MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

# Folic acid applications affect growth and metabolism of Spinaciaoleracea,Lactuca sativa and Corchorus olitorius

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Received: 7/11/2020
Accepted: 20/11/2020

**Abstract:** A pot experiment was conducted to assess the effects of three concentrations of folic acid (5,10&20 mM); through two applications, soaking or spraying on the growth and biological activities of spinach (*Spnancia oleracea*), lettuce (*Lactuca sativa*) and chorchorus (*Chorchorus olitorius*) during vegetative stage**.** The obtained results cleared that, Soil texture changed from clay silty in the untreated plants into clay loam by soaking the seeds of the used plants in folic acid the water holding capacity and porosity decreased by folic acid treatments. Although pH, Cl, Na and Ca decreased by folic treatments, EC, organic matter, $CaCO_3$, $HCO_3$, $SO_4$, K and Mg increased, as compared to the untreated soil. Fresh and dry weight of the test plants either decrease or increase but, water percentage, generally decreased by folic applications. At most, pigments, total carbohydrates, nitrogen, protein and phenol increased while alkaloids decreased, in response to folic treatments.

Treatment with folic acids decreased PPO and increased CAT and POX activity in *Spinancia oleracea* but in *Lactuca sativa*, no change detected in PPO activity but the of CAT and POX decreased. In *Chorchorus olitorius*, treatment with folic, more or less have no change; or slightly increase the activity of PPO, CAT and POX compared to the control value. An increase in K, Ca & Mg and decrease in Na content in the three used plants was observed in response to folic applications. Foliar application of folic acid (spraying) was most effective than pretreatments (soaking) also, the most response by folic addition was in case of *Chorchorus olitorius*.

**keywords**: Folic acid, Spnancia oleracea, Lactuca sativa, Chorchorus olitorius, soil, growth, metabolism.

## 1.Introduction

Vegetables are important part of healthy eating and provide a source of many beverages, in traditional medicine, in horticulture and in nutrients, including potassium, fiber, folate (folic acid) and vitamins A, E and C. Options like broccoli, spinach, tomatoes and garlic provide additional benefits, making them a superfood [1-3]. Furthermore, increasing the use of chemical fertilizers lead to the high cost of vegetable production and created pollution of agricultural environment as well as affects the soil fertility [4]. Therefore, there is an increasing interest in the use of organic N sources as fertilizers for the production of vegetable crops and particularly for the organic production of vegetables [5].

Folic acid (folate) is a water-soluble vitamin. It is also referred to as vitamin M, vitamin B9, vitamin Bc (or folacin), pteroyl-L-glutamic acid, and pteroyl-L-glutamate. Folate occur naturally in many foods and, among plants [6,7]. Folic acid is necessary for a healthy plant to grow. Most plants are able to produce their own folic acid. It can be found in high levels naturally in some plants, especially is present in dark green leafy vegetables. However, you can assist a plant that is deficient in this vitamin by giving it a fertilizer that contains these vitamin supplements [7]. Because folic acid exists in plants but decomposes rapidly when exposed to strong light, giving plants additional folic acid during the planting process is recommended [7].

*Spinacia oleracea* is an edible flowering (Family Chenopodiaceae), it may survive over winter in temperate regions [8], has a high nutritional value, especially when fresh, frozen, steamed, or quickly boiled. It is a rich source of vitamin A, C, E, K, magnesium, manganese, and folate. Spinach is a good source of the B vitamins riboflavin and vitamin B6, calcium, potassium, and dietary fiber [9,10].

*Lactuca sativa* is an annual plant of the daisy family Asteraceae. It is most often grown as a leaf vegetable, but sometimes for its stem and seeds, its oil-rich seeds [11]. Lettuce is considered as an excellent nutritive source of minerals and vitamins as it is consumed as a fresh green salad [12]. Also, lettuce leaves are a rich source of antioxidants, vitamins A and C [13] and phytochemicals which are anti-carcinogenic [14].

*Corchorus olitorius* L (Malukhiyah, family Malvaceae) is one of the most widely consumed traditional vegetables in Africa [15]. It is rich in minerals, vitamins and other nutritional factors [16]. Plant mucilage and/or acidic polysaccharides fractionated from the leaves, roots, and fruits have sometimes shown such interesting biological effects as anticomplementary activity [17,18] hypoglycemic activity [19] antitumoral activity, diuretic activity and antidiarrheic activity [20]. The phytochemistry of leaves revealed that the plants contained bioactive agents such as alkaloids, saponins, flavonoids, tannins, anthraquinone and cardiac glycosides [21,22].

The aim of the present investigation was to study further the effects of various concentrations of folic acid on growth and some metabolic activities of three leafy plants mainly; *Spinancia oleracea*, *Lactuca sativa* and *Corchorus olitorius* shoot, during vegetative stage.

## 2. Materials and methods

### Plants used

Pure strain of seeds was obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt and selected for apparent uniformity of size and shape. The used folic acid and the other chemicals were supplied from Sigma Chemical Company. In preliminary experiment, the effect of various concentrations of folic acid on spinach (*Spnancia oleracea*), lettuce (*Lactuca sativa*) and chorchorus (*Chorchorus olitorius*) plants was tested and in accordance the concentrations 5, 10 & 20 mM of folic acid was used in the present study.

### 2. Time course of experiment

A homogenously-sized lot of spinancia, lettuce and chorchorus seeds were selected and surface sterilized by soaking in 0.01% $HgCl_2$ solution for 3 minutes. After washing thoroughly with distilled water, the seeds of plant were divided into 3 equal groups treated as follow:

Group (1) treated with water to serve as a control.

Group (2) soaked separately in the used concentrations (5, 10&20 mM) of folic acid

Group (3) sprayed separately with the used concentrations (5, 10&20 mM) of folic acid.

Seeds of the three groups were cultivated in pots (20 cm in diameter) containing equal amounts of soil (sand: clay, 1:2 v/v). Before planting soaking the seeds of group (2) in the used concentrations of folic acid (as mentioned above) take place. At the same time super phosphate fertilizer (0.5 g/pot) was added to the soil before sowing. Meanwhile, N urea as nitrogen fertilizer (1 g/each pot) was added to the soil during the first week of cultivation. Ten seeds were sown in each pot and irrigated as usual practice by adding equal amounts of water to each pot when required. Plants were exposed to normal day night condition. After 15 days from sowing thinning takes places where 5 uniformed seedlings only left to grow in each pot. After another 15 days (30 days from sowing) spraying group (3) with the used concentrations of folic acid (as mentioned above).

Samples are taken during the vegetative stage; after 40 days from sowing the three tested plants. In addition, soil samples were collected from the three groups for soil analysis. The collected plant samples were used for assessment of growth parameters, as well as some metabolic contents (photosynthetic pigments, carbohydrates, nitrogen, total protein, total phenols, alkaloids). Also, the activity of some antioxidant enzymes; polyphenol oxidase, peroxidase & catalase as well as sodium, potassium, calcium and magnesium was carried out

Data were analyzed by least significant difference (L.S.D) test at probability of 0.05 to identify significant effect of a treatment. ANOVA analysis was done with the IBM SPSS-20 statics software [23].

## 3. Analytical Methods

### 3.1. Soil sampling and analysis:

Soil texture, porosity, water-holding capacity, and organic matter were determined according to Piper [24] and Jackson [25]. Soluble cations and anions in the soil paste extract were determined according methods described by Jackson [26]. Soil pH was determined in the saturated soil paste using a Gllenkamp pH-meter (Model pH Tester 2 TM) according to Richards [27]. Salinity was determined by measuring the electrical conductivity (EC) by the electrical conductivity meter (EC meter Model TD Scan 3) in saturation extract in ds $m^{-1}$ [27]. Calcium carbonate was determined using Collin's Calcimeter as described by Page et al. [28].

### 3.2. Estimation of photosynthetic pigments

The plant photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method as recommended by Arnon [29] for chlorophylls and Horvazth *et al*. [30] for carotenoids as adopted by Kissimon [31].

