



Impact of Acetyl Acetone on Zinc Uptake and Oxidative Stress in Cucumber (*Cucumis sativus*)

Usman Bashir Mahmud¹, Ishaq Yahaya Lawan² and M. S. Dagari²

¹Department of Chemistry, Nigerian Army University, Biu, Nigeria

²Department of Chemistry, Kano University of Science and Technology, Wudil, Nigeria

Abstract

The purpose of this research is to determine the impacts of acetyl acetone on zinc uptake and oxidative stress in cucumber (*Cucumis sativus*) seedlings grown in hydroponic solutions. Thirty seedlings of cucumber were collected from Kura local government area Kano, Kano state. Concentrations of Zn^{2+} in the hydroponics were varied from 0.000 to 0.025 mol dm⁻³ and of acetyl acetone were also varied from 0.000 to 0.025 mol dm⁻³. The seedlings were replanted and kept at Department of Agriculture Kano University of Science and Technology, Wudil garden. The weight of plants increased significantly ($p < 0.05$) with concentration of Zn^{2+} in the absence of acetyl acetone and highly insignificantly ($p > 0.05$) with concentration of acetyl acetone in the absence of Zn^{2+} . In the presence of acetyl acetone and Zn^{2+} plant weight decreased highly insignificant ($p > 0.05$). Leaf fall was observed from plants in all hydroponic, the number of falling leaves was insignificant ($p > 0.05$) with concentration of Zn^{2+} but it is insignificant ($p > 0.05$) with concentration of acetyl acetone and highly insignificant in the presence of acetyl acetone and Zn^{2+} ($p > 0.05$). The volume of the solution decreased highly significant ($p < 0.05$) with concentration of Zn^{2+} and significant with concentration of acetyl acetone ($p < 0.05$) the volume also increased insignificantly in the presence of acetyl acetone and Zn^{2+} ($p > 0.05$). The pH values of treated hydroponics before replanting and after harvest

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were insignificant with increased in the concentration of Zn^{2+} ($p>0.05$). Both shoot and root zinc accumulated were insignificant at lower concentration of Zn^{2+} ($p>0.05$) and significant at higher concentration of Zn^{2+} ($p<0.05$). The zinc translocation factor decreased highly insignificantly ($p>0.05$) at lower concentration of Zn^{2+} and significant at higher concentration of Zn^{2+} ($p>0.05$) in treated plants compared to control. The chlorophyll, carotenoid and proline content varied in the presence and absence of acetyl acetone and or combination of the two, with ($p<0.05$) or ($p>0.05$).

Introduction

Zinc is an essential mineral that is naturally present in some food, added to other and available as a dietary supplement. Zinc is also found in many cold lozenges and some over the counter drugs sold as cold remedies.

Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes (Sandstead [36]) and it plays a role in immune function (Solomons [38]), protein synthesis (Prasad [32]), wound healing (Heyneman [16]), DNA synthesis (IMFNB [18]) and cell division (Prasad et al. [31]). Zinc also supports normal growth and development during pregnancy, childhood, and adolescence (Simmer and Thompson [37]) and is required for proper sense of taste and smell (Prasad et al. [31]). A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Rink [35]).

Zinc is present in several products, include some labeled as homeopathic medications, sold over the counter for the treatment and prevention of cold. Numerous case reports to anosmia (loss of the sense of smell), in some cases long lasting or permanent, have been associated with the use of zinc containing nasal gels or sprays (Jafek et al. [19]). In June 2009 the FDA warned consumers to stop using three zinc containing intranasal products because they might cause anosmia (US Food and Drugs Administration). The manufacture recalled these products from the market place. Currently, these safety concerns have not been found to be associated with cold lozenges containing zinc.

Zinc is also present in some denture adhesive creams at levels ranging from 17-34 mg/g (Nations et al. [27]). While use of these products as directed (0.5-1.5 g/day) in not of concern, chronic, excessive use can lead to zinc toxicity, resulting in copper deficiency and neurologic disease. Such toxicity has been reported in individuals who

used 2 or more standard 2.4 oz tubes of denture cream per week (Nations et al. [27]), many denture creams have now been reformulated to eliminate zinc.

Zinc deficiency is characterized by growth retardation, loss of appetite and impaired immune function in more severe cases zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males and eye and skin lesions (Prasad [30]). Zinc nutritional status is difficult to measure adequately using laboratory tests (Hunt [17]) due to its distribution throughout the body as a component of various proteins and nucleic acid (Hambidge and Krebs [12]). Plasma or serum zinc levels are the most commonly used indices for evaluating zinc deficiency but these levels do not necessarily reflect cellular zinc status due to tight homeostatic control mechanism (Maret and Sandstead [25]). Clinical effect of zinc deficiency can be present in the absence of abnormal laboratory indices (Maret and Sandstead [25]). Clinicians consider risk factors (such as inadequate caloric intake, alcoholism, and digestive disease) and symptoms of zinc deficiency (such as impaired growth in infants and children). When determining the need for zinc supplementation (IMFNB [18]).

