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Original Contribution

INFLUENCE OF FOLIAR APPLICATION OF IRON, CALCIUM AND ZINC SULFATE ON VEGETATIVE GROWTH AND REPRODUCTIVE CHARACTERISTICS OF STRAWBERRY CV. 'PAJARO'

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ABSTRACT
In this study, effect of foliar application of iron, calcium and zinc sulfate reproductive growth, yield and some qualitative characteristics of strawberry fruit were investigated. The treatments included zinc sulfate at three levels (50, 100 and 150 mg l^{-1}), iron at three levels (250, 500 and 1000mg l^{-1}), calcium at two levels (5 and 10 mM) and distilled water as a control. As result has shown iron, calcium and zinc sulfate increased dry weight, leaf area, length of roots of strawberry. Sprays of zinc sulfate at 150 mg l^{-1}, iron at 1000 mg l^{-1} and calcium at 10 mM improved number of flowers, weight of primary and secondary fruit. The highest percentage of total soluble solids, titratable acidity and ascorbic acid was attained in fruits treated with zinc sulfate at 150 mg l^{-1} and the lowest was achieved in control. In general, spraying zinc sulfate at 150 mg l^{-1}, iron at 1000 mg l^{-1} and calcium at 10 mM concentration is recommended for increasing the strawberry yield.

Key words: Foliar Application, Strawberry, Iron, Zinc Sulfate, Calcium

INTRODUCTION

Strawberry (Fragaria ×ananassa Duch. Cv. Pajaro) is one of the most delicious fruits of the world. Strawberry is a very rich source of bioactive compounds including vitamin C, E, b-carotene and phenolic compounds (phenol acids, flavan-3-ols, flavonols, and anthocyanins) (1). Paramrters like total acids (TA), total soluble solids (TSS) and their ratio (TSS/TA) are very important in determining strawberry fruit quality (2-5). The foliar nutrition of micro-nutrients have very important role in improving fruit set, productivity and quality of fruits. Foliar nutrition at proper time improved quality and quantity strawberry. Fe has an important role as a micronutrient element. Iron deficiency chlorosis is a common nutritional disorder,mainly associated with high pH or calcareous soils also it is a limiting factor for fruit agricultural production in many areas of the world(6-8). Since Zn is component of many enzymes and proteins organisms, it is an essential metal for normal plant growth and development. It is also, required for the synthesis of tryptophan, a precursor of IAA which acts as a growth promoting substance. It was reported that flower yield, essential oil percentage and essential oil yield of chamomile (*Matricaria chamomilla*) increased by foliar application of Fe and Zn compared with control (9). It is well known that calcium plays an important role in maintaining quality of fruits and vegetable. Calcium treatment helps to retain fruit firmness, increase vitamin C content, decreased storage breakdown rotting and browning in apple. The beneficial effects of Ca on maintaining fruit quality are well documented by many researchers (10-11). Pre and postharvest application of Ca is effective on, quality, delaying senescence, reducing postharvest decay and controlling the physiological disorder in many fruits and vegetables, (10). A few days before harvest,

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foliar application of Ca on strawberry plants increased fruit Ca content and influenced several postharvest senescence changes involving free sugar, organic acid, anthocyanin contents texture and electrical conductivity (12).Calcium application usually leads to an increase in apoplastic Ca concentration that may affect the structure and functions of cell walls, membranes and certain aspects of cell metabolism, also it delays leaf senescence and fruit ripening (13-17). The aim of this research was to evaluate the effects of the foliar application of iron, calcium and zinc sulfate on vegetative growth and reproductive characteristics of strawberry cv. Pajaro.

