Effect of Different Levels of Catalysts and Chemicals Primer on Content of Induced Callus Tissue of Cotyledon Leaves of Spilanthes Acmella Seedlings on Some Chemical Traits in Vitro

Ekhlas Meteab Ahmed Marir
Department of Horticulture and Landscape Gardening, College of Agriculture, University of Diyala, Iraq.
Email: Ekhlas.Meteab86@yahoo.com

Abstract
This experiment was conducted in the Plant Tissue Culture Laboratory, College of Agricultural Engineering Sciences, University of Baghdad for the period from September / 2018 to July 2019. The induced callus from the cotyledon leaves of seedlings of the Spilanthes acmella plant was used in order to know the effect of chemical catalysts and Starters on the chemical content. After 4 weeks of planting, the primary callus was planted at 150 mg in the nutrient medium supplemented with auxin, 4-dichlorophenoxy acetic acid (2,4-D) 2.0 mg.L⁻¹ and cytokinin Benzyl Adenine (BA) 0.5 mg.L⁻¹ at constant concentrations in the first five medium, to which the catalyst was added salicylic acid at concentrations (25, 50, 75) μmol. The second medium was added to methyl jasmonate at concentrations (25, 50, 75 μmol) of the third medium was added to Casein hydrolysate at concentrations (25, 50, 75 μmol) of the fourth medium was added to Glutamine (250, 300, 350) mg.L⁻¹. The results showed that the treatment of nutritional medium with high concentrations of stimulants and primer led to a significant increase in the content of plant tissues (the induced callus from the cotyledons) of total carbohydrates, the percentage of protein, the content of callus from the carotene pigment and content of proline, while the comparison treatment was the most effective in vegetable tissue contents of total carbohydrates and protein percentage and content of callus from the carotene pigment and proline, as well as this confirms that all treatments led to a positive and direct increase of chemical compounds content of plant tissues of chemical traits, especially in the treatment of Salicylic acid, methyl jasmonate, casein hydrolysate, glutamine, and phenylalanine (75 micromoles, 75 micromoles, 75 micromoles, 350 mg.L⁻¹, 150 micromoles) respectively, were followed by the treatments of Salicylic acid, methyl jasmonate, casein hydrolysate, glutamine and phenylalanine (50 μmol, 50 μmol, 50 μmol, 300 mg.L⁻¹, 100 μmol), respectively. The aim of this study is to know the effect of Salicylic acid, methyl jasmonate, casein hydrolysate, glutamine, and phenylalanine in the induction and differentiation of callus of cotyledon leaves cotyledon leaves of Spilanthes acmella seedlings on some chemical traits in vitro.

Keywords: Salicylic acid, Methyl jasmonate, Casein hydrolysate, Glutamine, and phenylalanine, Spilanthes acmella.

1. Introduction

Spilanthes acmella L. is an important medicinal ornamental plant that belongs to the Asteraceae family, an annual herbaceous plant in most climate types. It was cultivated in warm areas as a perennial plant. It is one of the frost-sensitive plants, its height ranges 60.32 cm, its multi-stemmed, golden-eyed flowers are small, with a dark red eye at the Apex or in a conical head. Aromatic and hermaphrodite and their roots are cylindrical in shape, tapering gradually to its edges, it has hairs similar to rhizomes, when its roots dry out, it is dark brown on the outside and white on the inside. Its roots are plump, its surface is rough, it contains aromatic acids, and it tastes pungent. As for the stem, it contains glands and hairs (fluff) that taste stinging. Old or adult stems are greenish-yellow in color, all parts of the plant have a pungent taste. The plant is characterized by masked, opposite, regular leaves, simple feathers, with a broad oval shape, and narrow at the base. Sharp, serrated, obtuse, or circular at the top, with a long stalk, with thin wings containing bristles, the leaf blade is triangular in shape, wide and oval. The veins are thin and contain bristles on the upper and lower sides [1]. Its fruits are black in color. The seeds are sown in March/April [2]. The plant reproduces by seeds and stem cuttings, as the seeds of this genus are characterized by being small and very smooth, black or gray in color [3]. The genus Spilanthes known as (Toothache Plant) belongs to the Asteraceae family and the old name of the family (Compositae) was called in the past Pellitory and the new English name Para cress. acmella, S. oleracea. S. calva, S. paniculata, S. mauritiana [4]. The technology of plant tissue culture plays an important
role in the propagation of medicinal plants to obtain many numbers in a short period of time, it has used many growth regulators, including auxins and cytokinins, as well as proteins such as caseins (milk protein) in addition to catalysts and chemical precursors for many plants that work to induce encouraging changes to increase the total carbohydrate content of plant tissues. The percentage of protein, the content of callus from the carotene pigment and the content of proline. The first scientific studies in this field in 1988, [5], noticed the addition of milk protein (hydrolyzed casein) to the nutrient medium, which led to an increase in the content of carbohydrates and proteins in cultured tissues in vitro. Norhayati, et al., [6] found that increasing the casein hydrolysate protein concentration from 0.75-1.0 g.L⁻¹ in addition to the presence of 2.0 mg.L⁻¹ of 2,4-D and 1.0 mg.L⁻¹ of Kinetin for MS medium. It resulted in increased synthesis of α-tocopherol, ascorbic acid and carotenoid in callus cultures of Centella asiatica. In a study by [7], it was found that adding 1 micromole per litter of Methyl and Salicylic Acid (SA) to the media led to an increase in lycopene dye synthesis, which reached (57199.%) for Lycopersicon esculentum Mill.