Cucumber (*Cucumis sativas*)

Cucumber (*Cucumis sativas*) is a widely cultivated plant in the gourd family, cucurbitacea. It is a creeping vine that bears cylindrical fruits that are used as culinary vegetables. There are three main varieties of cucumber there are: slicing, pickling and burpless. Within these varieties, several different cultivars have emerged. The cucumber is many different varieties are traded in the global market.

Morphology of Cucumber (*Cucumis sativas*)

The cucumber is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiraling tendrils. The plants have large leaves that form a canopy over the fruit. The fruits of the cucumber is roughly cylindrical, elongated with tapered end, and may be as large as 60 centimeters (24 in) long and 10 centimeters (3.9 in) in diameter. Having an enclosed seed and developing from a flower, botanically speaking, cucumbers are classified as pepoes, a type of botanical berry much like tomatoes and squash they are often also perceived, prepared and eaten as vegetable cucumbers are usually more than 90% water.

Flowering and Pollination

A few cultivars of cucumber are parthenocarpic, the blossoms creating seedless fruit

without pollination for these cultivars degrades the quantity. In the united state, these are usually grown in greenhouses where bees are excluded. In Europe, they are grown outdoors in some region, and bees are excluded from these areas.

Most cucumbers cultivars, however, are seeded and require pollination. Thousands of hives of honey bees are annually carried to cucumber field just before bloom for this purpose. Cucumbers may also be pollinated by bumblebees and several other bee species. Most cucumbers that require pollination are self-incompatible, so pollen from different plants is required to form seeds and fruit (Nonnecke [28]). Some self-compatible cultivars exist that are related to the lemon cultivars (Nonnecke [28]). Symptoms of inadequate pollination include fruit abortion and misshapen fruit. Partially pollinated flowers may develops fruit that are green and develop normally near the stem end, but are pale yellow and withered at the blossom end.

Traditional cultivars produce male blossoms fruit, then female, in about equivalent numbers. Never gynocious hybrid cultivars produce almost all female blossoms. They may have a pollenizer cultivars interplanted, and the number of beehives per unit area is increased, but temperature changes induce male flowers even on these plants, which may be sufficient for pollination to occur (Nonnecke [28]).

Hydroponics

Hydroponics is a set of hydroculture and is a method of growing of plants using mineral nutrient solutions, in water, without soil. Terrestrial plants may be grown with their roots in the mineral nutrient solution only, or in an inert medium, such as perlite or gravel. Hydroponics offers opportunities to provide optimal conditions for plant growth and therefore, higher yields can be obtained using it compared to open field production. It offers a means of control over soil-borne diseases and pests, which is especially desirable in the tropics where the life cycles of these organisms continue uninterrupted and so do the threat of infestation. Under hydroponics, some plants can be grown closer together than in the field because roots are directly fed; and multiple cropping can be practiced (Jensen [20]).

Materials and Methods

Sample Collection

Thirty seedlings of cucumber where collected in Kura local government area, Kano state. On 16/11/2015 By 1:05pm.

Identification



Seedlings were identified by Baha'uddeen Said Adam of the Department of Plant Science, Bayero University, Kano.

Treatment

The cucumber seedlings were washed thoroughly with tap water and then rinsed with deionized water.

Reagents

Boric acid (H_3BO_3), Disodium dihydrogen pyrophosphate ($\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$), Hydrated calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), Hydrated iron (III) chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Hydrated manganese sulphate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$), Magnesium sulphate monohydrate ($\text{MgSO}_4 \cdot \text{H}_2\text{O}$), Nitric acid (HNO_3), Potassium iodide (KI), Potassium nitrate (KNO_3), Zinc nitrate ($\text{Zn}(\text{NO}_3)_2$), Acetyl acetone.

Preparation of Hydroponic Mixtures Control

The control contained $2.56 \times 10^{-6} \text{ mol dm}^{-3} \text{ KNO}_3$, $5.0 \times 10^{-4} \text{ mol dm}^{-3} \text{ MgSO}_4 \cdot \text{H}_2\text{O}$, $1.03 \times 10^{-3} \text{ mol dm}^{-3} \text{ FeCl}_3 \cdot 6\text{H}_2\text{O}$, $2.50 \times 10^{-6} \text{ mol dm}^{-3} \text{ KI}$, $2.31 \times 10^{-3} \text{ mol dm}^{-3} \text{ MnSO}_4 \cdot \text{H}_2\text{O}$, $2.27 \times 10^{-3} \text{ mol dm}^{-3} \text{ H}_3\text{BO}_3$, $3.57 \times 10^{-4} \text{ mol dm}^{-3} \text{ Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $5.0 \times 10^{-4} \text{ mol dm}^{-3} \text{ Na}_2\text{H}_2\text{P}_2\text{O}_7$, $0.10 \text{ mol dm}^{-3} \text{ HNO}_3$. 500 cm^3 of the control was prepared by pipetting 1.28 cm^3 of $0.10 \text{ mol dm}^{-3} \text{ KNO}_3$, 5.15 cm^3 of 0.10 mol dm^{-3}

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 11.35 cm^3 of 0.10 mol dm^{-3} H_3BO_3 , 5.00 cm^3 of 0.05 mol dm^{-3} $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 3.57 cm^3 of 0.05 mol dm^{-3} $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 5.00 cm^3 of 0.05 mol dm^{-3} $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$, 0.17 cm^3 of $0.0075 \text{ mol dm}^{-3}$ KI , 23.10 cm^3 of 0.05 mol dm^{-3} $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 5.00 cm^3 of 0.10 mol dm^{-3} HNO_3 into a 500 cm^3 volumetric flask. The volume was made to mark with deionized water.

