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Aspirin and Kinetin Pretreatments Counteract the Harmful Effect of Drought Stress on Lettuce Plant

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Abstract: A pot experiment was carried out to study how pretreatments in aspirin (0.3 &0.6m M) or kinetin (50&100 ppm) counteract the harmful effects of drought stress (by irrigation with 50% of water field capacity) on lettuce plant during vegetative stage. The present results revealed that drought stress significantly decreased shoot length, No. of leaves, No. of nodes, fresh & dry weights, chlorophyll a, chlorophyll b, chlorophyll a/b, carotenoids and total pigments, in lettuce plants, after 25 days from sowing. These deleterious effects that caused by drought mitigated; either partially by soaking lettuce seeds in 0.3 mM aspirin or 50 ppm kinetin or completely by soaking lettuce seeds in 0.6 mM aspirin or 100 ppm kinetin. In general, this investigation confirms the ability of aspirin and kinetin in improving growth of lettuce as well as nullifying the harmful effects of water shortage in irrigation.

Keywords: Lettuce, drought, aspirin, kinetin, growth parameters**,** photosynthetic pigments**.**

1.Introduction

Abiotic stress is a main factor in limiting plant growth and food production in many regions of the world **[1]**. Environmental stresses as salinity and drought reduce growth and agricultural productivity more than other factors **[2].** Plants exposed to mild drought stress exhibit a modified architecture that restricts shoot growth, while root growth remains unaffected. In dicots, a number of branches and leaves on the branches are sensitive to water-limited supply. This modified architecture is an adaptive strategy that inhibits photosynthesis productivity, thereby altering the carbon flow to various sink organs **[3]**.

Lactuca sativa commonly known as lettuce is a genus of flowering plants in the daisy family, Asteraceae. The genus includes at least 50 species, distributed worldwide, but most wild lettuces are xerophytes, adapted to dry habitat types. Some occur in moister areas, such as the mountains of central Africa **[4,5]** Lettuce (*Lactuca sativa* L.) is among the top five most commonly consumed vegetables in the United States **[6]**. On the other hand, leafy vegetables are characterized by high nitrate accumulation, constituting an important dietary source of nitrate for humans **[7].**

Aspirin (also known as acetylsalicylic acid) with a formula: $C_9H_8O_4$ is a synthetic copy of salicylic acid. This acid, resembling what's in modern-day aspirin, can be found in jasmine, beans, peas, clover and certain grasses and trees **[8].** Some signs hint that the drought tolerance of a given plant can improved with the use of aspirin this might actually be true **[9].** He stated that while salicylic acid is more often examined for this role, acetylsalicylic acid may also be effective at improving plant tolerances to various issues. The effectiveness of using aspirin in this way goes back to the systemic acquired resistance (SAR). Just as aspirin triggers the plant's defense systems against bacterial infections, it can trigger the plant's defense systems to stimulate it to protect itself from other conditions.

Exogeneous application of kinetin (N6furfuryladenine) behaves as a strong natural antioxidant and protects proteins and DNA from oxidative damage **[10]** and positively affect grain filling in rice **[11].** On the other hand, in kinetin- treated rice seedlings with 2 mg/liter by spraying, kinetin

significantly increased plant fresh & dry weights, plant height, daily growth rate and chlorophyll content as compared with untreated seedling **[12].** Kinetin enhanced shoot growth and grain yield, and also reduced membrane injury by dehydration and improved the water status of plant under both aerobic and anaerobic conditions. Kinetin had a dominant effect on the stability of chlorophyll and soluble sugar contents **[13].** Moreover, kinetin stimulated the synthesis of polysaccharides and materials of the new cell walls in kinetin –treated *Phaseolus vulgaris* plants **[14**].

This investigate was conducted to study the effect of pretreatment of aspirin (0.3 & 0.6 mM) or kinetin (50&100 ppm) on ameliorating the harmful effects of drought stress (by irrigation with 50% WFC) on lettuce plants by following up growth and the contents of pigments of *Lactuca sativa* during vegetative stage (25 days old).