2. Materials and Methods

The study experiments were conducted in the plant tissue culture laboratory for postgraduate studies - College of Agricultural Engineering Sciences - the University of Baghdad for the period from September 2018 to July 2019. Callus induced from the developing top of the seedlings of Spilanthes acmella (L.) One month age was used in order to know the effect of catalysts and Starters on the total carbohydrate content in plant tissues, protein percentage, the content of carotene pigment and the proline in callus. As 150 mg of callus was planted in nutrient media, which consisted of inorganic salts, MS, sucrose 30 g.L⁻¹, Vitamins and growth regulators had added 0.5 mg.L⁻¹ of Benzyl Adenine (BA) and 2.0 mg.L⁻¹,4 dichloro phenoxy acetic acid (2,4-D). Then different concentrations of salicylic acid were added at concentrations (25, 50, 75) micromoles, methyl jasmonate at concentrations (25, 50, 75) micromoles, casein hydrolysate (25, 50, 75) micromoles, and glutamine (250, 300, 350) mg.L⁻¹ and phenylalanine (50, 100, 150) micromoles each separately in addition to the presence of one control treatment for all experiments, then the acidity of a media was adjusted to pH 5.7 after, then agar was added by 7 g. L⁻¹. The seeds or plant parts were sown in glass bottle which content 250 ml of media nutrient All experiments were conducted under sterile conditions in a Laminar air flow hood. Each treatment represented ten replications,

The study included the following:

- Estimation of total carbohydrates (µg.g⁻¹)
- The carbohydrate content was calculated according to the method [8].
- Estimated of protein percentage (%)
- The protein percentage was calculated according to the method [9]
- estimation of the carotene pigment in callus (mg-100g⁻¹)
- The carotene pigment was estimated according to Goodwin's method [10] with a weight of 0.5 mg of fresh callus for each treatment
- Estimation of proline (µg.g⁻¹)
- The protein percentage was calculated according to the method by Bates et al. [11].

Experimental design and statistical analysis

The statistical program Statistical Analysis System SAS [12] was used in data analysis to study the effect of different treatments on the studied traits, and the significant differences between the means were compared with the Least Significant Difference-LSD test. Each treatment included 10 replicates