Hydroponics Containing $0.0025 \text{ mol dm}^{-3}$ and $0.025 \text{ mol dm}^{-3}$ $\text{Zn}(\text{NO}_3)_2$

500 cm^3 of a hydroponic containing $0.0025 \text{ mol dm}^{-3}$ $\text{Zn}(\text{NO}_3)_2$ was prepared by pipetting 5 cm^3 of 0.25 mol dm^{-3} $\text{Zn}(\text{NO}_3)_2$ into a 500 cm^3 volumetric flask. The volume was made to mark with deionized water after adding the other components as in the control. Similarly, hydroponics containing $0.025 \text{ mol dm}^{-3}$ zinc nitrate was prepared by pipetting 50 cm^3 of 0.25 mol dm^{-3} $\text{Zn}(\text{NO}_3)_2$ into a 500 cm^3 volumetric flask. The volume was made to mark with deionized water after adding the other components.

Hydroponics Containing $0.0025 \text{ mol dm}^{-3}$ $\text{Zn}(\text{NO}_3)_2$ and $0.005 \text{ mol dm}^{-3}$ Acetyl Acetone

500 cm^3 of a hydroponic containing $0.0025 \text{ mol dm}^{-3}$ $\text{Zn}(\text{NO}_3)_2$ and $0.005 \text{ mol dm}^{-3}$ acetyl acetone was prepared by pipetting 5 cm^3 of 0.25 mol dm^{-3} $\text{Zn}(\text{NO}_3)_2$ and 10 cm^3 of 0.25 mol dm^{-3} acetyl acetone into a 500 cm^3 volumetric flask. The volume was made to mark with deionized water after adding the other components. Other hydroponics mixtures containing different concentrations of $\text{Zn}(\text{NO}_3)_2$ and acetyl acetone were prepared by adding appropriate volumes of reagent and diluting to 500 cm^3 with deionised water.

$1000\text{mg}/\text{dm}^3 \text{ Zn}^{2+}$ Solution

2.8970g of $\text{Zn}(\text{NO}_3)_2$ was dissolved in a 400 cm^3 beaker containing 100 cm^3 deionised water and 10 cm^3 of 10% nitric acid. The solution was transferred into a 1000 cm^3 volumetric flask and made to mark with deionized water.

$100\text{mg}/\text{dm}^3 \text{ Zn}^{2+}$ Solution

10cm^3 of $1000\text{mg}/\text{dm}^3 \text{ Zn}^{2+}$ solution was pipetted into a 100cm^3 volumetric flask, diluted and made to mark with deionised water.

Replanting of Cucumber Seedlings

Pre-treated *cucumber* seedlings were separately replanted in 500cm³ of hydroponics containing 0.0000, 0.0025, 0.025 mol dm⁻³ Zn(NO₃)₂ with 0.000, 0.005, 0.250 mol dm⁻³ acetyl acetone respectively in clean 750cm³ table water plastic bottles on the 5th August, 2015 around 10:00am. Each treatment was replicated three times. The replanted seedlings were kept in the garden of fishery and wildery department of agriculture in Kano University of Science and Technology Kano.

Harvesting of Seedlings

All the seedlings were harvested at the same time i.e. on 19/11/2015 at 12:47pm. The harvested seedlings were washed with 1% nitric acid and rinsed thoroughly with deionized water and dried.

Atomic Absorption Spectrometric Analysis

Two grams of each vegetables will was weighed in different crucibles. One milliliter of concentrated nitric acid was added and then pre-ashed by placing the crucibles on a heater until the contents turned black. The pre-ashed samples were then transferred into a muffle furnace with a temperature of 480°C for 3 hours, after which they cooled to room temperature. The cooled samples were dissolved using 5ml of 30% HCl and then filtered using whatman filter papers. The filtrates were individually poured in to 50ml standard flask and made up to the mark with deionized water. These were then transferred into prewashed sample bottles for analysis of the zinc metal using GBC atomic absorption spectrophotometer.