2. Materials and methods

2.1. Time course of the experiment

Pure strain of lettuce (*Lactuca sativa* L; green cultivars) is obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. A green house experiment was conducted in growth conditions at 25-30°C temperature under 16 h light and 8 h dark photoperiodic cycle with a light intensity of 350 μmol m^{-2} s^{-1} and with a relative humidity of 50%. Seeds were surface sterilized with soaking in 0.01% $HgCl_2$ solution for 3 minutes. After washed thoroughly with distilled water, the seeds were divided into 5 equal groups each contains 100 seeds which soaked for 12 hours; group 1(in water, to serve as control), group 2&3 (in 0.3 &0.6 mM aspirin) and group 4&5 (in 50 & 100 ppm kinetin). Each group divided into two subgroups according to water irrigation; the first subgroups irrigated with 100% water field capacity (WFC) of soil and the second subgroups irrigated with 50% WFC. *Lettuce* seeds were cultivated in pots (30 cm in diameter) containing equal amounts of sand: clay (1:2 v/v) soil. Ten seeds were sown in each pot with three replicates for each treatment. At the same time super phosphate fertilizer (0.5 g/pot) was added to the soil before sowing. The pots were divided into ten subgroups representing the following treatments,

T1: irrigated with 100% WFC+ pretreatment in water

T2: irrigated with 50% WFC+ pretreatment in water

T3: irrigated with 100% WFC + pretreatment in 0.3 mM aspirin

T4: irrigated with 100% WFC + pretreatment in 0.6 mM aspirin

T5: irrigated with 50% WFC + pretreatment in 0.3 mM aspirin

T6: irrigated with 50% WFC + pretreatment in 0.6 mM aspirin

T7: irrigated with 100% WFC + pretreatment in 50 ppm kinetin

T8: irrigated with 100% WFC + pretreatment in 100 ppm kinetin

T9: irrigated with 50% WFC + pretreatment in 50 ppm kinetin

T10: irrigated with 50% WFC + pretreatment in 100 ppm kinetin

During vegetative stage (25 days from sowing) samples were collected to assess growth parameters (shoot length, number of nodes/plants, number of leaves/plants, shoot fresh weight, shoot dry weight and shoot water percentage as well as pigments content.

2.2. Determination of photosynthetic pigments:

Using the spectrophotometric approach described by **[15]** for chlorophylls and **[16]** for carotenoids as adopted by **[17]**, the plant photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were estimated in the leaves sample.

Statistical analysis: All data obtained from the experiments are expressed as the mean ± standard error of three replications. All statistical analyses were performed with CoStat statistical package version 6.311. One-way ANOVA was conducted to compare the means. Differences between treatments were separated by the least significant difference test at 0.05 probability level.

3. Results and Discussion

3.1 Effect of drought, aspirin or kinetin n growth parameters

The results given in figure 1 indicated that, compared to control (irrigated with 100 WFC + 0 aspirin) shoot length *Lactuca sativa* decreased significantly by irrigation with 50% WFC but this decline became non-significantly by aspirin pretreatment; especially 0.6 mM that record 4,0 cm. Meanwhile, aspirin pretreatment (0.3 &0.6 mM) to that irrigated with 100 WFC increased lettuce shoot length significantly with the maximum value by 0.6 mM (4.8 cm). Fresh weight significantly decreased by irrigation with 50% WFC and by 0.3 mM aspirin+ irrigation with 50% WFC but, irrigation with 50% WFC + 0.6 mM aspirin decreased this parameter non-significantly. Whereas, aspirin pretreatment (0.3 &0.6 mM, respectively) to that irrigated with 100% WFC increased lettuce shoot fresh weight non-significantly and significantly with the maximum value by 0.6 mM (6 g). As comparing to control value of shoot dry weight, no difference recorded by irrigation with 50% WFC, this parameter increased by the other used treatments non-significantly; in the plant irrigated with 100 WFC + 0.6 mM aspirin and irrigated with 50% WFC + 0.3 mM aspirin and significantly; in the plant irrigated with 100% WFC + 0.3 mM aspirin and irrigated with 50% WFC + 0.6 mM aspirin with the same value (1.4 g). The water percentage of lettuce shoot increased non-significantly; in the plant irrigated with 100 WFC + 0.6 mM aspirin (79.81%) and decreased by the other used treatments; significantly by irrigation with 50% WFC, 50% WFC + 0.3 mM aspirin and 50% WFC + 0.6 mM aspirin or non-significantly by irrigation with 100% WFC + 0.3 mM aspirin. The number of leaves/plant and the number of nodes/ plant non-significantly affected by the used treatments; increased by irrigation with 100% WFC + 0.3 mM aspirin and by irrigation with 100% WFC + 0.6 mM aspirin and decreased by the other treatments.