### 3.3. Estimation of some metabolites

Carbohydrates content was analyzed by the method of Hedge and Hosreiter [32]. The total nitrogen was determined by the conventional semi-micropropagation of Kjeldahl method of Rees and Williams [33] and described by Haroun [34]. Protein content in the plant extract was estimated by using spectrophotometer according to Bradford [35]. Total phenols could be estimated by the Folin- Ciocalteau reagent [36]. Quantitative determination of alkaloid was according to the methodology by Harborne [37].

### 3.4. Extraction and estimation of antioxidant enzymes activity

For CAT, POX and PPO, the extraction medium was 0.1 M phosphate buffer at PH 6.8 was used where all the operations were carried out at 4ºC [38]. Polyphenol oxidase (PPO) and peroxidase (POX) activities were assayed according to Devi [39] while the catalase activity was assayed by the method of Sinha [40] (1972) as modified by Gopalakrishnan and Starlin [41].

### 3.5. Estimation of K, Na, Mg and Ca, ions:

Concentrations of the cations Na and K in leaf content was estimated using Flame photometer (M7D) according to Allen et al. [42]. Ca and Mg were determined by using atomic absorption spectrophotometer.

## 3. Results and Discussion

### 1. Effect of folic on soil analysis

Soil texture changed from clay silty in the untreated plants into clay loam by soaking the seeds of the used plants in folic acid the water holding capacity and porosity decreased but, increase with increasing folic acid concentrations (Table 1).

Analysis of the soil where seeds of the three tested plants untreated and treated with different concentrations of folic acid showed that, soil pH increase with increasing folic acid concentrations in soil where the seeds treated with (either soaking or spraying) but, decrease as compared to the untreated soil. While EC $dsm^{-1}$ and organic matter (OM %) increase with increasing folic acid concentrations in soil where the seeds treated with folic acid (either soaking or spraying), comparing to the control theses parameters increased (Table 2).

Although the concentration of the determined cations ($Na^+$, $K^+$, $Ca^{++}$ and $Mg^{++}$) and the determined $SO_4^{--}$, $Cl^-$ and $CaCO_3$ were decrease with increasing concentrations in soil where the seeds treated with folic acid (either soaking or spraying), Na and Ca as well as $Cl^-$ and $HCO_3$ decreased as compared to the untreated soil, in contrast, K and Mg as well as $SO_4^{--}$, and $CaCO_3$ increased as compared to the untreated soil (Table 2).

In this respect, the increments in the determined in soil parameters may be due to the improvement of the element's availability in response to folic acid treatments. In accordance, an increase in membrane permeability would in turn facilitate the absorption and utilization of mineral nutrients and the transport of assimilate in plants [43,44].

**Table 1.** Physical parameters of soil samples in response to different folic acid concentrations. Abbreviation: WHC: water holding capacity

| **Folic acid** | | **Physical characteristics** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Soil texture %** | | | Texture class | WHC % | Porosity % |
| Treatment | Conc.(mM) | Sand | Silt | Clay | | | |
| Control | | 18.0 | 27.0 | 53.5 | Clay silty | 63.7 | 41.71 |
| Soaking | 5 | 47.5 | 36.79 | 15.71 | Sandy loam | 34.8 | 21.3 |
| | 10 | 44.31 | 38.09 | 17.6 | Sandy loam | 37.2 | 22.9 |
| | 20 | 31.58 | 39.3 | 29.12 | Clay loam | 48.8 | 31.4 |
| Spray | 5 | 50.66 | 35.8 | 13.54 | Sandy loam | 32.6 | 19.8 |
| | 10 | 36.05 | 41.02 | 22.93 | Sandy clay loam | 42.7 | 26.6 |
| | 20 | 33.14 | 40.07 | 25.79 | Clay loam | 45.6 | 29.2 |

**Table 2**. Chemical parameters of soil samples enriched with different folic acid concentrations.

| **Folic acid** | | **Chemical characteristics** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | EC $dsm^{-1}$ | OM % | $CaCO_3$ % | **Anions %** | | | | **Cations %** | | | |
| Treat ment | Conc. (mM) | | | | | $CO_3^{--}$ | $HCO_3^-$ | $Cl^-$ | $SO_4^{--}$ | $Na^+$ | $K^+$ | $Ca^+$ | $Mg^{++}$ |
| Control | | 7.71 | 0.15 | 1.5 | 1.98 | 0 | 0.20 | 0.24 | 0.33 | 0.34 | 0.05 | 0.22 | 0.16 |
| Soaking | 5 | 7.08 | 1.07 | 1.42 | 4.98 | 0 | 0.074 | 0.132 | 0.78 | 0.08 | 0.23 | 0.09 | 0.78 |
| | 10 | 7.11 | 0.96 | 1.54 | 4.79 | 0 | 0.064 | 0.124 | 0.74 | 0.05 | 0.20 | 0.07 | 0.74 |
| | 20 | 7.32 | 0.63 | 1.92 | 3.77 | 0 | 0.049 | 0.063 | 0.46 | 0.05 | 0.14 | 0.05 | 0.46 |
| Spray | 5 | 6.96 | 1.19 | 1.33 | 5.17 | 0 | 0.079 | 0.121 | 0.85 | 0.10 | 0.25 | 0.11 | 0.85 |
| | 10 | 7.23 | 0.79 | 1.68 | 4.26 | 0 | 0.066 | 0.085 | 0.60 | 0.07 | 0.17 | 0.07 | 0.60 |
| | 20 | 7.29 | 0.75 | 1.81 | 3.98 | 0 | 0.052 | 0.069 | 0.54 | 0.05 | 0.15 | 0.05 | 0.54 |

Abbreviation: EC: electrical conductivity; OM: organic matter

## 2. Effect of folic acid on growth parameters

Treatments of *Spinancia oleracea* with folic acid (either soaking or spraying) increased fresh and dry weight of this plant shoot, meanwhile water percentage showed increase by spraying with 5mM and decrease in response to other folic acid treatments, as compared to control value (Fig. 1).

As regard the effect of different concentrations of folic acid on growth parameters of *Lactuca sativa* shoot, a variable decrease in shoot fresh weight was observed. Dry weight of *Lactuca sativa* shoot decreased by soaking in 20 mM folic but, increased by the other folic treatments. Meanwhile water percentage showed decrease in its value, comparing to the value of control (Fig. 2).

The pattern of changes in the growth parameters of *Chorchorus olitorius* shoot, as affected by folic acid treatments cleared that, soaking in 10&20 mM and spraying with 5 mM increased fresh weight but the other treatments decreased this parameter. Dry weight decreased by soaking in 5 mM and spraying with 20 mM but increased by the other treatments. Only spraying with 10&20 mM folic increased water percentage of *Chorchorus olitorius* shoot, but the other folic treatments decreased this percentage, comparing to the value of control (Fig. 3).

In this respect, folic acid has positive effect on growth of soybean [45] and on strawberry [46]. Essentially, folic acid helps plants to grow more heavily and healthy [47]. Moreover, results of Youssif [48] led to demonstrate that application of vitamins; cobalamin (B12), folic acid (B9) or ascorbic acid (V.C) whether foliar spray solely or in combination had a significant positive effect on growth, yield and chemical composition, of potato plants grown in sandy soil. The results of folic acid are in agreement with those obtained by Stakhova et al. [79] on peas, Al-Said & Kamal [50] on sweet potato.

In the current study, the increment; in some cases, in fresh and dry weight of the test plants in response to the used treatments was accompanied with improvements in pigments, total carbohydrates and protein. In support, Ibrahim et al. [51] found that folic acid improved significantly growth parameters including plant length of potato plants, leaf area, haulm dry weight and chlorophyll compared to the controls in both seasons. Additionally, total soluble carbohydrates and total soluble protein were increased significantly in the leaves.