Chlorophyll and Carotenoid Analysis

Chlorophyll estimation of leaves of treated and control plants were done according to the method of Arnon (1949). Two hundred milligram (0.2g) of fresh leaf tissues of each sample were homogenized using chilled acetone in a test tubes. The homogenate was centrifuge for 10 minutes and the supernatant was collected. The residue was again extracted with 80% acetone and centrifuged. The supernatant was pooled together and the extraction process was repeated until the residue become colourless. The volume of the combined supernatant was noted. The absorbance of the solution was measured against the solvent (80% acetone) at 645 nm, 663 nm and 470 nm for chlorophyll A, chlorophyll B and carotenoid respectively.

Proline Analysis

The plant material was homogenized in 3% aqueous sulfosalicylic acid (0.01 g /0.5 ml) and the residue is removed by centrifugation at 3000r for 10 minutes. 1 ml of homogenized tissue reacts with 1 ml acid-ninhydrin and 1 ml glacial acetic acid in a test tube and heated for 1 hour at 100°C and the reaction is terminated in an ice bath.

The reaction mixture was extracted with 2 ml toluene, mixed vigorously and left at room temperature for 30 minutes until separation of two phases.

The chromophore-containing toluene (1 ml, upper phase) is warmed to room temperature and its optical activity is measured at 520 nm using toluene as a blank.

The proline concentration is determined from a standard curve using D-proline.

Data Analysis

The data were analyzed through one-way analysis of variance (ANOVA) to determine the effect of treatments, and least significant difference (LSD) tests were performed to determine the statistical significance of the differences between means of treatments.

Results and Discussion

Monitoring the Growth of Replanted Seedlings

Table 3.1A. Changes in physiological parameters observed during the growth of seedlings.

Zn	AA	$\Delta W P$	ΔV
0.0000	0.0000	-21.600±2.343	-106.667±15.275
0.0025	0.0000	-19.000±3.251	-96.667±25.166
0.0250	0.0000	-11.933±2.875	-76.667±5.774
0.0000	0.0050	-29.500±9.528	-76.667±15.275
0.0000	0.0250	-17.500±2.088	-93.333±5.774
0.0025	0.0050	-14.633±3.037	-88.333±12.583
0.0025	0.0250	-16.367±2.984	-88.667±5.774

0.0250	0.0050	-10.733±3.089	-73.333±15.275
0.0250	0.0250	-7.600±2.883	-71.666±7.638

Abbreviations: AA = acetyl acetone, Δ WP = change in plant weight, Δ V = change in volume of solution.

Table 3.1B. ANOVA output for changes in physiological parameters.

Parameter	Source	DF	SS	MS	F	P-value
Δ WP	Δ Zn ²⁺ + 0.000 AA	1	239.6433	239.6433	0.048349	0.048349
	Δ AA + 0.000 Zn ²⁺	1	552.9552	552.9552	15.35982	0.059366
	Δ Zn ²⁺ + Δ AA	2	406.5757	203.2879	39.32648	3.56E-05
NFL	Δ Zn ²⁺ + 0.000 AA	1	0.000189	0.000189	1.493827	0.346118
	Δ AA + 0.000 Zn ²⁺	1	0.000225	0.000225	2.25	0.272393
	Δ Zn ²⁺ + Δ AA	2	0.000554	0.000277	2.751724	0.116807
Δ V	Δ Zn ²⁺ + 0.000 AA	1	7513.495	7513.495	75.13485	0.013049
	Δ AA + 0.000 Zn ²⁺	1	9027.85	9027.85	3249.909	0.000308
	Δ Zn ²⁺ + Δ AA	2	17072.8	8536.4	337.2906	3.45E-09

Abbreviations: AA = acetyl acetone, Δ WP = change in plant weight, NFL = number of falling leaves, Δ V = change in volume.

Table 3.1B shows the changes in plant weight, number of fallen leaves and volume of hydroponic solutions.

They were evaluated using the relations;

- $\Delta WP(g) = (\text{Weight of plant after harvest}) - (\text{Weight of plant before replanting})$.
- NFL = Total number of fallen leaves.
- $\Delta V = (\text{volume of solution after harvest}) - (\text{volume of solution before replanting})$.

Change in Plant Weight (ΔWP)

The change in plant weight for all treatments including the control was determined by taking the difference between the weight of plant before replanting and the weight of plant after harvest. The change in plant weight (ΔWP) for the control was $-21.600 \pm 2.343g$. Values of ΔWP for 0.0025 and $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ were $-19.000 \pm 3.251g$ and $-11.933 \pm 2.875g$ respectively. The correlation of Zn^{2+} with ΔWP was significant ($p > 0.05$). For a given concentration of Zn^{2+} at different concentrations of acetyl acetone, the change in plant weight was also determined, For $0.0025 \text{ mol dm}^{-3}$, the values of ΔWP were -29.500 ± 9.528 , -17.500 ± 2.088 and $-14.633 \pm 3.037g$, for 0.000 , 0.005 and $0.025 \text{ mol dm}^{-3}$ acetyl acetone respectively. The corresponding values of ΔWP for $0.025 \text{ mol dm}^{-3}$ were -16.367 ± 2.984 , -10.733 ± 3.089 and -7.600 ± 2.883 for 0.000 , 0.005 and $0.025 \text{ mol dm}^{-3}$ acetyl acetone respectively. However, the correlation of acetyl acetone with ΔWP was insignificant ($p > 0.05$).