MATERIALS AND METHODS

Plant Growth Conditions and Treatments

The experiment was conducted during 2011 and 2012 on strawberry plants at the experimental greenhouse, Ilam, Iran (Elevation 1339 m, Latitude East 33.638, Longitude North 46.431). Strawberry plants (*Fragaria* × *ananassa* Duch. cv. Pajaro) were grown under natural light conditions. The temperature conditions were 24 ± 5°C and 15 ± 4°C, during days and nights respectively; with relative humidity of 70%. Daughter plants of Pajaro were potted in 3 plastic pots filled with 2:1 sandy loam soil and compost. After 2 weeks of establishment, in the beginning of November, the treatments, included: zinc sulfate at three levels (50, 100 and 150 mg l^{-1}), iron at three levels (250, 500 and 1000mg^{-1}), calcium as $CaCl_2$ at two levels (5 and 10 mM) and distilled water as control. During the experimental period plants were fertilized with Hogland solution. Spray materials were used as follows: after plant establishment, at the beginning of flowering, and 15 days after the second time.

Measurements

In the end of experiment, plants were carefully taken out of their pots, roots were washed with distilled water, and the whole plants were oven dried for 72 hours at 70° c then. The following quality parameters of harvested fruits were determined: dry weight, number of runners, leaf area, number of flowers, length of the roots, length of flowering period, weight of primary and secondary fruits and number of their achenes, pH, TSS, TA, and vitamin C of ‘Pajaro’ strawberry. Dry weights were expressed as gram. Length of roots was measure by using a ruler and was expressed as cm. Number of runners and flowers were counted throughout the experimental period. Leaf area was measured using a ΔT^- leaf area meter and expressed as cm^2. Length of flowering period was calculated and expressed as days between the first appearing flowers till end of experiment. Primary and secondary fruits were weighed in order to measure their weight as gram. Number of their achenes was counted afterwards.

Ascorbic acid content (Vitamin C)

Ascorbic Acid (AA) content of strawberry was determined by the 2,6-dichlorophenolindophenol method (18). An aliquot of 10 mL strawberry fruit juice extract was diluted to 50 mL with 3% metaphosphoric acid in a 50 mL volumetric flask. The aliquot was filtered and titrated with the standard dye to a pink endpoint (persisting for 15 sec). The pH value was measured with a pH meter.

Total soluble solids and titratable acidity

To characterize the maturity and quality of the fruit total soluble solids (TSS), titratable acidity (TA) were determined. A sample of 15 Strawberry was randomly harvested selected for quality measurements from each replicate of each treatment. TSS, expressed as Brix, was measured with a portable refract meter (19). Titratable acid (TA) was determined by diluting each 5 ml aliquot of strawberry juice to 100 ml with distilled water, then titrating to pH 8.2 by using 0.1 N NaOH. Acidity was expressed as mg citric acid/100 ml juice.

Statistical Analysis

The experiment was conducted as a factorial experiment in a completely randomized design with 4 replications, each consisting of 3 pots with each pot containing one plant. Data were analyzed by SPSS 16 software and and comparing averages was done by Duncan’s test and a probability value of %5.

RESULTS AND DISCUSSION

Application of zinc sulfate significantly influenced plant dry weight ($p \leq 0.05$) **(Table 1).** The highest dry weight and length of roots were obtained at 150 mg l^{-1} zinc sulfate. Iron and calcium had not significant change in dry weight, number of runners and length of roots. Besides the function of zinc in CO_2