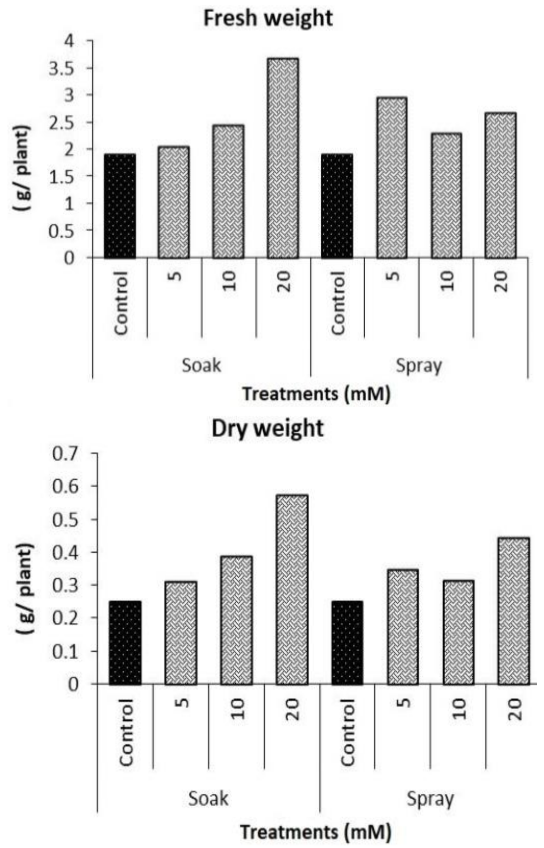
From other side of view, in the current study, it can be concluded that an adequate amount of nutrients in response to the doses of folic acid that being applied in the soil, were taken up in the root tissues as such, and consequently translocated and assimilated within the used plants and hence caused the observed responses; either decrease or increase in the determined growth parameters. This in agreement with Haroun [52] who stated that, the importance of vegetative growth in controlling the plant dry matter and growth has been appreciated. Thus, it could be concluded that, the rate of plant growth expansion has great influence on dry matter production.

As it clear from the changes that detected in the pH of the soil where the treated *Spinancia oleracea, Lactuca sativa* and *Chorchorus olitorius* planted, in response to folic applications that led to increase in the availability of nutrients that reflected on growth and metabolism of the test plants. In accord, Ayas and Gulser [53] reported that the efficiency of the use of organic additives strongly depended the quality of organic matter. Moreover, soil reaction has a significant effect on plant nutrient availability and the plant nutrients are usually the most available among 6.5-7.5 pH ranges.

### 3. Effect of folic acid in pigments content

Quantitative proportions of chlorophyll a, b and carotenoids are important for controlling photosynthesis intensity [54]. As can see from the results of this study, the effect of different concentrations of folic acid on photosynthetic pigments of *Spinancia oleracea* leaves cleared that, chlorophyll a, chlorophyll a+b, carotenoids and total pigments increased by soaking in 5 mM folic acid and decreased by the other treatments. On the other hand, chlorophyll b decreased by all the used folic acid treatment as compared to control value (Fig. 4).

As cleared in (Fig. 5), folic acid treatments to *Lactuca sativa* increased chlorophyll a, chlorophyll a+b and total pigments by the used treatments, except for soaking in 10 mM which decreased these pigments. Chlorophyll b, either decreased by soaking or increased by spraying. Carotenoids of *Lactuca sativa* decreased only by soaking in 5 mM and increased by the other treatments, as compared to control value.



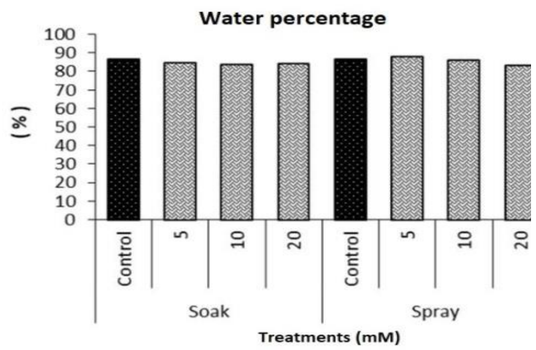
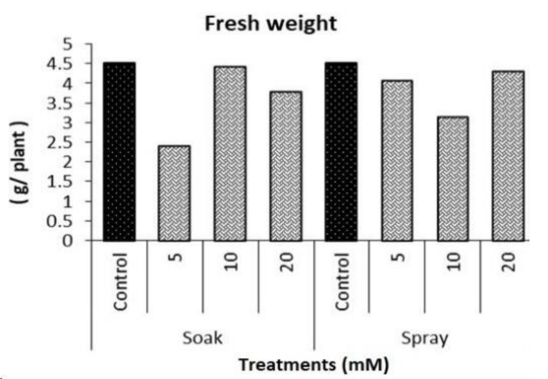




**Figure 1.** Effect of different concentrations of folic acid on growth parameters of *Spinancia oleracea* shoot.



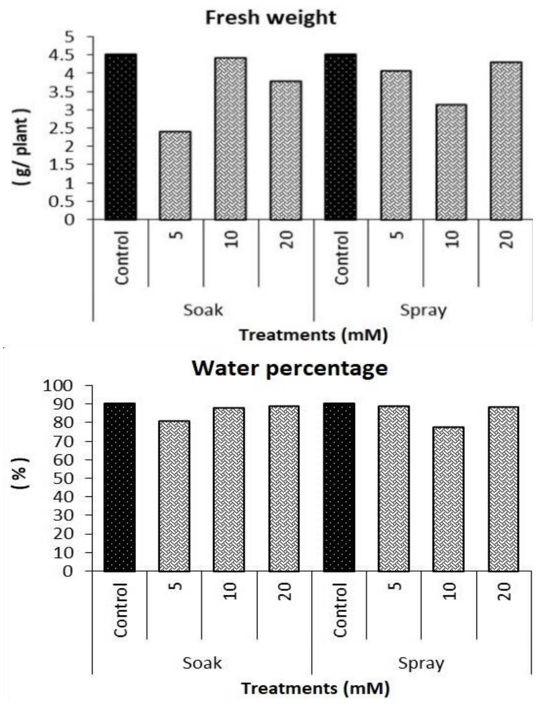



**Figure 2**. Effect of different concentrations of folic acid and humic acid on growth parameters of *Lactuca sativa*.

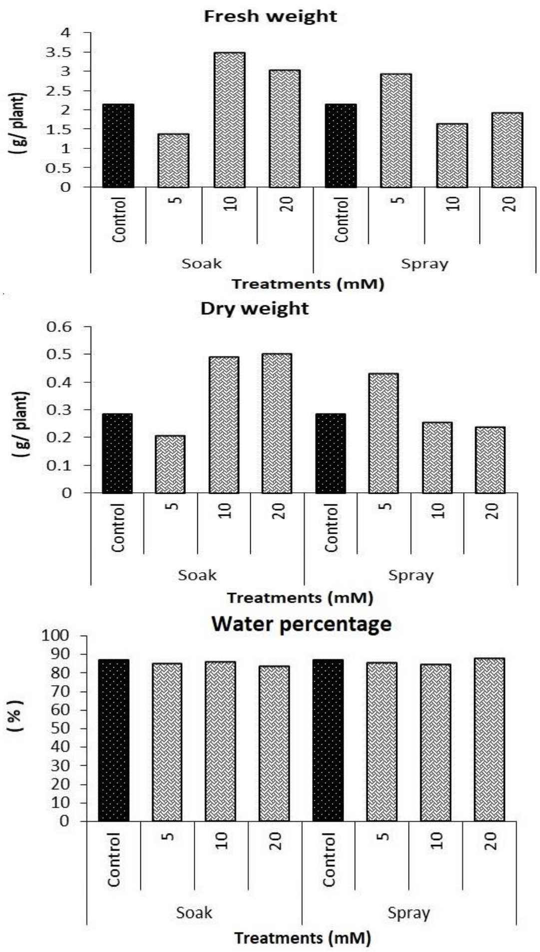



**Figure 3.** Effect of different concentrations of folic acid and humic acid on growth parameters of Chorchorus olitorius shoot.

As regards the effect of different concentrations of folic acid on photosynthetic pigments of *Chorchorus olitorius* leaves, an increment was detected in chlorophyll a, chlorophyll a+b, carotenoids and total pigments but chlorophyll b decreased by the used folic treatments, as compared to control value (Fig. 6).

In this respect, Stakhova et al. [49] and Burguieres et al. [55] obtained that folic acid treatment increased the content of chlorophyll in the leaves of *Pisum sativum*. Moreover, Stakhova et al. [49] and Burguieres et al. [55] stated that folic acid treatment increases the productivity of *Pisum sativum* by increasing the content of chlorophyll in the leaves.