As comparing to control, shoot length of *Lactuca sativa* plant affected significantly by the used treatments; decreased by irrigation with 50% WFC either alone or + 50 & 100 ppm kinetin pretreatment. Meanwhile, pretreatment with 50 & 100 ppm kinetin to that irrigated with 100% WFC increased lettuce shoot length significantly with the maximum value by 100 ppm (5.2 cm). Irrigation with 50% WFC decreased fresh weight either significantly by soaking in 0 & 50 ppm kinetin or non-significantly by soaking in 100 ppm kinetin, irrigation with 100% WFC + pretreatment with 50 & 100 ppm kinetin increased lettuce fresh weight significantly with the maximum value by pretreatment with 100 ppm to that irrigated with 100% WFC (5.2 g). Dry weight of lettuce plant decreased non- significantly by irrigation with 100% WFC + 50 & 100 ppm kinetin pretreatment but this parameter increased significantly by the other used treatments; the maximum value by pretreatment with irrigation with 50% WFC + soaking in 0 & 50 ppm kinetin (1.1 g). In case of the calculated water percentage of lettuce plant, irrigation with 50% WFC decreased this ratio either significantly by soaking in 0 & 50 ppm kinetin or non-significantly by soaking in 100 ppm kinetin, irrigation with 100% WFC + pretreatment with 50 & 100 ppm kinetin increased lettuce water percentage non-significantly with the maximum value by pretreatment with 100 ppm to that irrigated with 100% WFC (86.7 %). The number of leaves/plant and the number of nodes/plant non-significantly affected by the used treatments; increased by irrigation with 100% WFC + 50 &100 ppm kinetin and decreased by irrigation with 50% WFC + soaking in 0 & 100 ppm kinetin, irrigation with 100% WFC + soaking in 50 &100 ppm kinetin record 5, the maximum values (Figure 2).

One effect of the application of SA is an improvement in plant growth under normal conditions as well as under drought stress, due to (i) the stimulation of biosynthesis of phytohormones, (ii) the stimulation of soil nutrient absorption and solubilization, and (iii) the stimulation of root hardness and growth **[18].** Also, SAare considered multifunctional plant-growth regulators. They help alleviate the oxidative damage from various stresses **[19]** and increase gene expression involving cell division, cell expansion, photosynthesis, metabolism, and hormonal balance **[20,21].** In the current results, the significant increments in the determined growth parameters by aspirin and kinetin

pretreatments which was decreased by the drought treatment may be due to the fact that, aspirin treatments suggested to inverse the loss of water by acting as antitranspirant agent as well as inhibiting the opening of stomata. In harmony, different levels of acetylsalicylic acid appeared to function as antitranspirant in leaves of *Phaseolus vulgaris* **[22]** and inhibiting the opening of stomata in epidermal strips of *Commelina communis* **[23].** In addition, **[24]** proved that, Kinetin-induced modification in growth criteria, ion contents and water relations of sorghum plants treated with cadmium chloride of sorghum plant. On the other hand, kinetin acts as anti-stress and as a strong antioxidant *invitro* and *invivo,* with potential beneficial uses in agriculture and human health care **[25].**

3.2. Effect of drought, aspirin or kinetin on photosynthetic pigments content:

Examining the data in figure 3 showed that treatment with 0.6 mM and 0.3 mM aspirin under irrigation with 100% WFC increased chlorophyll a content in leaves of *Lactuca sativa* plant significantly with the maximum value in case of treatment with 0.6 mM aspirin (3.46). On the other hand, other treatments decreased this pigment significantly. The pattern in chlorophyll b content changes is more or less similar to that recorded in chlorophyll a, with the maximum value in case of irrigation with 100% WFC + 0.6 mM aspirin (2.84). As regarding carotenoids, treatment with 0.6 mM and 0.3 mM aspirin under irrigation with 100% WFC increased carotenoids content significantly with the maximum value in case of treatment with 0.6 mM aspirin (2.12). But other treatments decreased this pigment; either significantly by treatment with 0.3 mM aspirin under irrigation with 50% WFC and under irrigation with 50% WFC or non- significantly by treatment with 0.6 mM aspirin under irrigation with 50% WFC. The response of total chlorophylls content of lettuce leaves to the used treatments has, consequently similar to that observed in case of chlorophyll a and chlorophyll b with the maximum value in case of treatment with 0.6 mM aspirin (6.3) under irrigation with 100% WFC and a minimum value in case of irrigation with 50% WFC (2.5). Chlorophyll a/ chlorophyll b ratio increased significantly and non- significantly by 50% WFC alone and + 0.6 mM aspirin pretreatment, respectively but decreased non- significantly by the other used treatments. The maximum value of this ratio (2.35) recorded in case of irrigation with 50% WFC and a minimum value in case of 100% WFC + o.6 mM aspirin (1.20). The calculated values of total pigments showed that, the used treatments affect this parameter significantly; either increment in response to the plants pretreated with 0.3 & 0.6mM aspirin under irrigation with 100% WFC or decrease by the other used treatments. Where the maximum value in case of treatment with 0.6 mM aspirin (8.42) under irrigation with 100% WFC and a minimum value in case of irrigation with 50% WFC (3.0), comparing to the control value.