3. Results and discussion

3.1 The callus tissue content

In Tables (1, 2, 3, 4) showed an increase in the content of plant tissues (the induced callus tissue from cotyledons) of total carbohydrates with an increase in the concentrations of Salicylic acid, methyl jasmonate, casein hydrolysate, glutamine and phenylalanine added after four weeks of planting for culture medium. Record the concentration of 75 micromoles of salicylic acid and 75 micromoles of methyl jasmonate, 75 micromoles of casein hydrolysate, 350 mg.l of glutamine and 75 micromoles of phenylalanine had the highest tissue content of total carbohydrates, which amounted to (7.160, 8.663,9.217, 7.957, 11.633) micrograms., respectively, with a significant difference from all other treatments, followed by the effect of concentrations (50, 50, 50, 300, 100) respectively. Including the comparison treatment, the lowest carbohydrate content in plant tissues was recorded, with a content of 2.105 μg.g.D.W. for all experiments, The reason for this that dehydration reduces the speed of carbohydrate conversion for the growth of plant cells or their transformation into primary metabolites by inhibiting carbohydrate-dissolving enzymes, considering that enzymes are protein substances and insufficient proteins under stress conditions. That stress is a cause of an increase in carbohydrate contents in plant tissues of and another group of
compounds that accompany them in response to adaptation to drought conditions [13] Carbohydrates and sugars work to balance the osmosis between cytoplasm and cellular structures inside the cells, thus preserving the components of plant cells [14], or the reason for this may be that salicylic acid is one of the plant hormones that work according to reducing the tension that the plant was exposed to the works on the regulation of growth, physiological processes, nutrient absorption, protein synthesis, inhibiting the biosynthesis of ethylene, the process of breathing, protecting the functions of some cellular organelles, and increase the concentration of carbohydrates to provide the necessary energy for these vital processes, where the presence of carbohydrates is necessary for all metabolic processes [15, 16] There are many scientific studies that they conducted show the active role of the casein hydrolysate protein in tissue cultures and cell suspensions in vitro of different plants, whether it is the generation of organs or embryos, the formation of callus tissue, the production of artificial seeds or production of pharmaceutically active compounds. As mentioned in previous and various topics. In this result didn’t find scientific studies on the effect of adding Casein hydrolysate to the content of plant tissues grown in synthetic medium from a few carbohydrates from research, and from this research, the study of Al-Ali [17] on date palm tissues Phoenix dactylifera L. in vitro noted an increase in the accumulation of carbohydrates when including media culture with casein hydrolysate. Also Haroun, et al. [18] showed that 1-2 micromoles of L-glutamine and L-asparagine added to the multiplication medium of vulgaris Phaseolus gave the best response to the production of carbohydrates.

3.2 Protein percentage (%)

The results of the statistical analysis in Tables (1,2,3,4) showed that the inclusion of media culture with concentrations (75 μmol of Salicylic acid, 75 μmol of methyl jasmonate, 150 μmol of Phenylalanine, 350 μmol of Glutamine) increased the percentage of protein in tissues plant (induced callus from shoot apex), which amounted to (17,499, 14,477, 11,460, 10,998)% respectively. However, it differed with concentrations of Casein hydrolysate added to the food medium, where the concentration of 50 micromol recorded the highest percentage of protein, which amounted to 10.318%, with an insignificant difference from the rest of the concentrations added to the culture medium, while the control treatment recorded the lowest percentage of protein content in plant tissues, which amounted to 1.647 %.

3.3 Protein percentage content in callus (%)

The results of statistical analysis in Tables (1,2,3,4) showed that the inclusion of the media culture with concentrations (75 μmol of Salicylic acid, 75 μmol of methyl jasmonate, 150 μmol of Phenylalanine and 350 μmol of Glutamine) increased the percentage of protein in tissues plant (induced callus from shoot apex), which amounted (17,499, 14,477, 11,460, 10,998)% respectively. However, it differed with the concentrations of Casein hydrolysate added to the food medium, where the concentration of 50 micromol recorded the highest percentage of protein, which amounted to 10.318%, with an insignificant difference from the rest of the concentrations added to the culture medium, while the control treatment recorded the lowest percentage of protein content in plant tissues, which amounted to 1.647 %. The results explain that glutamine is one of the most important amino acids involved in process of building proteins that work on the formation of enzymes that play a role in most biological processes, as well as in the construction of both RNA and DNA. It also leads to an increase in the concentration of endogenous glutamine and its accumulation, which constitutes a basic component involved in protein synthesis and thus contributes to the process of activating proteins formation in addition to the fact that glutamine was a storehouse for amino groups and can move from one site to another within the plant tissues and members of amino groups of cells that need in protein formation [19]. This agree with Haroun et al. [18] that the concentration of 1-2 μmol of L-glutamine and L-asparagine that added to branching media of vulgaris Phaseolus gave best response for protein production. The increase in growth using phenylalanine may be related to its function in proteins formation and performing a number of additional functions in regulating the metabolism, transporting and storing nitrogen [20] and amino acids play an important role in stimulating cell growth [21]. In addition, it can serve as a source of carbon, energy, and the manufacture of other organic compounds such as purines, vitamins, alkaloids, terpenes, protein, amines, and others [22]. The use of phenylalanine in most of the studied traits may be due to the role of amino acids in increasing plant cell growth and the efficiency of absorption nutrients, As amino acid ions were released easily for cells to benefit from quickly and easily enter the cytoplasm of plant cells, which leads to an increase in the process of photosynthesis as a result of its entry into the synthesis of a number of enzymes, this process and as a result of its rapid processing of nitrogen and activation of carbon metabolism process [23]. The increase in the protein content of the callus tissue at a concentration of 50 micromoles of casein hydrolysate may be due to the nature and composition of this substance, in addition to the composition of the nutrient medium, especially when adding levels of casein hydrolysate. This addition increases the energy units of the cultivated plant tissues. This leads to an increase in the active manufacturing process of proteins, as well as the casein hydrolysate and its distinctive combination of vitamins and essential amino acids, which play a major role in all processes within plant tissues that were grown in vitro.
3.4 Proline content in callus tissue (µg·g⁻¹·dry weight)