In this investigation, the stimulation in the pigment contents by the application of the used treatments to the tested plants (*Spinancia oler acea, Lactuca sativa* and *Chorchorus olitorius*) are coincided with the increments in the available nutrients in soil and in consequence in the determined metabolites, in this connection, the chlorophyll synthesis is depending on mineral nutrition [56]. In general, the photosynthesis and related physiological processes are directly linked to the plant nutrients content [57,58].

In the current study, the observed increments, in some cases in the photosynthetic pigments of folic-treated test plants are supported by the stimulation of shoot fresh and dry weight. In this concern, Long *et al*. [59] showed a close relationship between enhanced photosynthesis, biomass and yield, which suggests that increasing photosynthesis increasing yield when other genetic factors are not altered. On the other hand, the decline in photosynthetic pigments of some treated plants may suggest that these treatments inhibit pigment biosynthesis.

From other side of view, the present variable changes in the photosynthetic pigments of *Spinancia oleracea, Lactuca sativa* and *Chorchorus olitorius* in response to various used treatments may be attributed to (a) increased photosynthetic electron transport [60], (b) stimulation of pigment biosynthesis [61] and (c) interaction with the thylakoid membrane surface [62].

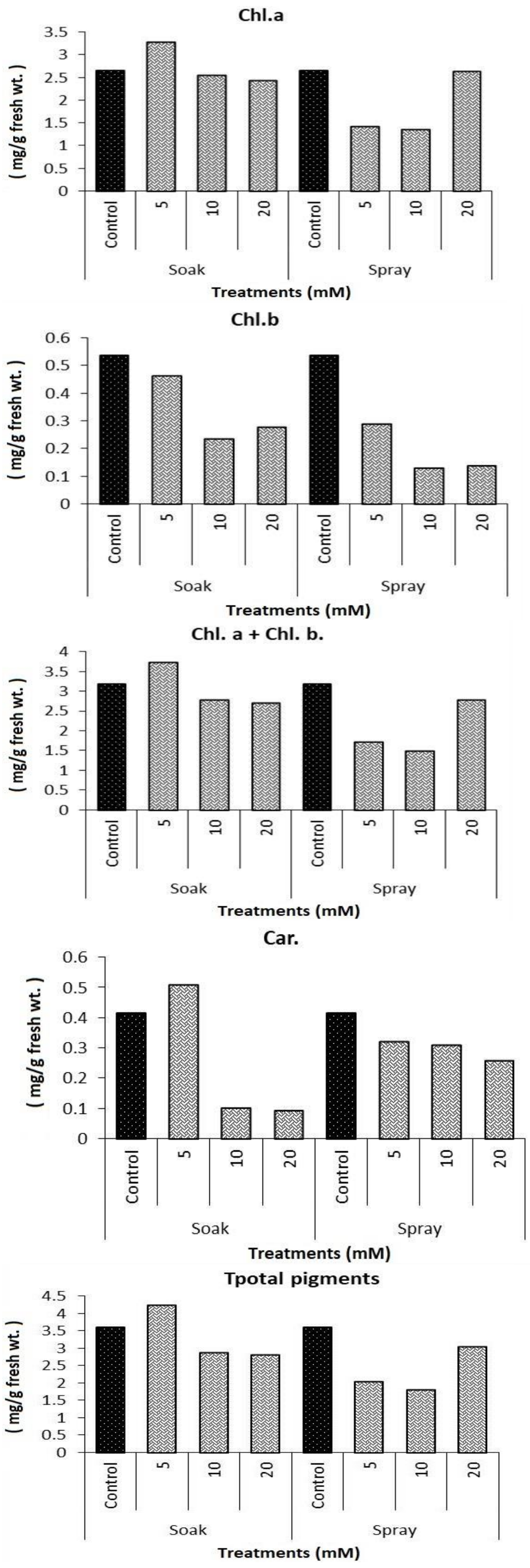



**Figure 4**. Effect of different concentrations of folic acid on photosynthetic pigments of *Spinancia oleracea* leaves.