Number of Fallen Leaves (NFL)

The fallen of leaves was observed during growth of the Cucumbar (*Cucumis sativus*) seedlings in both control and other hydroponic treatments. The addition of Zn^{2+} in the absence of acetyl acetone shows an insignificant fallen of leaves ($p > 0.05$), while the addition acetyl acetone could not cause insignificant fallen of leaves ($p > 0.05$). However, the changes due to addition of Zn^{2+} with acetyl acetone were insignificant ($p > 0.05$).

Change in Volume of Solutions (ΔV)

Volume of hydroponic solution was taken before replanting and after harvesting of replanted Cucumber (*Cucumis sativus*) seedlings for both the control and other hydroponic treatments. The presence of Zn^{2+} and absence of acetyl acetone showed a significant decrease in volume ($p < 0.05$). Only In the presence of acetyl acetone, the volume decreased significantly ($p < 0.05$). However, the addition of Zn^{2+} with acetyl acetone gives insignificant change in volume ($p > 0.05$).

Changes in Chlorophyll

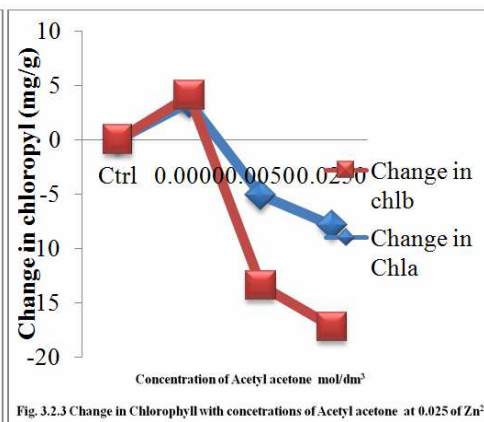
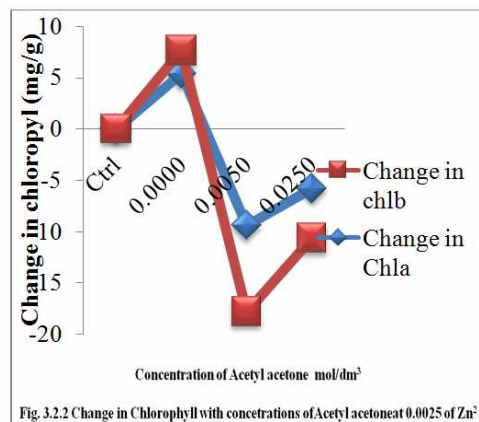
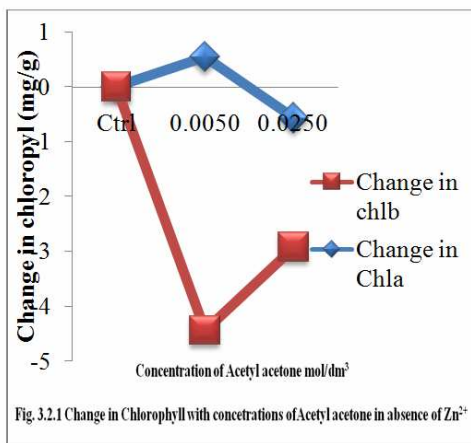
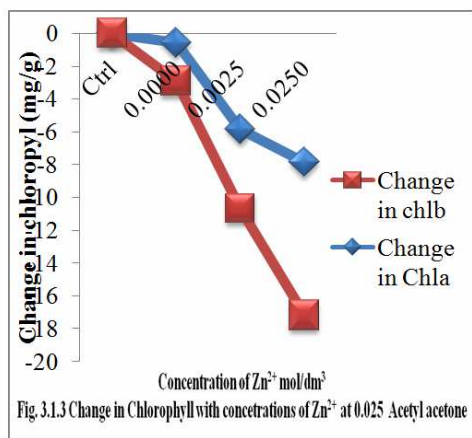
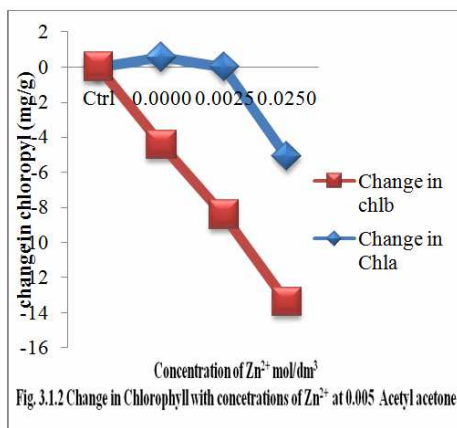
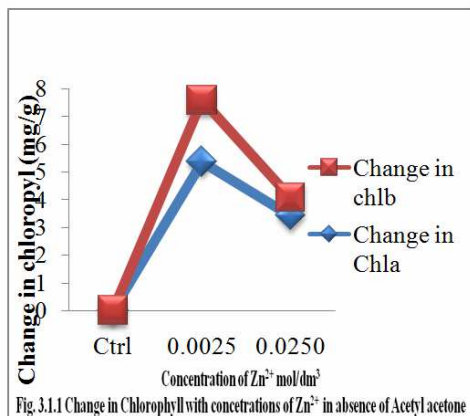


Table 3.1C. ANOVA output for changes in chlorophyll a and chlorophyll b.

