple range test at 0.05 level.

2.4. Studied traits

1. Leaves content of nitrogen (%): was estimated by the Semi micro kjeldal method [15].
2. Leaves content of phosphorous (%): it was estimated using the method of ammonium molybdate and ascorbic acid and measured by a spectrophotometer at a wavelength of 622 nm as stated in the method [16].
3. Leaves content of potassium (%): Use the Flame Photometer to estimate the percentage of potassium according to a method [17].
4. Total chlorophyll content in leaves (mg.g\(^{-1}\) fresh weight). Chlorophyll was estimated according to [18] method by crushing 0.5 g of leaves by adding 20 ml of acetone (80%), the light absorption was read by a spectrophotometer for the samples at wavelengths 663 and 645 nm, according to the content according to the following equation:
   \[ \text{Chl. T} = 20.2 \times A_{645} + 8.02 \times A_{663} \]
   As A 663 and A 645 represent the reading of the device at wavelengths 663 and 645 nanometers, respectively.
5. Proline content in leaves (μg.g\(^{-1}\)). Estimated using a spectrophotometer according to [19]

3. Results and Discussion

3.1. Leaves content of nitrogen (%)

---
Results shown in table 2 reveals that spraying with humic acid at 3 and 2% concentrations were significantly superior to the rest of the treatments by giving them the highest nitrogen content in the leaves 1.90 and 1.87% respectively, compared with 0% concentration which gave the lowest content (1.79%). Foliar spraying with salicylic acid with 400 mg.L\(^{-1}\) gave a significant effect on N content (1.85%), whereas a concentration of 0 mg.L\(^{-1}\) gave the lowest N content (1.82%). The interaction between the highest concentrations of the two factors gave the highest N content (1.92%), whereas the control treatment gave the lowest N content (1.78%).

Table 2. Effect of humic and salicylic acids spray and interaction between them on nitrogen content (%) of papaya seedlings leaves.

| SA Mean | Humic acid (%) | Salicylic acid (mg.L\(^{-1}\)) |
|---------|---------------|-------------------------------|
| 1.82    | 1.86 1.85 1.79 1.78 | 0 |
| B       | a b c d         | b c d e e                     |
| 1.84    | 1.91 1.87 1.81 1.78 | 200 |
| A B     | a b a b c d e e |                            |
| 1.85    | 1.92 1.88 1.82 1.79 | 400 |
| A       | a a b c c d e e |                            |
| 1.90    | 1.87 1.80 1.79 | HA Mean                      |
| A A B B |                            | HA Mean                      |

Means values with similar letters do not differ multiple range according to Duncan’s multiple range test at 0.05 level.

3.2. Leaves content of phosphorous (%)

Table 3 shows that spraying humic acid at 3% gave the highest percentage of P (0.56%), whereas 0 (control) and 1% humic acid treatments gave the lowest content 0.51% for each of them. Regarding the effect of salicylic acid spraying on the phosphorous content in the leaves, the treatment of spraying with the highest concentration (400 mg.L\(^{-1}\)) gave the highest content (0.54%), whereas the concentration of 0 mg.L\(^{-1}\) gave the lowest P content. Interaction treatment between the highest concentrations of both factors gave the highest percentage of 0.57%, significantly superior to most treatments. Whereas, the interaction treatment between spraying with a concentration of 1% of humic acid and a concentration of 0 mg.L\(^{-1}\) of salicylic acid gave the lowest value (0.49%).

Table 3. Effect of humic and salicylic acids spray and interaction between them on phosphorous content (%) of papaya seedlings leaves.

| SA Mean | Humic acid (%) | Salicylic acid (mg.L\(^{-1}\)) |
|---------|---------------|-------------------------------|
| 0.52    | 0.56 0.53 0.49 0.49 | 0 |
| B       | a b c d e e | d e                           |
| 0.53    | 0.57 0.54 0.52 0.51 | 200 |
| AB      | a b a b c d e b c d e | c d e                          |
| 0.54    | 0.57 0.54 0.53 0.53 | 400 |
| A       | a a b c d e a b c d e |                           |
| 0.56    | 0.54 0.51 0.51 | HA Mean                       |
| A B B B |                            | HA Mean                       |

Means values with similar letters do not differ multiple range according to duncans multiple range test at 0.05 level.