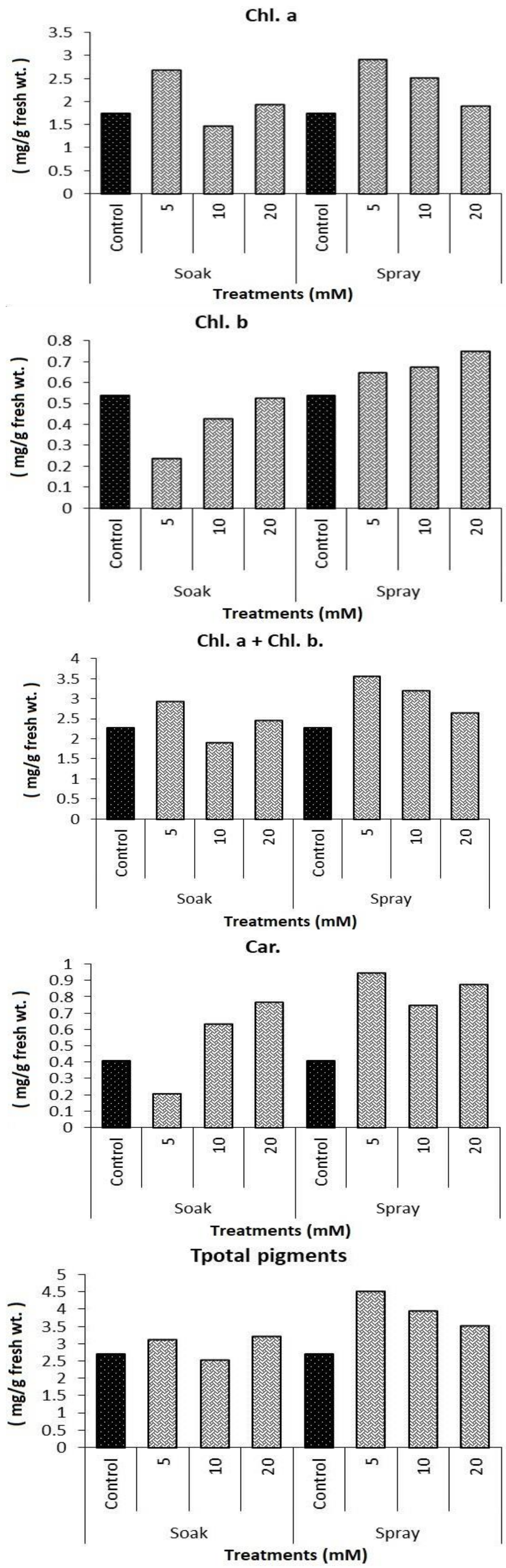



**Figure 5.** Effect of different concentrations of folic acid on photosynthetic pigments of *Lactuca sativa* leaves.

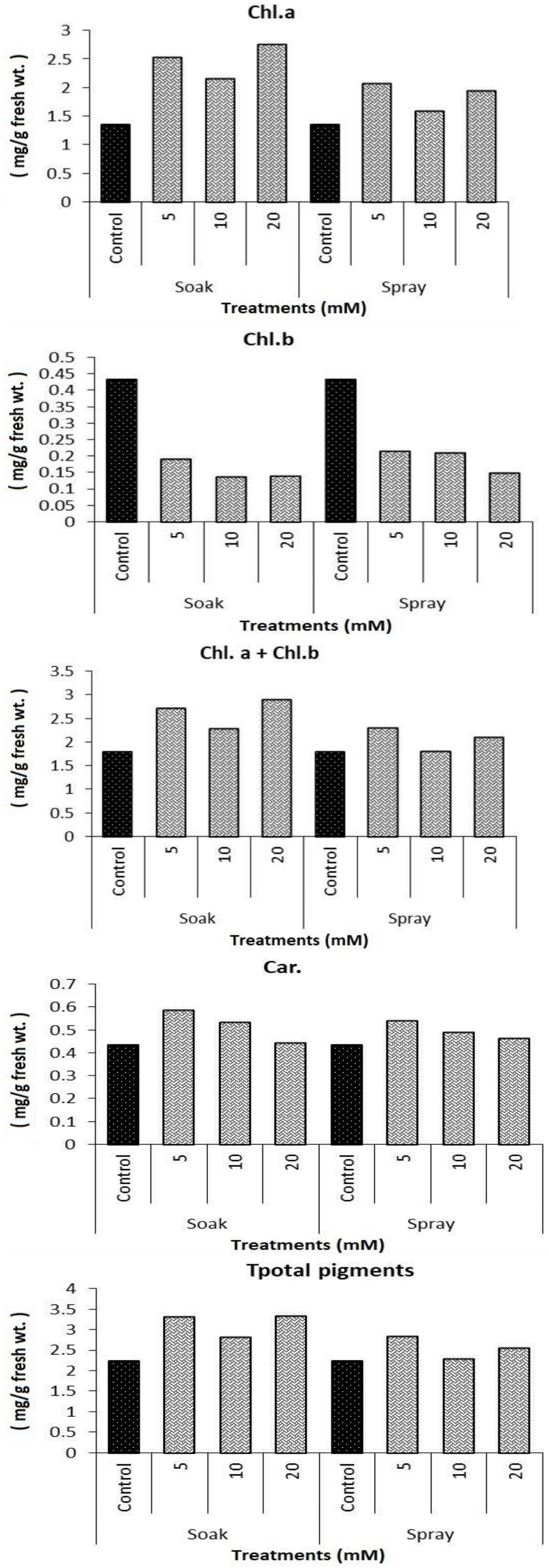



**Figure 6.** Effect of different concentrations of folic acid on photosynthetic pigments of *Chorchorus olitorius* leaves.

## 4. Effect of folic acid on some metabolites

With respect to the effect of different concentrations of folic acid and on some metabolites of *Spinancia oleracea* shoot, total carbohydrates, total nitrogen and protein increased by the used folic treatments, except for spraying with 5 mM folic which decreased these parameters. Also, total phenols and alkaloids increased by all the used folic treatments (Fig. 7).

The determined metabolites of *Lactuca sativa* shoot as affected by folic acid treatments (Fig. 8) showed that, total carbohydrates increased by all the used folic treatments, total nitrogen and protein decreased by the used folic treatments, except for spraying with 10 mM folic which increased these parameters. While total phenols increased by soaking in 10&20 mM and spraying with 10 mM but, decreased by the other treatments. Alkaloids increased by soaking in 10 mM and spraying with 10 mM but, decreased by the other treatments, as compared to control value.

Concerning the effect of different concentrations of folic acid on the determined metabolites of *Chorchorus olitorius* shoot, it was found that, total carbohydrates decreased by soaking in 20 mM but, increased by the other treatments. Total nitrogen and protein decreased by soaking in 10&20 mM but, increased by the other treatments. Total phenols increased by the used treatments except by spraying with 5&10 mM that decreased this metabolite. Also, alkaloids increased by the used treatments except when spraying with 5 mM folic that decreased this metabolite, comparing to the value of control (Fig. 9).

In this concern, folates were reported to play important roles in signaling cascades [63], as well as in nitrogen and carbon metabolism [64,65]. In support, Li et al. [46] reported that folate is one of the most important micronutrients and has cofactor activity. Results of Youssif [48] led to demonstrate that, the highest significant value of starch, total sugar % and total chlorophyll were obtained by the treatment of highest level 150 ppm folic acid in both seasons. These results are in conformity with those obtained by Li et al. [46] on strawberry.

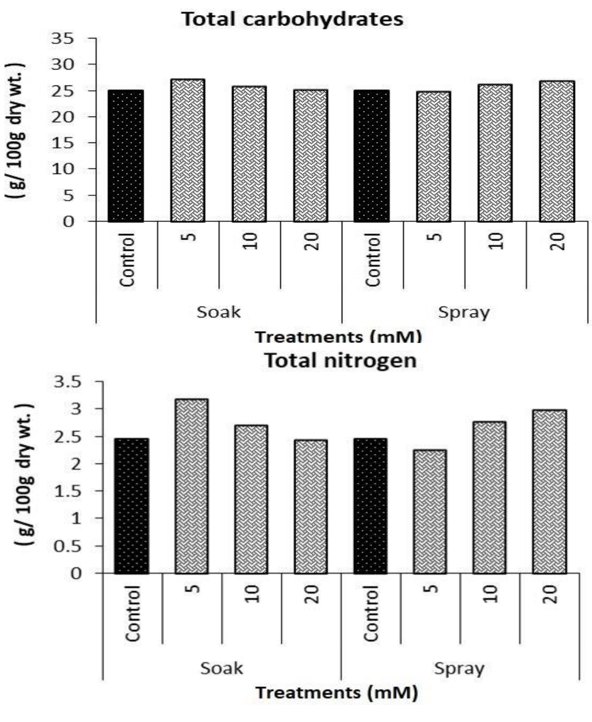
Folates are indispensable components of metabolism in all living organisms [66] are not only of primary importance in regulation of gene expression, but are also necessary for the synthesis of lipids, proteins, chlorophyll and lignin.

In accord with the results of this study that recorded increments in pigments, total carbohydrates as well as protein contents in the shoot of the test plants, in response to folic acid, Ibrahim *et al*. [51] found that folic acid improved significantly total chlorophyll, total soluble carbohydrates and total soluble protein compared to the controls in both seasons.

### 5. Effect of folic on antioxidant enzymes activity

As regarding the effect of various concentrations of folic acid on some antioxidant enzymes activity of *Spinancia oleracea* shoot, it is apparent that, polyphenol oxidase activity decreased by all the used folic treatments. On the other hand, peroxidase and catalase activity increased by all the used folic treatments (Fig. 10).

In studying the effect of different concentrations of folic acid on some antioxidant enzymes activity of *Lactuca sativa* shoot (Fig. 11), it was detected that, polyphenol oxidase activity increased by the folic treatments but decreased by soaking in 20 mM and spraying with 20 mM. On the other hand, peroxidase and catalase activity increased by spraying with 5 mM but decreased by the other treatments, comparing to the value of control.



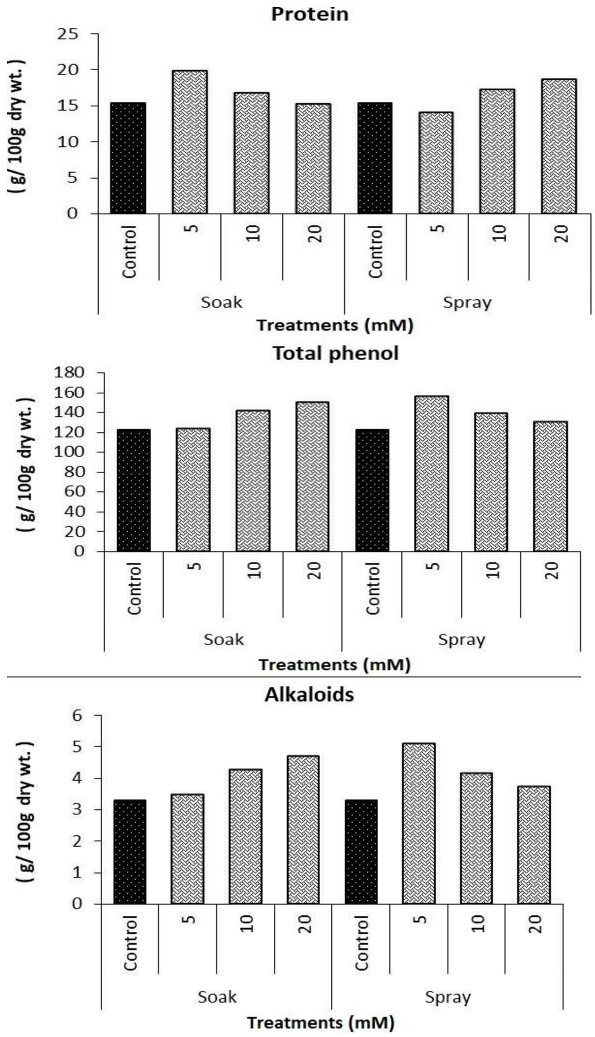
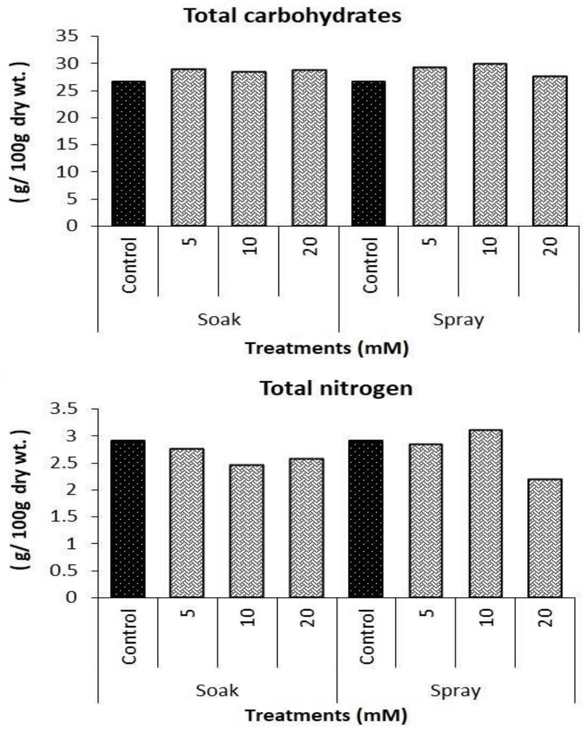
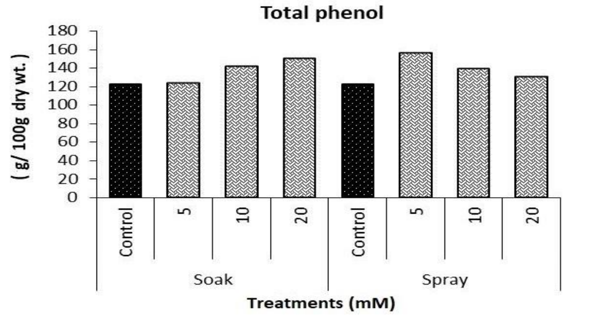