As compared to control (irrigated with 100% WFC + 0 kinetin) treatment that record 2.4 in chlorophyll a content, a significant decrease was recorded by irrigation with 50% WFC either alone or + 50 & 100 ppm kinetin pretreatment, this pigment increased significantly by both kinetin under irrigation with 100% WFC; with a maximum value by pretreatment with 100 ppm kinetin to that irrigated with 100% WFC (3.96). The pattern of changes in chlorophyll b and carotenoids content are more or less similar to that recorded in chlorophyll a, with a maximum value in case of treatment with 100 ppm kinetin under irrigation with 100% WFC (2.84 & 2.12, respectively) and a minimum value in case of irrigation with 50% WFC (0.77 & 0.5, respectively). Chlorophyll a/ chlorophyll b ratio increased by the used treatments, either significantly with 50% WFC alone and + 50 ppm kinetin pretreatment or non- significantly by the other used treatments. The maximum value of this ratio (2.29) recorded in case of irrigation with 50% WFC and a minimum value in case of control (1.23). Total chlorophylls and total pigments consequently, showed a pattern of changes comparable to that detected in chlorophyll a so. the maximum values of total chlorophylls and total pigments (6.8 and 8.92, respectively) recorded in case of treatment with 100 ppm kinetin under irrigation with 100% WFC and a minimum value in case of irrigation with 50% WFC+ 0 kinetin (2.51 and 3.01, respectively) (See figure 4).

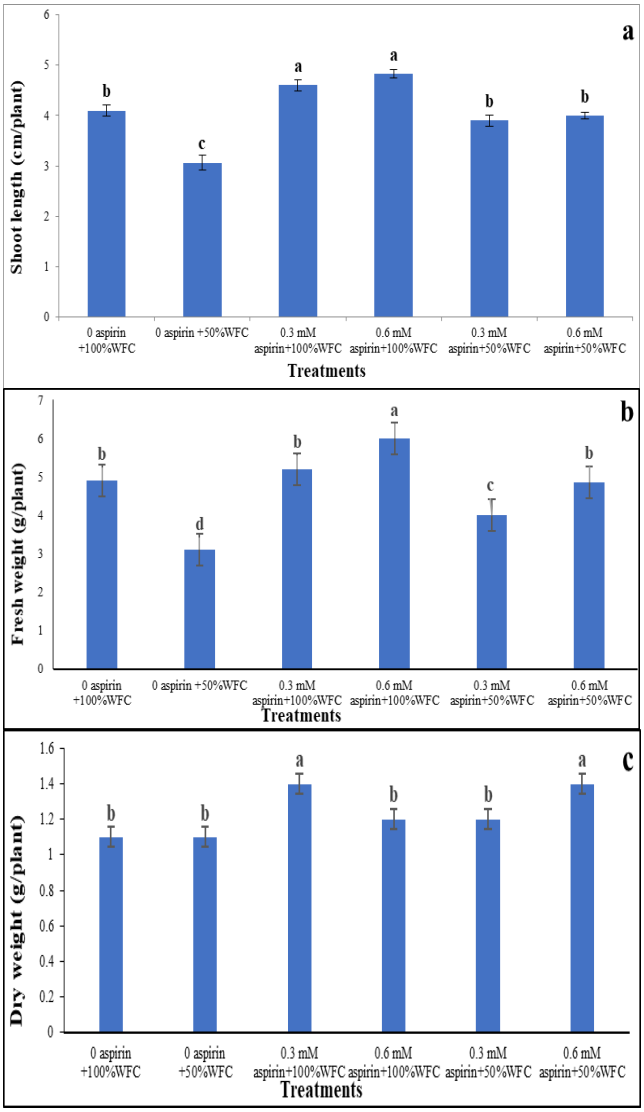
Exposure of the lettuce plant to moderate drought stress caused oxidative stress, which reduced chlorophyll content, including chl a, chl b, carotenoid content and total pigments. This reduction may be due to the pigment photo-oxidation and the degradation of chlorophyll. Similar results are reported in cotton and soybean plants **[26 , 27]** respectively. Plants respond to drought stress by closing stomata to limit water loss, reducing carbon flow **[28]** and reducing ATP synthase and rubisco activities **[29].** Treatment with aspirin caused enhancement in the photosynthetic pigments in lettuce plants in the present study. In this connection, down-regulated chlorophyllase activity and up-regulated expression of chlorophyll biosynthetic genes may result in increased pigment synthesis by the application of antioxidants **[30].**