assimilation; Zinc is a component of carbonicanhydrase, as well as several dehydrogenises and auxin production which in turn enhanced the elongation processes.
Consequently, the fresh and dry weights of herb could be increased(20). Norvell & Welch (21) reported that adequate supply of Zn is important in controlling root uptake, shoot accumulation of Na and vegetative growth. Zinc sulfate, Iron and Calcium significantly increased leaf area ($p \leq 0.05$). The highest leaf area was obtained at 150 mg l^{-1} zinc sulfate, 1000 mg $^{-1}$ and 10 mM calcium. It is evident that increasing the leaf area was due to increasing in the concentration of zinc, iron and calcium **(Table 1).** Iron deficiency inhibited leaf growth, cell number, size and cell division, as well as chlorophyll, protein, starch and sugar content (20). Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions (22). Hence, iron fills many essential roles in plant growth and development; including chlorophyll synthesis, thylakoid synthesis and chloroplast development. Iron is required at several steps in the biosynthetic pathways. Zinc is an essential element for plant that act as a metal component of various enzymes,a functional structural, regulatory cofactor protein synthesis, photosynthesis, synthesis of auxin, cell division, maintenance of membrane structure,function, and sexual fertilization (20). Nazarpur (23) reported that $ZnSO_4$ increased strawberry leaf number cultivar Camarosa and Armore, respectively. Sing *et al.* (24), found that the application of Zn as foliar spray not only increased the number of leaves but also reduced the leaf drop and hastened the flowering in papaya plant. Foliar spray application of 50g iron EDDHA per tree, by adding two rows, leads to a significant increase in leaf chlorophyll content of peach (25). The beneficial effect of calcium in increasing fruit set might be due to the higher availability of photosynthesis and these chemicals are also associated with hormone metabolism which promotes synthesis of auxin, essential for fruit set and growth.

Table 1. *Effect of pre-harvest application of zinc sulfate, iron and calcium on dry weight, number of runners, leaf area, number of flowers and length of the roots*

Treatment		Plant dry weight (g)	Number of runners	Leaf area (cm^2)	Length of roots (cm)	Number of flowers
Control	0	8.6 j	2.11 fg	18.14 fg	11.36 g	7.2 bc
zinc sulfate (mg l^{-1})	50	10.8 i	2.54 de	29 de	16 f	6.8c
	100	12.5 ef	3.45 c	33.65 c	29.7 b	7 bc
	150	17.08a	5.1 a	46.3 a	33.11 a	15.7a
Iron (mg/l)	250	12.8 e	2.89 f	23.5 f	17.8 e	10 b
	500	13 d	3 d	31 d	20.3 d	10b
	1000	16.4 b	3.4 c	45 a	30 b	15 a
Calcium (mM)	5	11.4 f	3.16 ef	23.5 ef	18 e	7.3 bc
	10	14.8 c	3.3 cd	44.12 a	18.5 e	15.4a

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Reproductive Growth

Table 1 and 2 show that iron and zinc sulfate increased length of flowering period, weight of primary and secondary fruits and number of their achenes **(Table 1 and 2).** Calcium increased fruit number, although the length of flowering period was significantly reduced ($p \leq 0.05$) **(Table 2).** Positive effect of $ZnSO_4$ on fruit number is well documented (26). Growth of the receptacle is controlled primarily by auxin, which is synthesized in achenes (27), Therefore $ZnSO_4$ is applied to increase fruit number, size and quality. Iron improves photosynthesis, yield and assimilates transportation to sinks and finally increased seed yield (28). Zaiter *et al.* (29) showed that foliar feeding of iron chelate on strawberry increased the Performance by increasing the number of fruits. Irons have a positive effect on the synthesis and activity of chlorophylls; thereby it increases the Photosynthesis. Ability to photosynthesize and produce more food increases the generative power; whereby the tree can hold more fruits. Calcium is an important nutrient that plays a key role in the structure of cell walls,cell membranes, fruit growth, and development, as well as general fruit quality(30).

Table 2. *Effect of pre-harvest application of zinc sulfate, iron and calcium on length of flowering period, weight of primary and secondary fruits and number of their achenes*

Treatment		Length of flowering period (days)	Weight of primary fruit (g)	Weight of secondary fruit (g)	Number of achenes of primary fruit	Number of achenes of secondary fruit
Control	0	15.7c	9.11 c	7 c	134.1 c	108 c
zinc sulfate(mg l^{-1})	50	16 c	12.3 b	8.6 b	156.7bc	115.6 bc
	100	21.5b	12 b	9 b	175.8 b	159.8 b
	150	35a	18.3 a	16.3 a	221.8 a	210.4 a
iron (mg/l)	250	13.6 c	11.83 b	10.2 b	136.5 c	101.8 c
	500	20.7 b	12 b	8.7 b	186.7b	163.8 b
	1000	33a	18 a	15.8 a	220.5a	20.9.8 a
Calcium (mM)	5	20.5 b	14.7ab	10.9ab	159.2 bc	134.5 bc
	10	27.3 ab	17.8 a	15.3a	223.5 a	210.4 a