**Figure 7**. Effect of different concentrations of folic acid on some metabolites of *Spinancia oleracea* shoot.





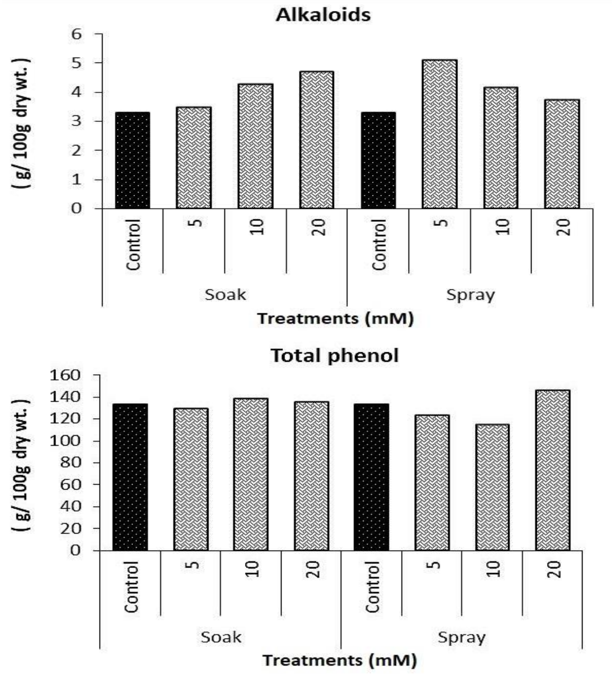
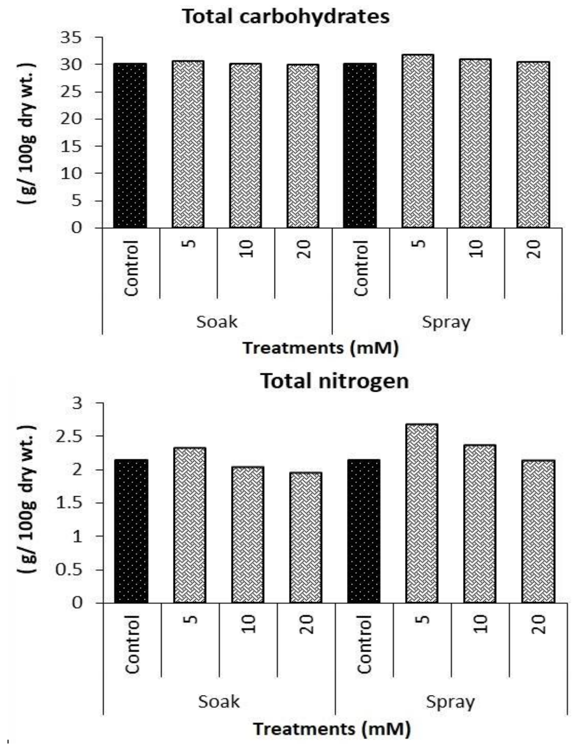




**Figure 8.** Effect of different concentrations of folic acid on some metabolites of *Lactuca sativa* shoot.



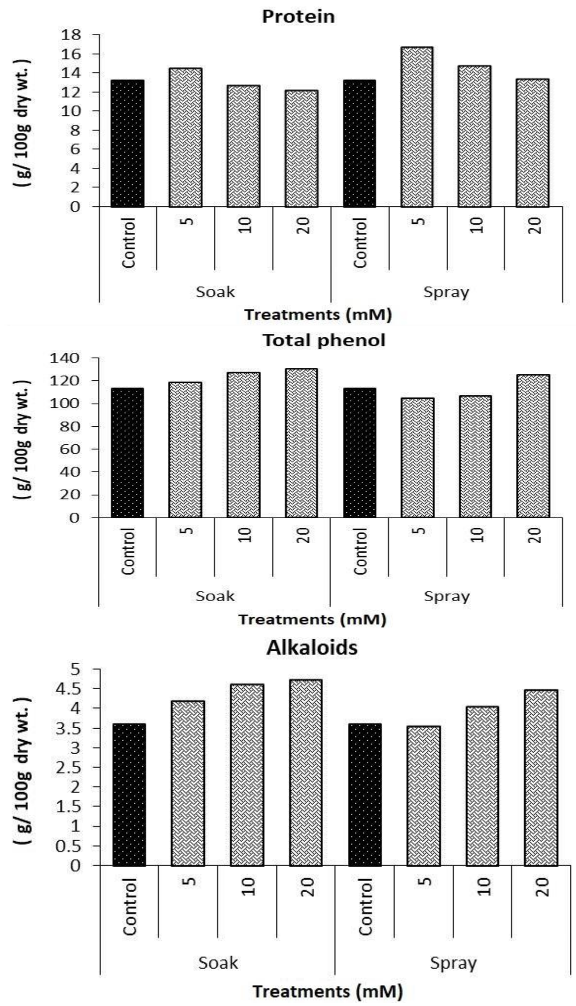




**Figure 9.** Effect of different concentrations of folic acid on some metabolites of *Chorchorus olitorius* shoot.

The determined antioxidant enzymes activity of *Chorchorus olitorius* shoot as affected by different concentrations of folic acid (Fig. 12) showed that, polyphenol oxidase activity increased by the used folic treatments except when soaked in 20 mM and spraying with 10 mM folic that decreased the activity of this enzyme. On the other hand, peroxidase and catalase activity increased and decreased by all the used folic treatments, respectively.

Various enzymes working in defense mechanism in plants are synthesized and protect the plant if necessary. Free electron as a reaction to stress and accordingly a significant increase in free radical levels have occurred. The primary enzyme that is actively included in the functioning of the defense mechanism in all of them is peroxidase, that has a role in the plant defense reactions. Peroxidase is synthesized in chloroplasts in many plants [67].

In this respect, FA differently enhanced the growth of *Phaseolus vulgarus* [68]. Moreover, they are responsible for the growth patterns of the plants such as leaf development, stem and root elongation, flowering etc. and they are included in some biological process such as the production of plant growth-influencing substances as free enzymes [69].