In this study, in comparison with aspirin treatment alone, advancements in the contents of photosynthetic pigments, such as chlorophyll a and chlorophyll b, carotenoid and total pigments, were significant in lettuce plants treated with aspirin, which was similar to emerging research results demonstrated in the literature **[31].** Photosynthetic pigments are the carrier of leaf photosynthesis and the chlorophyll content is an important index of plant drought resistance that is closely correlated with photosynthetic intensity, largely determining plant growth condition and leaf photosynthetic capacity. Plants responding to stressful environments, such as drought, have been documented to lead to low chlorophyll levels, thereby resulting in the retardation of plant overall growth.

Canakci and Munzuroğlu **[32]** study the effects of different concentrations of aspirin; acetylsalicylic acid (ASA) on germination various growth parameters and chlorophyll (a+b) amount of cucumber (*Cucumis sativus*). They also stated that, one-week seedlings exposed to ASA for 48 h: 0.5x10(-2) M ASA increased chlorophyll (a+b) amount with an increase in fresh weight. In addition, recent studies indicate that SA can regulate many different responses, such as tolerance to abiotic stress, plant growth and development, and soil microbiome **[18].**

Kinetin enhanced shoot growth and grain yield, and also reduced membrane injury by dehydration and improved the water status of plant under both aerobic and anaerobic conditions. Kinetin had a dominant effect on the stability of chlorophyll and soluble sugar contents **[13].** Moreover, kinetin stimulated the synthesis of polysaccharides and materials of the new cell walls in kinetin –treated *Phaseolus vulgaris* plants **[14].**

In conclusions: Drought stress (50%WFC) has a negative effect on lettuce growth, chlorophyll synthesis. The deleterious effect of drought stress in plants treated with two concentrations of aspirin (0.3 and 0.6 mM) or kinetin (50&100 ppm); especially 0.6 mM aspirin and 100 ppm kinetin has been improved the growth by positively affecting the determined parameters. These results strongly nominated to construct a hypothesis that aspirin or kinetin as a good cheap safe candidate improves the resistance of plants to drought.



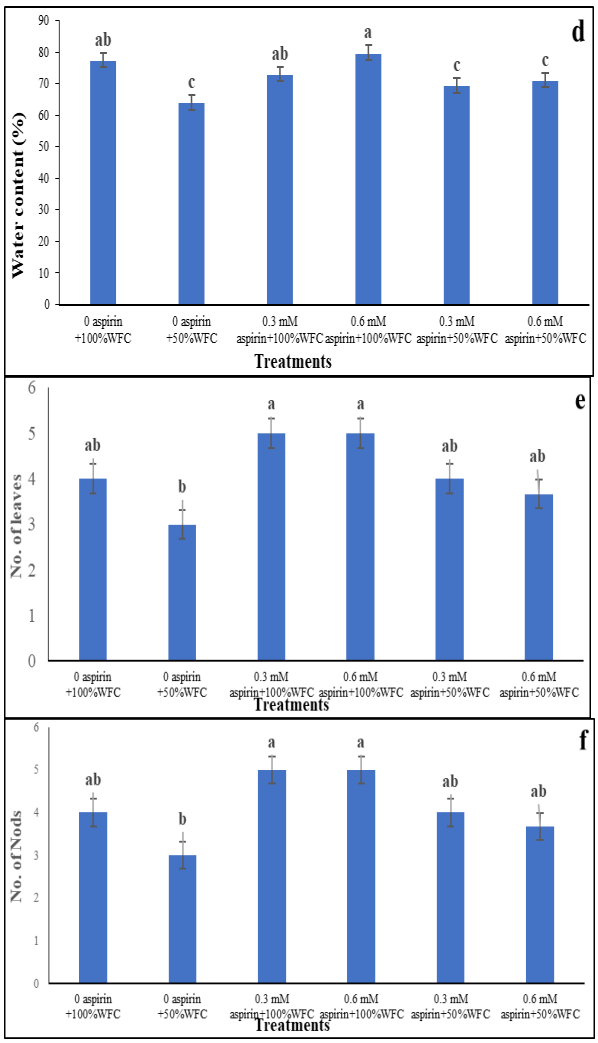
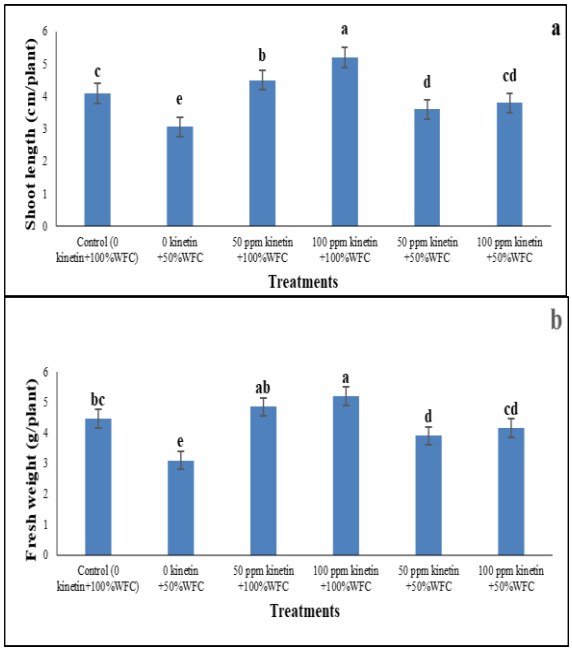