Parameter	Source	DF	SS	MS	F	P-value
$\Delta\text{chl a and b}$	ΔZn^{2+} + 0.000AA	2	13.41458	6.70729	2.307729	0.180567
	ΔZn^{2+} + 0.005AA	2	100.0229	50.01145	2.894997	0.106963
	ΔZn^{2+} + 0.025AA	2	40.27132	20.13566	1.945457	0.198525
	ΔAA + 0.000 Zn^{2+}	2	11.98614	5.99307	2.753551	0.141761
	ΔAA + 0.0025 Zn^{2+}	2	18.47028	9.235138	0.424553	0.666508
	ΔAA + 0.025 Zn^{2+}	2	36.38524	18.19262	1.016237	0.400003

Abbreviations: ΔAA = change in concentration of acetyl acetone, Δchl = change in chlorophyll, ΔZn^{2+} = change in concentration of zinc

Table 3.1C shows the changes in chlorophyll (a) and chlorophyll (b).

Figure 3.1.1 shows the changes in chlorophyll (a) and chlorophyll (b). The change in plant chlorophyll (a) and chlorophyll (b) for all treatments including the control was determined by comparing with the corresponding control value. Concentration of Zn^{2+} was varied from 0.000, 0.0025 and 0.025 mol dm⁻³ in the absence of acetyl acetone, addition of 0.0025 mol dm⁻³ Zn^{2+} causes the chlorophyll (a) and the chlorophyll (b) to increase, further addition of Zn^{2+} concentration to 0.025 mol/dm³ decreases the chlorophyll (a) and (b) content. Changes in chlorophyll (a) and chlorophyll (b) contents of the plant in the absence of acetyl acetone were insignificant ($p > 0.05$).

Figure 3.1.2 shows the changes in chlorophyll (a) and chlorophyll (b), concentration of Zn^{2+} was varied from 0.000, 0.0025 and 0.025 mol dm⁻³ at 0.0050 mol dm⁻³ acetyl acetone. At 0.000 mol dm⁻³ Zn^{2+} , there was slightly increase in the chlorophyll (a) and subsequent decrease in the chlorophyll (b), addition of 0.0025 mol dm⁻³ causes slightly decrease in the chlorophyll (a) and chlorophyll (b) also decrease, further addition of Zn^{2+}

to $0.025 \text{ mol dm}^{-3}$ decreases the content of chlorophyll (a) and (b). Changes in chlorophyll (a) and chlorophyll (b) contents of the plant at $0.0050 \text{ mol dm}^{-3}$ of acetyl acetone were insignificant ($p>0.05$).

Figure 3.1.3 shows the changes in chlorophyll (a) and chlorophyll (b), concentration of Zn^{2+} was varied from 0.000, 0.0025 and $0.025 \text{ mol dm}^{-3}$ at $0.025 \text{ mol dm}^{-3}$ acetyl acetone. At $0.000 \text{ mol dm}^{-3}$ Zn^{2+} causes decrease in chlorophyll (a) and (b), addition of $0.0025 \text{ mol dm}^{-3}$ decreases the chlorophyll (a) and (b), further addition of Zn^{2+} to $0.025 \text{ mol dm}^{-3}$ decreases the chlorophyll (a) and (b) content. Changes in chlorophyll (a) and chlorophyll (b) contents of the plant at $0.0250 \text{ mol dm}^{-3}$ of acetyl acetone were insignificant ($p>0.05$).

Figure 3.2.1 shows the changes in chlorophyll (a) and chlorophyll (b), with concentration of acetyl acetone in the absence of Zn^{2+} . Addition of $0.005 \text{ mol dm}^{-3}$ acetyl acetone increases the chlorophyll (a) and chlorophyll (b) decreases drastically, further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone decreases the content of both the chlorophyll (a) and (b). Changes in chlorophyll (a) and chlorophyll (b) contents of the plant in the absence of Zn^{2+} were insignificant ($p>0.05$).

Figure 3.2.2 shows the changes in chlorophyll (a) and chlorophyll (b), with concentration of acetyl acetone at $0.0025 \text{ mol dm}^{-3}$ Zn^{2+} . At $0.000 \text{ mol dm}^{-3}$ acetyl acetone, increases but chlorophyll (a) and chlorophyll (b), addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone decreases the chlorophyll (a) and (b), further addition of $0.0025 \text{ mol dm}^{-3}$ acetyl acetone increases but chlorophyll (a) and chlorophyll (b). Changes in chlorophyll (a) and chlorophyll (b) contents of the plant at $0.0025 \text{ mol dm}^{-3}$ of Zn^{2+} were insignificant ($p>0.05$).