The results showed an increase in proline content in plant tissues with different levels. The concentration of 50 µmol Salicylic acid, 50 µmol of methyl jasmonate, and 300 mg·L⁻¹ of Glutamine recorded the highest average content of proline, which amounted to 3.770, 3.910, 3.770, µg·gm·dry weight, respectively, which were significantly excelled on all other treatments. Followed by the concentrations of 150 micromoles of Phenylalanine and 75 micromoles of Casein hydrolysate, was a highest average of proline was 4.070, 3.845 µg·gm and dry weight, respectively. The results of the statistical analysis of all the traits mentioned in the study indicate close proportions between the high concentrations added to media culture, while control treatment was same for all the experiments, which had the most effect on the traits, which gave the lowest proline content in plant tissues, it was 1.647 µg·gm dry weight. The increase in the concentration of proline is due to the role of glutamine acid, which is the primer of proline construction and it is possible to increase glutamine acid in the effective contribution to the proline synthesis process by providing effective concentrations of glutamine acid. It enhances the possibility of proline synthesis inside the plant cell [24]. These results are consistent with the findings of Al-Dulaimi, [25] in increasing the production of proline when using different concentrations of glutamine and salicylic acid.

3.5 Carotene pigment (mg/100g)

Regarding to The carotene pigment, the results from the previous tables (1,2,3) indicated that there was a significant increase in the content of plant tissues (the induced callus from the cotyledons) in the carotene pigment with an increase in the concentrations of the additives to media culture. The treatment of the medium with concentrations of 75 µmol of Salicylic acid, 50 µmol of methyl jasmonate, 75 µmol of Casein hydrolysate, 300 mg·L⁻¹ of Glutamine and 100 µmol of Phenylalanine gave the highest content of carotene pigment, which amounted to 27.702, 26,000, and 31.100,68,733, respectively. All the statistical data for this trait showed the relative closeness between the high concentrations added to media, while the most influential for this trait was the control treatment, which gave the lowest percentage of carotene dye amounted to 10.333 mg / 100 g. The results agreed with [18] that the concentration of 1-2 µmol of L-glutamine and L-asparagine added to the branching media of *Vulgaris phaseolus* gave the best response to production of carotenoids.

### Table 1. Effect of different levels of Salicylic acid carbohydrate, proline content of callus tissue (µg·g⁻¹), The percentage of protein (%) and carotene pigment (mg·100g⁻¹) after four weeks of planting on MS media.

| Salicylic acid (µmol) | total carbohydrates | Proline | percentage of protein | carotene pigment |
|-----------------------|---------------------|---------|-----------------------|------------------|
| 0                     | 2.105               | 2.560   | 1.647                 | 10.333           |
| 25                    | 2.663               | 3.555   | 7.471                 | 15.000           |
| 50                    | 4.110               | 3.910   | 9.501                 | 23.000           |
| 75                    | 7.160               | 3.305   | 17.499                | 27.702           |
| LSD(0.05)             | 3.891               | 0.579   | 6.414                 | 10.947           |

### Table 2. Effect of different levels of methyl jasmonate (MeJa) carbohydrate, proline content of callus tissue (µg·g⁻¹), The percentage of protein (%) and carotene pigment (mg·100g⁻¹) after four weeks of planting on MS media.

| MeJa (µmol) | total carbohydrates | Proline | percentage of protein | carotene pigment |
|-------------|---------------------|---------|-----------------------|------------------|
| 0           | 2.105               | 2.560   | 1.647                 | 10.333           |
| 25          | 3.380               | 3.580   | 5.173                 | 19.333           |
| 50          | 5.203               | 3.770   | 6.870                 | 26.000           |
| 75          | 8.663               | 3.858   | 14.477                | 21.000           |
| LSD(0.05)   | 4.410               | 0.680   | 3.407                 | 4.067            |