Figure 1 (a-f): Effect of different concentrations of aspirin under 100% and 50% WFC on growth parameters of *Lactuca sativa* L; green cultivars at the vegetative stage (25days old). The Fisher's test revealed that different letters differ significantly from each bar (p-value < 0.05).



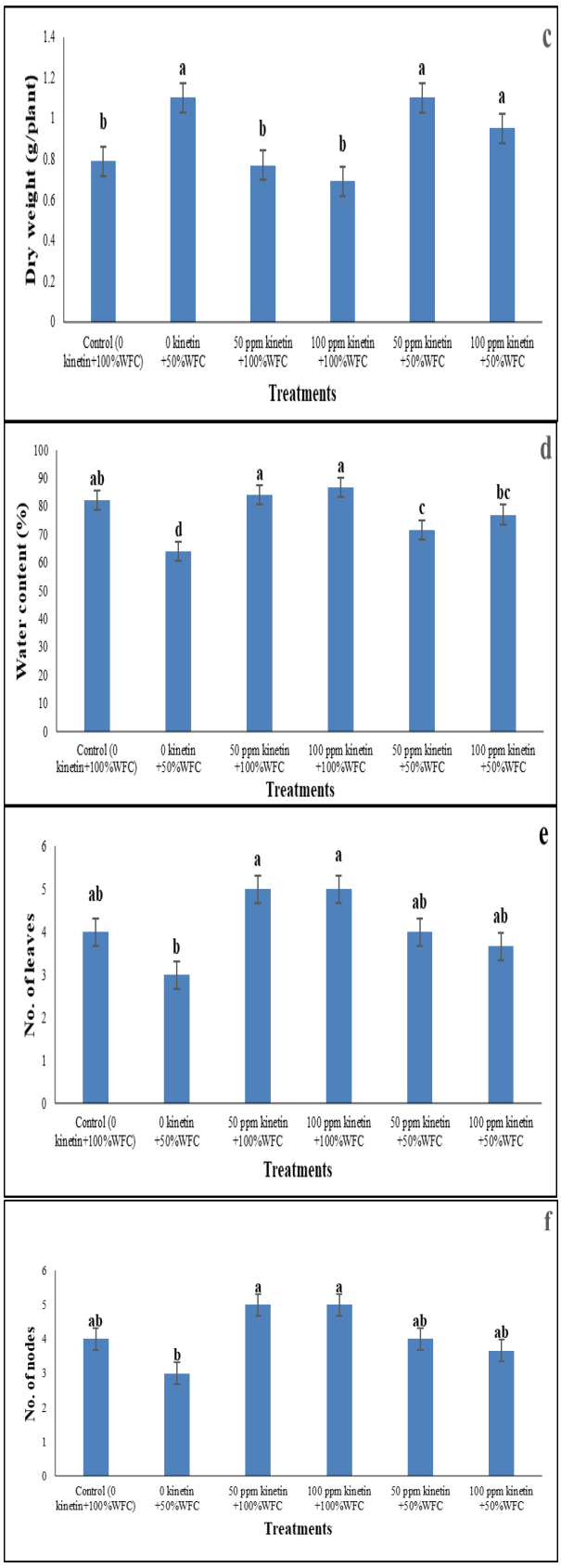