Figure 3.2.3 shows the changes in chlorophyll (a) and chlorophyll (b), with concentration of acetyl acetone at $0.025 \text{ mol dm}^{-3}$ Zn^{2+} . At $0.000 \text{ mol dm}^{-3}$ Zn^{2+} with different concentration of acetyl acetone gives an increase of chlorophyll (a) and (b), at $0.000 \text{ mol dm}^{-3}$ acetyl acetone results in decrease of chlorophyll (a) and (b), further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone maintained the decrease of the chlorophyll (a) and (b) contents Changes in chlorophyll (a) and chlorophyll (b) contents of the plant at $0.025 \text{ mol dm}^{-3}$ of Zn^{2+} were insignificant ($p>0.05$).

Changes in Carotenoid

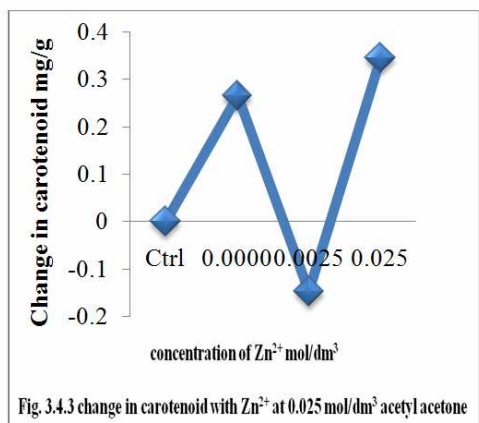
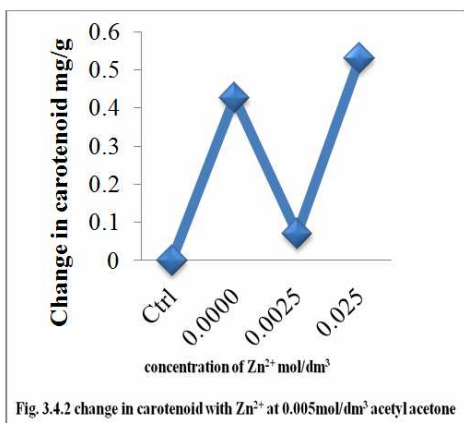
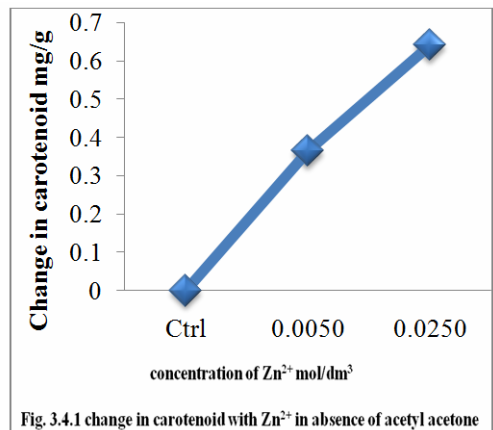
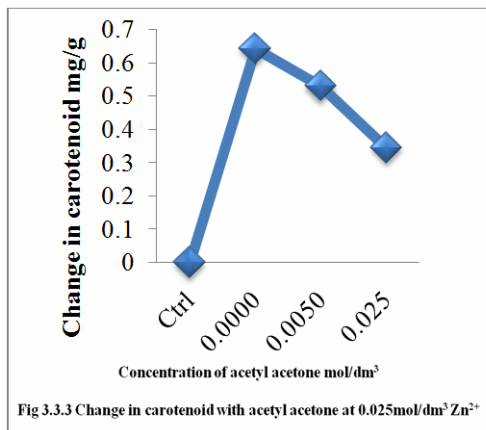
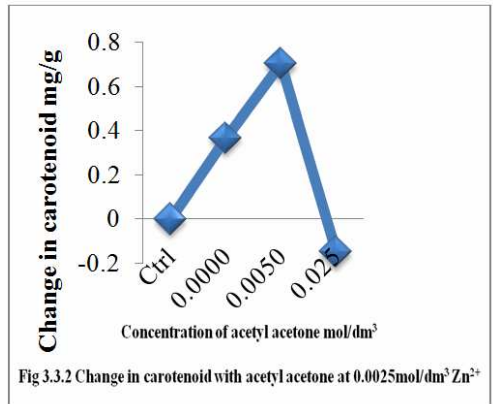
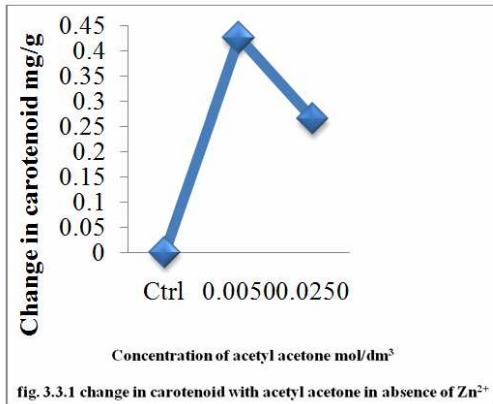


Table 3.1D. ANOVA output for changes in carotenoid.