### Table 3. Effect of different levels of Casein hydrolysate on carbohydrate, proline content of callus tissue (µg·g⁻¹), The percentage of protein (%) and carotene pigment (mg·100g⁻¹) after four weeks of planting on MS media.

| CH (((µmol)) | total carbohydrates | Proline | percentage of protein | carotene pigment |
|-------------|---------------------|---------|-----------------------|------------------|
| 0           | 2.105               | 2.560   | 1.647                 | 10.333           |
| 25          | 3.307               | 3.630   | 2.570                 | 17.333           |
| 50          | 5.263               | 3.605   | 14.307                | 27.667           |
| 75          | 9.217               | 3.780   | 10.630                | 31.000           |
| LSD(0.05)   | 4.128               | 0.550   | 8.498                 | 10.857           |
Table 4. Effect of different levels of Glutamine on carbohydrate, proline content of callus tissue (µg.g⁻¹). The percentage of protein (%) and carotene pigment (mg.100g⁻¹) after four weeks of planting on MS media.

| Glutamine Con. mg L⁻¹ | total carbohydrates (µg) | Proline (µg) | percentage of protein (%) | carotene pigment (mg.100g⁻¹) |
|-----------------------|--------------------------|-------------|---------------------------|-----------------------------|
| 0.0                   | 2.105                    | 2.560       | 1.647                     | 10.333                      |
| 250                   | 2.567                    | 3.525       | 5.844                     | 19.173                      |
| 300                   | 4.917                    | 3.845       | 7.675                     | 28.533                      |
| 350                   | 7.957                    | 2.640       | 10.998                    | 23.990                      |
| LSD(0.05)             | 3.762                    | 0.801       | 8.498                     | 2.364                       |

Table 5. Effect of different levels of Phenylalanine on carbohydrate, proline content of callus tissue (µg.g⁻¹). The percentage of protein (%) and carotene pigment (mg.100g⁻¹) after four weeks of planting on MS media.

| Phenylalanine Con. (µmol) | total carbohydrates (µg) | Proline (µg) | percentage of protein (%) | carotene pigment (mg.100g⁻¹) |
|--------------------------|--------------------------|-------------|---------------------------|-----------------------------|
| 0.0                      | 2.105                    | 2.560       | 1.647                     | 10.333                      |
| 50                       | 3.377                    | 3.740       | 3.333                     | 11.667                      |
| 100                      | 6.093                    | 3.455       | 7.933                     | 29.667                      |
| 150                      | 11.633                   | 4.070       | 11.460                    | 24.000                      |
| LSD(0.05)                | 7.872                    | 0.506       | 7.504                     | 4.981                       |

Conclusion

The positive role of Salicylic acid, methyl jasmonate, casein hydrolysate, glutamine, and phenylalanine in this study leads to the recommendation to include them in on some chemical traits Of which (total carbohydrates, Proline, percentage of protein,and carotene and concluded from the present study also that cotyledon leaves of Spilanthes acmella plants have ability of growth and induction of indirect callus when they are cultured in the right medium and concentration of BA, 2,4-D