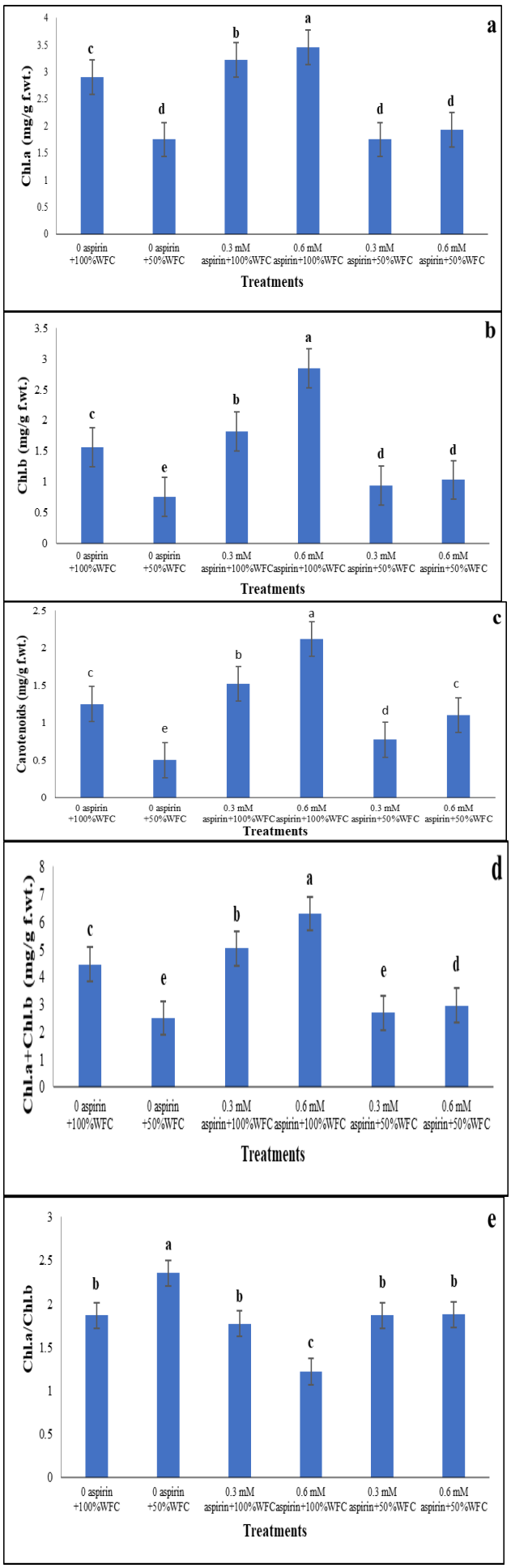


Figure 2 (a-f): Effect of different concentrations of kinetin under 100% and 50% WFC on growth parameters of *Lactuca sativa* L; green cultivars at the vegetative stage (25 days old). The Fisher's test revealed that different letters differ significantly from each bar (p-value < 0.05).