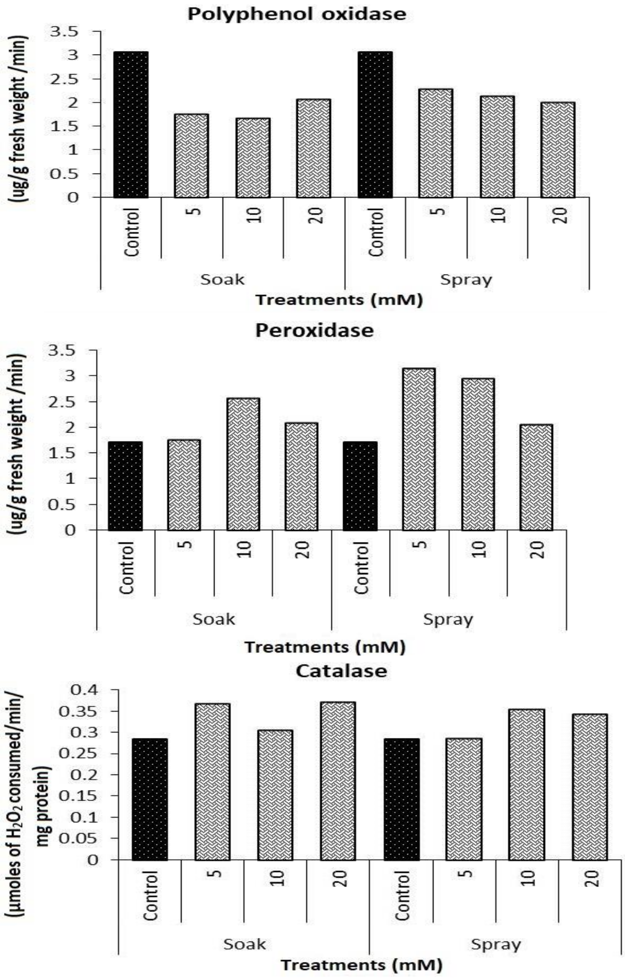
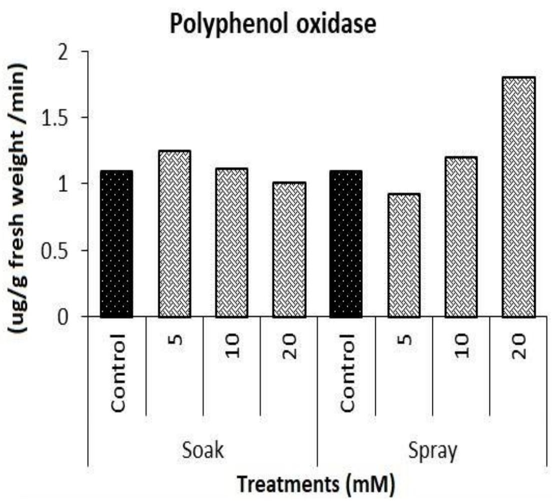



**Figure 10**. Effect of different concentrations of folic acid on some antioxidant enzymes activity of *Spinancia oleracea* shoot



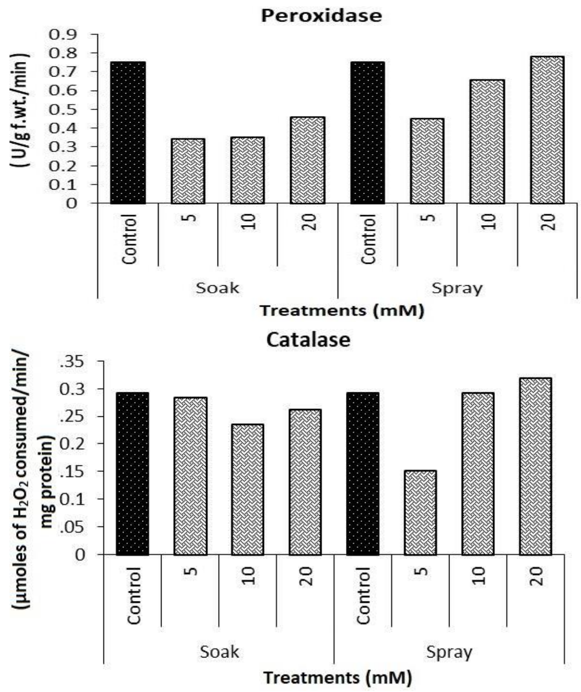




**Figure 11.** Effect of different concentrations of folic acid on some antioxidant enzymes activity of *Lactuca sativa* shoot

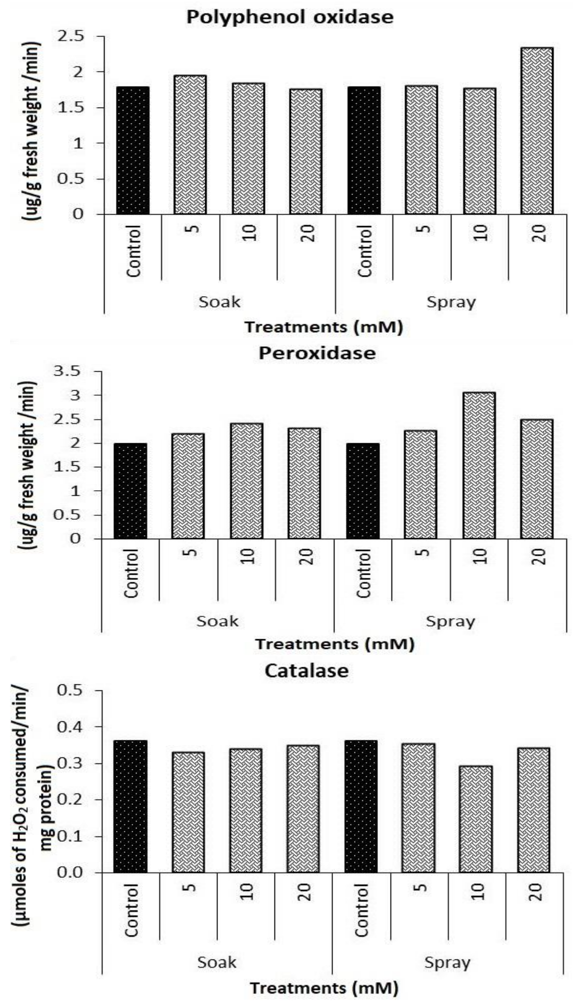



**Figure 12.** Effect of different concentrations of folic acid on some antioxidant enzymes activity of *Chorchorus olitorius* shoot.

## 6. Effect of folic acid on some elements

It was detected by examining the effect of different concentrations of folic acid on some elements of *Spinancia oleracea* shoot that, sodium decreased by all the used folic treatments. Potassium also, increased by the used folic treatments, except when spraying with 10 mM folic that decreased this element. Also, calcium increased by the used folic treatments, except when spraying with 5&10 mM folic that decreased this element. On the other hand, magnesium increased by spraying with 5&10 mM folic and decreased by the other used folic treatments, comparing to the value of control (Fig. 13).

As cleared in (Fig. 14) that, folic acid treatments decreased sodium in *Lactuca sativa* shoot. On the other hand, the other determined elements (potassium, calcium and magnesium) increased by all the used folic treatments.

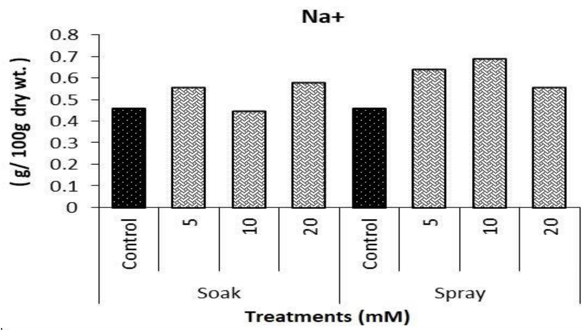
Sodium decreased in *Chorchorus olitorius* shoot in response to folic acid treatments. Potassium and calcium increased by the used folic treatments, except when spraying with 20 mM folic that decreased these elements. Also, magnesium increased by the used folic treatments, except when soaking in 5 mM folic that decreased this element, comparing to the value of control (Fig. 15).

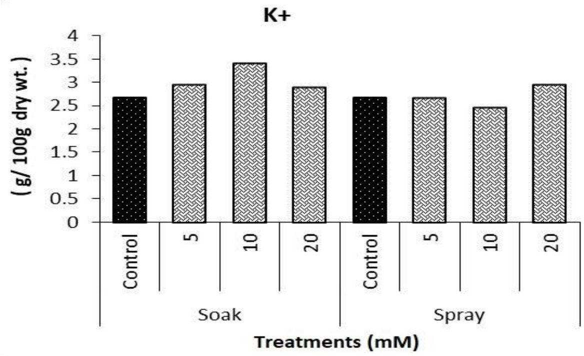
In accordance, an increase in membrane permeability would in turn facilitate the absorption and utilization of mineral nutrients and the transport of assimilate in plants [43,44]. This would also contribute toward enhancing the capacity of biomass production by plants as reflected by the increase in fresh and dry weights of plants in the present investigation.