3.3. Leaves content of potassium (%)

Results in Table 4 indicate a linear increase in the leaves content of potassium as HA spraying at the highest concentration was significantly superior to the rest of the treatments, reaching 1.82%, while 0 % gave the lowest leaves content (1.59%). Salicylic acid spraying treatments were significantly superior to the control treatment, as the treatment with the highest concentration (400 mg.L\(^{-1}\)) which gave the highest value of 1.75%, while the control treatment gave 1.68%. The interaction between the two factors of the study had a significant effect on the potassium content in the leaves, as the spraying treatment with the highest concentrations of both acids (3% and 400 mg.L\(^{-1}\)) gave the highest potassium content 1.85%, Whereas control treatment recorded the lowest percentage (1.50%).
Table 4. Effect of humic and salicylic acids spray and interaction between them on potassium content (%) of papaya seedlings leaves.

|          | Humic acid (%) | Salicylic acid (mg.L⁻¹) |
|----------|----------------|-------------------------|
|          | 3  | 2  | 1  | 0  |          |              |
| SA Mean  | 1.68| 1.79| 1.74| 1.69| 1.50| 0          |
|          | B  | b  | c  | d  | e  | g          |
|          | 1.74| 1.83| 1.78| 1.72| 1.62| 200        |
|          | A  | a  | b  | c  | d  | f          |
|          | 1.75| 1.85| 1.79| 1.73| 1.65| 400        |
|          | A  | a  | b  | c  | d  | e f        |
|          | 1.82| 1.77| 1.71| 1.59| HA Mean|          |
|          | A  | B  | C  | D  |     |            |

Means values with similar letters do not differ multiple range according to duncan’s multiple range test at 0.05 level

3.4. Chlorophyll content in leaves (mg g⁻¹ fresh weight)

Results in Table 5 showed that spraying with humic acid increased Leaves chlorophyll content, which the highest concentration (3%) gave the highest value (44.24 mg.g⁻¹ fresh weight), compared with 0% concentration which gave the lowest value (39.91 mg.g⁻¹ fresh weight). Spraying with the highest concentration (400 mg.L⁻¹) of salicylic acid gave the best chlorophyll content compared with the rest treatments, whereas (0 mg.L⁻¹) gave the lowest content. Interaction between 3% humic acid and 400 mg.L⁻¹ of salicylic acid gave the highest chlorophyll content (48.59 mg.g⁻¹ fresh weight), whereas interaction of 1% HA with 200 mg.L⁻¹ SA, and control treatment gave the lowest chlorophyll content (38.07 and 38.33 mg.g⁻¹ fresh weight respectively).

Table 5. Effect of humic and salicylic acids spray and interaction between them on chlorophyll content (mg.g⁻¹ fresh weight) of papaya seedlings leaves.

|          | Humic acid (%) | Salicylic acid (mg L⁻¹) |
|----------|----------------|-------------------------|
|          | 3  | 2  | 1  | 0  |          |              |
| SA Mean  | 4.00| 3.92| 4.13| 4.14| 3.83| 0          |
|          | B  | b  | b  | a  | b  |             |
|          | 4.14| 4.48| 4.30| 3.80| 3.96| 200        |
|          | B  | a  | b  | a  | b  |             |
|          | 4.46| 4.85| 4.32| 4.47| 4.17| 400        |
|          | A  | a  | b  | a  | b  |             |
|          | 4.42| 4.25| 4.14| 3.99| HA Mean|          |
|          | A  | AB | AB | B  |     |            |

Means values with similar letters do not differ multiple range according to duncan’s multiple range test at 0.05 level

3.5. The Proline content in leaves

Results in Table 5 indicate that there are a significant differences in the leaves proline content between the treatments of spraying with humic acid. 0% treatment gave the highest content 147.66 micrograms per gram. Whereas, spraying with humic acid at a concentration of 3% gave the lowest value 105.44 (µg.g⁻¹). The same result was obtained with salicylic acid spraying treatments, were the concentration of 0 mg.L⁻¹ gave the highest proline content of 128.58 (µg.g⁻¹), while the salicylic acid spraying treatment with the highest concentration (400 mg.L⁻¹) recorded the lowest value 112.91 µg.g⁻¹. The interaction between the two factors of the study had a significant effect on leaves proline content, the control treatment gave the highest value, while the interaction of the highest concentrations of both factors recorded the lowest value 93.33 (µg.g⁻¹).