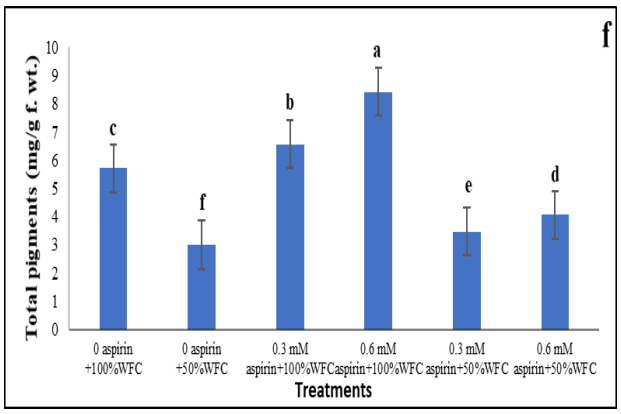
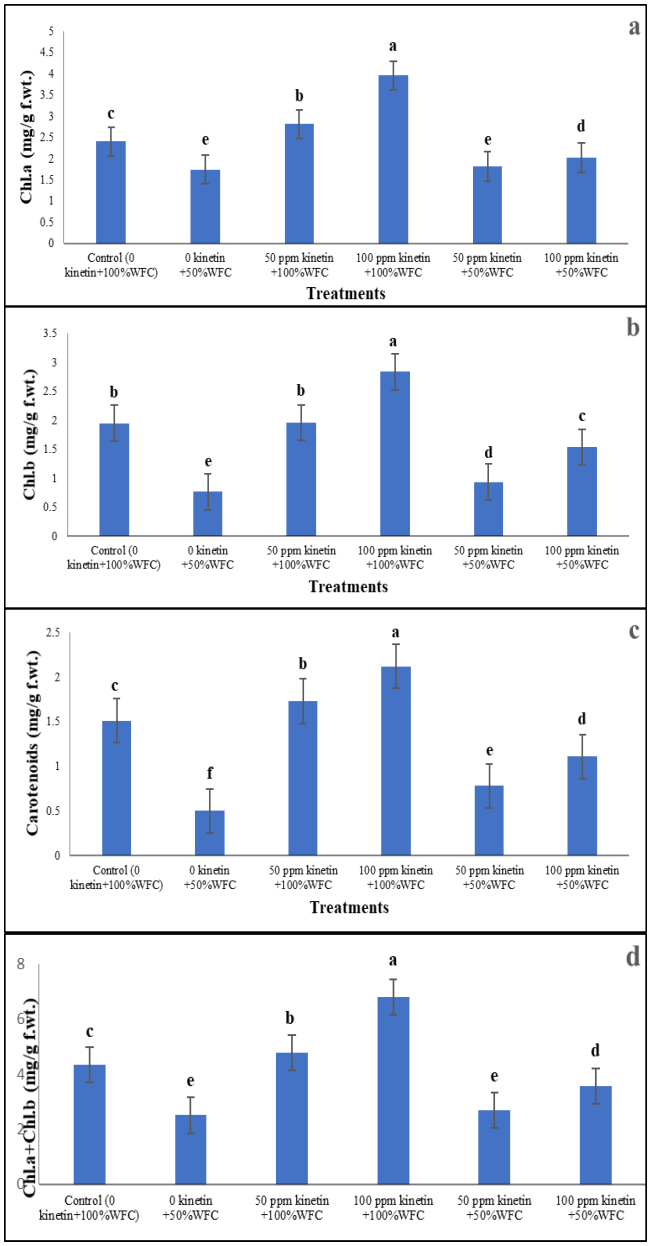


Figure 3 (a-f): Effect of different concentrations of aspirin under 100% and 50% WFC on photosynthetic pigments of *Lactuca sativa* L; green cultivars at the vegetative stage (25 days old). The Fisher's test revealed that different letters differ significantly from each bar (p-value < 0.05).



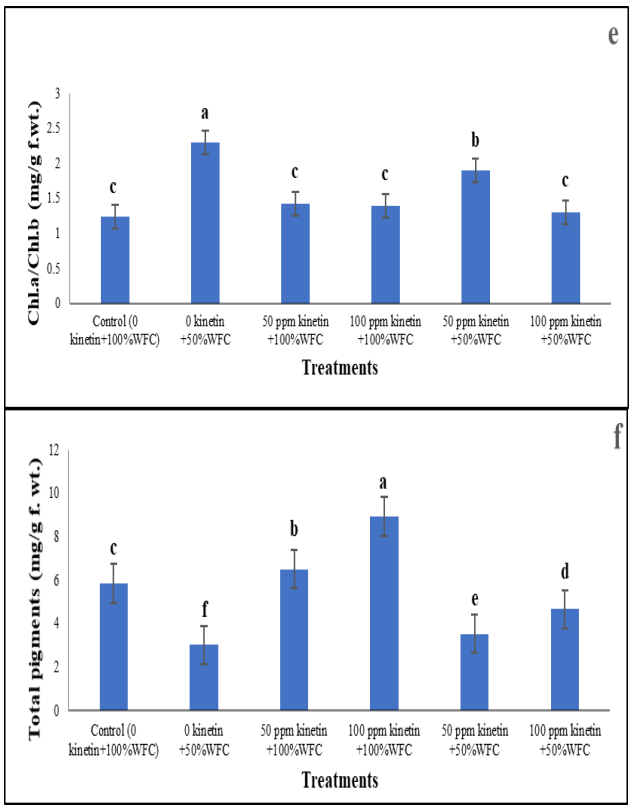


Figure 4 (a-f): Effect of different concentrations of kinetin under 100% and 50% WFC on photosynthetic pigments of *Lactuca sativa* L; green cultivars at the vegetative stage (25 days old). The Fisher's test revealed that different letters differ significantly from each bar (p-value < 0.05).

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