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Fruit characters and quality attributes

The highest pH was attained in fruits treated with 150 mg l^{-1} of foliar Zn concentration and the lowest was observed in control group. Iron foliar concentrations had no statistically significant differences from controls ($p \leq 0.05$) **(Table 3).** Result shown that zinc sulfate (150 mg l^{-1}) and iron (1000 mg l^{-1}) higher concentrations increased TSS, TA and vitamin C **(Table 3).** Application of zinc sulfate can increase TSS in fruit of guava(31). Since zinc has an important role in photosynthesis and enzymes responsible for plant metabolism, the increased TSS could be attributed to $ZnSO_4$. Rath *et al.* (32) reported, foliar application of zinc sulfate (0.8 %) increased vitamin C. Dixi and Gamdagin (33) claimed that a foliar spray application of $ZnSO_4$ on March and April increased size, TSS and juice of oranges. The effect of iron on the amount of soluble solids may be due to its availability in foliar feeding of plants and the role of iron in photosynthesis; that cause higher photosynthetic rate. This finding is in accordance to report of Yogeesha (34)on the grapes. The results have shown that calcium (10 mM) increased TSS, TA and vitamin C (Table 3). This indicates that calcium may be more critical for the TSS contents of strawberry fruit. These results could be ascribed to increasing the soluble matter in the juice by the penetrated calcium chlorides. Higher ascorbic acid content in such fruit may primarily be due to the reason that Ca has promotory influence on vitamin C content (30). In general, Ca pre-harvest treatment, fruit of ‘Pajaro’ strawberry have higher TSS and ascorbic acid content, lower acidity than control.

CONCLUSONS

As a whole, Zinc, iron and calcium increased length of roots, plant dry weigh as well as such reproductive growth as number of fruits. Besides the function of zinc in CO_2 assimilation; Zinc is a component of carbonicanhydrase, as well as several dehydrogenises and auxin production which in turn enhanced the elongation processes. Consequently, the fresh and dry weights of herb could be increased. Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions. Hence, iron fills many essential roles in plant growth and development, including chlorophyll synthesis, thylakoid synthesis and chloroplast development. The beneficial effect of calcium in increasing fruit set might be due to the higher availability of photosynthesis and; these chemicals are also associated with hormone metabolism which promotes synthesis of auxin, essential for fruit set and growth. In general the application of $ZnSO_4$ at 150 mg l^{-1} is recommended to improve reproductive growth in strawberry, cultivar Pajaro.

Table 3. *Effect of pre-harvest application of zinc sulfate, iron and calcium on pH, TSS, TA and vitamin C of 'Pajaro' strawberry*

Treatment		pH	TSS	TA g(citric acid)/(g/L)	Vitamin C (mg/100 g)
Control	0	3.18 abc	5.4 fg	4.17 g	22j
zinc sulfate(mg l^{-1})	50	3.17 bc	6.47de	5 f	28.9i
	100	3.15bcd	7.33 c	7.99 c	52.36 c
	150	3.39 a	9.87 a	9.7 a	67.12a
iron (mg/l)	250	3cd	5.34 f	6.8 e	46.31e
	500	3.18 abc	7 d	7 d	47 d
	1000	3.2 ab	8.67b	8.7 b	56.7 b
Calcium (mM)	5	3.11 d	5.9 ef	6.6 e	41.89f
	10	3.15 bc	9.8 a	9.6 a	65.7a

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Abbreviations: TSS-- total soluble solids; TA-- titratable acidity; Zn-- Zinc Sulfate; Ca-- Calcium; Fe-- Iron

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