References

[1] Grubben, G.J.H. and Denton, O.A. 2000 Plant resources of Tropical Africa 2 Vegetables. PROTA Foundation, Wageningen, Backhuys, Leiden, CTA, Wageningen.
[2] Savadvi,RV, yadav R, yadav N. 2010. Study on immunomodulatory activity of ethanolic extract Spilanthes acmella Murr. Leaves. Indian journal of natural Products and Resources; 1(2): 207.
[3] Saha J, Jain K, Jain B, Sahu R. K. 2011. A review on phytopharmacology and micro propagation of Spilanthes acmella. Pharmacology online newslett. 2:1105-10.
[4] CSIR (Council of Scientific andIndustria Research). 1989. The Wealth of India: a dictionary of Indian raw materials and industrial products, CSIR, New Delhi. 10: 11–12.
[5] Steward, F. C.; Pollard, J. K.; Patchett, A. A. and Witkop, B. 1958. The effects of selected nitrogen compounds on the growth of plant tissue cultures. Biochemical et Biophysical Acta, 28:308- 317.
[6] Norhayati, Yusuf, Misri Kusnan, Nor’aini mohd Fadzillah and Maziah Mahmood. 2016. the effect of medium compositions and light on the production of ascorbic acid, α-tocopherol and carotenoids in centella asiatica callus, j. trop. plant physiol. (8):12-22.
[7] Andleeb Zehra, Manish Kumar Dubey, Mukesh. Meena. Mohd. Aamir, Laxmi.Ahirwa and R.S. Upadhyay. 2016. Improvement of Lycopene, Ascorbic Acid and Total Phenol Content of Postharvest Tomato Fruits by Exogenous Application of Salicylic Acid and Methyl Jasmonate, Har Krishan Bhalla & Sons, FPI 1 (1) pp 1–7.
[8] Herbert, D.P., J.Philips and R.E.Stange.1971. Method in microbiology. Narri, J.R. and D.W. Robbin (eds.). Acad. Press., London, New York, Chap.3p.513.
[9] Gresser, M.S. and J. W.Parson. 1979. Sulfuric – Perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium and magnesium, Analytical chemical Acta. 109:431-436.
[10] Goodwin,T.W. 1976. Chemistry and Biochemistry of Plant Pigments 2nd Ed Academic Press London. New York. San Francisco, p.373.
[11] Bates, L.S.R. Walden andD.Tear. 1973. Rapid determination of free proline for water stress studies. Plant and soil, 39:205-207.
[12] SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1 th ed. SAS. Inst. Inc. Cary. N.C. USA.
[13] Gill, S.S.and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 48:909–930.
[14] Cales, B ; Dekeyser, R; Villarroel, R; Vanden Bulcke, R; Van Montagu, M. and Caplan, A.1990. Characterization of rice gene showing organ-specific expression in response to salt stress and drought. Plant Cell, 2 : 19–27.
[15] Mohammed, M.A., Salman, S.R., (2017). Structural and surface roughness effects on sensing properties of ZnO doping with Al thin films deposited by spray pyrolysis technique, Journal of Engineering and Applied Sciences, 12 (Specialissue6), pp. 7912-7918.
[16] Taiz, L, Zeiger E. 2006. Plant physiology, 4th edn. Sinauer Associates Inc. Publishers, Massachusetts.

[17] Al-Ali, Ziad Tarq Safy Abd. 2014. Effect of paclobutrazol and caseinhydrolysate on somatic embryos fromation and Germination of Date plam (phoenix dactylifera L.) CV.NERSY, Ph D. thesis, College of Agriculture, University of Basrah

[18] Haroun, S. A, W. M. Shukry and O. El-Sawy. 2010. Effect of asparagine or glutamine on growth and metabolic changes in phaseolus vulgaris under in vitro conditions, Bioscience Research, 7(1): 01-21.

[19] Vickery, H.B.; Pucher, G.W. and Clark, H. E. 2017. Glutamine Metabolism of the Beet. American Society of Plant Biologists, 11: 413-420.

[20] Davies, D. D. 1982. Physiological aspects of protein turn over. Encycl., Plant Physiology, New Series, 14A (Nucleic acids and proteins: structure biochemistry and physiology of proteins). 190-288 – Ed., Boulter, D. and Par.

[21] Nahed, G.A., Mazher, A.A. and Farhat, M.M. 2010. Response of vegetative growth and chemical constituents of Thuja orientalis L. plant to foliar application of different amino acids at Nubaria. J. of American Sci. 6 (3): 295-301.

[22] Abd El-Aziz, G.N. and Balbaa, L.K. 2007. Influence of tyrosine and zinc on growth, flowering and chemical constituents of Salvia farinacea plant. World J. Agric. Sci. 75:1479-1489.

[23] Allawi Luabi Dagher Al-Khauzai. The Effect of Crossbreeding on Some Economic Traits for Chicks of Lohmann Chicken. Al-Qadisiyah Journal For Agriculture Sciences, 10, 1, 2020, 221-226. doi: 10.33794/qjas.2020.167069

[24] Forde, B. G. and Lea, P. J. 2007. Glutamate in plants: metabolism, regulation, and signalling. Journal of Experimental Botany, 58 (9): 2339–2358.

[25] Al- Dulaimi. Rehab Jomaa Mansour,2018. The Effect of Salicylic acid and Glutamine in growth and yield of Cucumis sativus L.(cucumber) exposed to drought stress, M.SC. Degree in Biology, College of Sciences, University of Diyala,