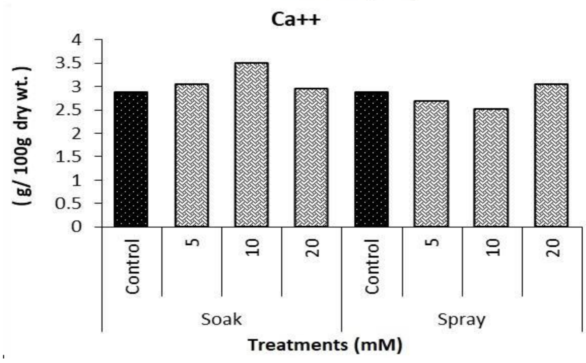
It is worthy to mention herein that, *Corchorus olitorius* showing; at most, the best responses in relation to the used treatments especially with the highest concentration of folic acid (20mM), this may be due to the fact that this plant being contains low concentrations of folic acid. In accord, folic acid essentially helps plants to grow more heavily and healthy, because folic acid exists in plants but decomposes rapidly when exposed to strong light, giving plants additional folic acid during the planting process is recommended [47].

In confirm, many literatures reported that *Spinancia oleracea* and *Lactuca sativa* contain considerable amounts of folic acid whereas, *Chorchorus olitorius;* contain very low folic; if present hence, the most response by folic addition was in case of *Chorchorus olitorius*. In this concern, folate is necessary for a healthy plant to grow and most plants are able to produce their own folic acid. It can be found in high levels naturally in some plants, especially is present in dark green leafy vegetables. However, you can assist a plant that is deficient in this vitamin by giving it a fertilizer that contains these vitamin supplements [7].

By way of comparison in this study, foliar application of folic acid (spraying) was most effective than pretreatments (soaking). In support, the foliar application of organic fertilizers can supply nutrients more rapidly than methods involving root uptake which made the local growers use foliar fertilizers to supplement soil applied nutrients to compensate for decreased root activity [70].



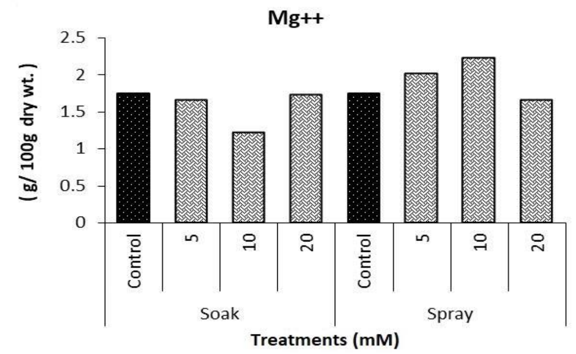



**Figure 13**. Effect of different concentrations of folic acid on some elements of *Spinancia oleracea* shoot

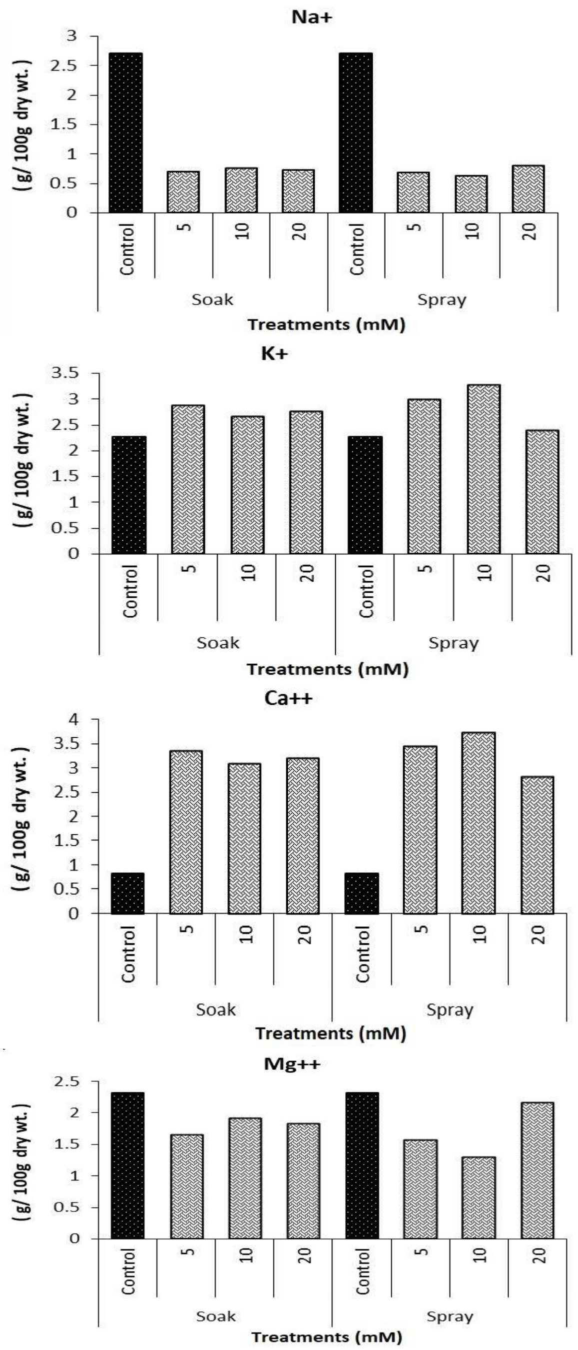



**Figure 14**. Effect of different concentrations of folic acid on some elements of *Lactuca sativa* shoot

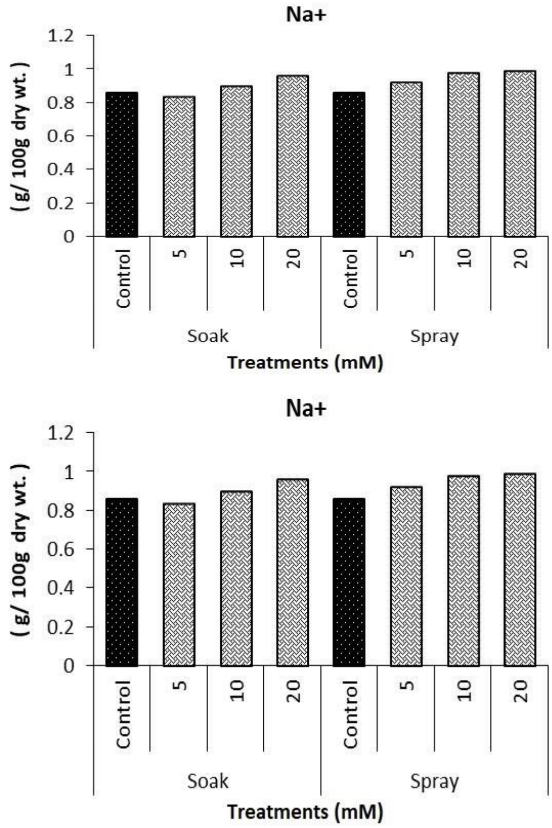



**Figure 15**. Effect of different concentrations of folic acid on some elements of *Chorchorus olitorius* shoot

## Conclusion

It became evident from this study that exogenous application (by soaking the seeds or spraying the plant) of folic acid; mainly spraying, appeared to supply extra quantities that being implicated in the observed improvement of the vegetative growth of *Spinancia oleracea*, *Lactuca sativa* and *Chorchorus olitorius* plants. But the three plants appeared to differ with respect to the optimum concentration of the used compounds required for best growth. Thus, under the present experiment conditions; although *Spinancia oleracea* and *Lactuca sativa* showing the optimum responses in relation to the low concentrations of folic acid (5&10mM), *Corchorus olitorius* showing the optimum responses in relation to the highest concentration of folic acid (20mM), this may be due to the fact that this plant being contain low concentrations of folic acid. In addition, the disparity of changes in the determined metabolite contents, antioxidant enzymes activity and in the associated growth parameters of the three test plants, are consequences of contents and several roles played by folic acid which are known to cause change in the soil and its components as well as nutrients availability and uptake.

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