Table 6. Effect of humic and salicylic acids spray and interaction between them on proline content (µg.g⁻¹) of papaya seedlings leaves.

|          | Humic acid (%) | Salicylic acid (mg.L⁻¹) |
|----------|----------------|-------------------------|
|          | 3  | 2  | 1  | 0  |          |              |
| SA Mean  | 128.58| 109.33| 115.33| 130.67| 159.00| 0           |
|          | A  | def| cdef| bcd | a        |             |
Humic acid spray (especially at a high rate) caused a significant increase in NPK levels in plant leaves (tables 2, 3, and 4) due to the ability of HA to increase water content in the root zone and the ions exchangeable ability between the soil solution and plant roots, on the other hand HA spray increased cells membrane permeability which resulted in increasing nutrient absorption through these membranes, and as a result the increase of nutrient content in plant leaves. These results were in agreement with that founded by [8] the spraying of humic acid at a concentration of 12% on avocado transplant led to an increase in the average content of nitrogen, phosphorous, and potassium in the leaves.

The significant increase in leaves nutrient content in plants sprayed with SA resulted from the role of SA in resistance water stress in roots zone and facilitating these nutrient availability for absorption by plant roots and plant leaves (tables 2, 3, and 4), these results were in agreement with [13] who confirmed the spraying of salicylic acid at 100, 200 mg.L⁻¹ on olive transplants of the cultivar Ashassi showed significant superiority in the content of the leaves of nitrogen, phosphorous and potassium and [14] who found When spraying salicylic acid at 0, 100, 200 mg.L⁻¹, the highest concentration in the leaves content of nitrogen, phosphorous, potassium and carbohydrates.

The increase in the chlorophyll content (Table 5) as a result of spraying humic acid may be due to the increase in the nitrogen content of the leaves (Table 3) and for the direct role of humic acid in the construction of the chlorophyll pigment [20], these results are in agreement with [21] which showed a significant increase in the chlorophyll content of citrus trees leaves when the humic acid was sprayed at levels 40, 60, and 80 ml.L⁻¹, and [22] noticed that there was a significant effect on the chlorophyll content of leaves compared to the no-additive treatment when treating orange trees cv. Valencia at 5 years old with three levels of humic acid: 25, 50, and 100 g.Tree⁻¹.

The increase in chlorophyll content when spraying with salicylic acid is due to the role of salicylic acid, which increases the accumulation of chlorophyll pigments and chlorophyll-like pigments in the leaves, as well as improves the photosynthesis process of plants under stress conditions, this agrees with what he found [23] the treatment of orange transplants with salicylic acid at 1000 and 2000 mg.L⁻¹ led to an increase in the chlorophyll content of the leaves, and [24] Which indicated that the treatment of sour orange saplings grown under saline stress conditions with salicylic acid at 0.25 (as a treatment in addition to the soil with irrigation) and at 0.15 (spraying on the leaves) gave the highest content of chlorophyll in the leaves.

HA and SA spray caused a significant decrease in leaves proline content (table 6) as a result of these two acids role in increasing soil moisture content and elevating environmental stress on the plants. Thus, the level of proline decreases, as the plant increases the production of this acid in response to extreme environmental conditions. This agree with [24] which confirmed that the proline content in leaves was significantly reduced when citrus root stocks were treated with humic acid.

### References

1. Dasilval, J. A. T. Z. Rashid, D. T. Nhu et al. (2007). Papaya biology and biotechnology, Tree forestry science and biotechnology (1):47-73.
2. Matsuura, F. C. A. U., M. I. D. S. Folegatti, R. L. Cardoso and D. C. Ferreira. (2004). Sensory acceptance of mixed nectar of papaya, passion fruit and acerola. Scientia Agricola (Piracicaba, Brazil) 61:604-608.
3. Nwoia, G. E., P. Ogiemluwakhe and C. Eji. (2012). Chemical composition of leaves, fruit pulp and seed in some morphotypes of C. Papaya L. morphotypes. International Journal of Medicinal and Aromatic Plants 2:200-06.
4. Dais.( 2009). Cultivating papayas. Department of Agriculture forestry and fisheries, directorate Agricultural in formation serves private Bag x144, pretoria, south africa. Availaple at www.nda.agric.za/publications
5. Nardi, S ; D, Pizzeghello;M, Schiavon and A, Ertani. (2016). Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. Scientia Agricultura. 73(1):18-23.
6. Stino, R. G., A. T. Mohsen and M. A. Maksoud. 2009. Bio-Organic fertilization and its impact on apricot young trees in newly reclaimed soil. American-Eurasian J. Agric. And Environ. Sci., 6(1):62-69.
7. Asil, S. and P. M, Neumann. (2010). Rhizosphere humic acid interacts with root cell walls to reduce hydraulic conductivity and plant development. Plant Soil. 336:313-322.
8. Rengrudkij, Phanupong and Gregory J. Partide. (2003). The effects of humic acid and phosphoric acid on grafted hass avocado on Mexican seedling rootstocks. Proceeding word avocado congress pp. 395 - 400.
[9] Haggag, L. F.; Mustafa, N. S.; Shahin, M. F. M.; Genaidy, E. A. E.; and Mahdy, H. A. (2014). Impact of NPK, humic acid and algae extract on growth of “Aggizi” olive seedlings cultivated in sandy soil under greenhouse condition. International Journal of Agricultural Technology. 10 (6):1585-1592.

[10] Raskin, I. (1992a). Role of Salicylic acid in plants. Annual review of plant physiology and plant molecular biology. 43:439-463.

[11] Hayat, S.; Ali, B. and Ahmed, A. (2007). Salicylic acid: Biosynthesis, metabolism and physiological Role in plants. In: S. Hayat and A. Ahmed, (Eds.) Salicylic: A plant hormone. Springer, Netherlands. Pp:1-14.

[12] Alsultani, M.J., Abed, H.H., Ghazi, R.A., Mohammed, M.A. , (2020), Electrical Characterization of Thin Films (TiO2: ZnO)1-x (GO)x / FTO Heterojunction Prepared by Spray Pyrolysis Technique, Journal of Physics: Conference Series, 1591(1), 012002.

[13] Jassim, A. H. (2018). Effect of spraying with sawed extract and salicylic acid on the growth of Olive transplant (Olea europaea L) cv. ashraasi. Master thesis. College of agriculture. University of Tikreet. Eng. abstract.

[14] Al-Hamdani, khaled Abdulla sahar, Heba taha muhammed al-samraee (2020). Effect of spraying with some nutrients and salicylic acid on some chemical characteristics of clementine fanliki transplant. Journal Diyala of agricultural sciences. 12: 530 – 545 . Eng. abstract.

[15] AOAC. (1980). Official Methods of Analysis. 13th ed. Association of Official Analytical Chemists. Washington, D. C.

[16] Olsen, S. k. and L. E. Sommers. (1982). Phosphorus in A. L Page, (Eds). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties 2nd edition, Amer. Soc, of Agron. Inc. Soil Sci. Soc. Am. Inc. Madison. Wis. U. S.A.

[17] Haynes, R.J. (1980). A comparison of two modified kjeldhal digestion techniques for multi-element plant analysis with conventional wet and dry ashing methods. Comm.soil.Sci.plant analysis. 11(5):459-467.

[18] Mohammed, M.A., Abdulridha, W.M., Abd, A.N., (2018), Thickness effect on some physical properties of the Ag thin films prepared by thermal evaporation technique, Journal of Global Pharma Technologythis . 10(3), pp. 613–619.

[19] Al-Sahaaf, Faddel hussein (1989). applied plant nutrition. Al-hikmma house for publishing and distribution. University of Baghdad.Iraq.Eng .abstract.

[20] Abbas, T; Ahmed, S; Ashrafi, M.; Shahid, M. A.; Yasin, M.; Valal, R. M.; Pervez, M. A.; and Abbas, S. (2013). Effect of humic acid Application at different growth stages of Kinnow mandarin (Citrus reticulata Blanco) on the basis of physio-biochemical and reproductive responses. Academia Journal of Biotechnology. 1 (1) : 14 - 20.

[21] Abobatta, W.F.R.2014. Effect of humates compound and magnetic iron on growth and fruiting of Valencia orange trees (Citrus sinensis L.). Ph.D. Dissertation, College of Agriculture. Minoufiya University, Egypt.

[22] Al-Taey, D.K.A.(2010). Effect of acetyl salicylic acid in increasing the tolerance of plants & reducing the damage effects by saline water on olive transplants (Olea europaea L.) J. B. Univ. P. A. S. 18(5).

[23] El-Shazly, samy M.; Khalil, Hoda A.; Abd El-Hamed, shaimaa F. (2015). Effect of salicylic acid on growth and physiological status salt stressed sour orange seedling.( Citrus aurantium L). Alex. J. Agric. 60(3):229-239

[24] Hathal, Nessrin muhammed .(2014). Effect of Humic Acid Spray on Some Citrus Rootstocks Tolerance to Irrigation Water Salinity. master thesis. College of agriculture. University of Diyala.Eng .abstract.