Parameter	Source	DF	SS	MS	F	P-value
Δ crtn	Δ Zn ²⁺ + 0.000AA	1	0.109207	0.109207	16.57388	0.055372
	Δ Zn ²⁺ + 0.005AA	1	0.13207	0.13207	1.438785	0.296527
	Δ Zn ²⁺ + 0.025AA	1	0.369024	0.369024	32.38833	0.004708
	Δ AA + 0.000 Zn ²⁺	1	0.239317	0.239317	12.42552	0.071907
	Δ AA + 0.0025 Zn ²⁺	1	0.16642	0.16642	5.698143	0.075407
	Δ AA + 0.025 Zn ²⁺	1	0.031435	0.031435	0.896088	0.397434

Abbreviations: Δ AA = change in concentration of acetyl acetone, Δ crtn = change in Carotenoid, Δ Zn²⁺ = change in concentration of zinc

Table 3.1D shows the changes in carotenoid.

Figure 3.3.1 shows the changes in carotenoid, with concentration of acetyl acetone in the absence of Zn²⁺. The change in plant carotenoid for all treatments including the control was determined by comparing with the corresponding control value. Addition of 0.005 mol dm⁻³ acetyl acetone increases the carotenoid content drastically, further addition of 0.025 mol dm⁻³ acetyl acetone causes decrease of carotenoid content. Changes in carotenoid contents of the plant due to the absence of Zn²⁺ were insignificant ($p > 0.05$).

Figure 3.3.2 shows the changes in carotenoid, with concentration of acetyl acetone at 0.0025 mol dm⁻³ Zn²⁺. At 0.000 mol dm⁻³ acetyl acetone, the carotenoid content increases, addition of 0.005 mol dm⁻³ acetyl acetone, carotenoid increases, further addition of 0.025 mol dm⁻³ acetyl acetone carotenoid decreases. Changes in carotenoid contents of the plant at 0.0025 mol dm⁻³ Zn²⁺ were insignificant ($p > 0.05$).

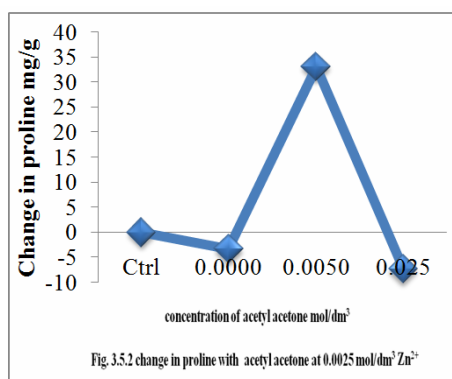
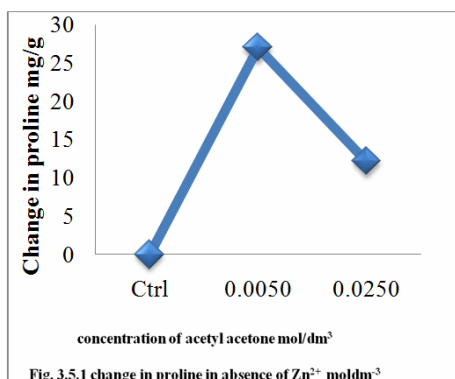
Figure 3.3.3 shows the changes in carotenoid, with concentration of acetyl acetone at $0.0250 \text{ mol dm}^{-3} \text{ Zn}^{2+}$. At $0.000 \text{ mol dm}^{-3}$ of acetyl acetone the carotenoid content increases, subsequent addition of $0.005 \text{ mol dm}^{-3}$ and $0.025 \text{ mol dm}^{-3}$ of acetyl acetone decreases the carotenoid content. Changes in carotenoid contents of the plant at $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ were significant ($p < 0.05$).

Figure 3.4.1 shows the changes in carotenoid, with concentrations of Zn^{2+} in the absence of acetyl acetone. Addition of $0.005 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ increases the carotenoid content, further addition of $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ increases the carotenoid content. Changes in carotenoid contents of the plant due to the absence of acetyl acetone were insignificant ($p > 0.05$).

Figure 3.4.2 shows the changes in carotenoid, with concentration of Zn^{2+} at $0.005 \text{ mol dm}^{-3}$ of acetyl acetone. Addition of $0.000 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ increases the carotenoid content, addition of $0.0025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ decreases the carotenoid, further addition of 0.025 Zn^{2+} increases the carotenoid content. Changes in carotenoid contents of the plant at $0.005 \text{ mol dm}^{-3}$ acetyl acetone were insignificant ($p > 0.05$).

Figure 3.4.3 shows the changes in carotenoid, with concentration of Zn^{2+} at $0.025 \text{ mol dm}^{-3}$ of acetyl acetone. At $0.000 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ the carotenoid content increase, addition of 0.0025 Zn^{2+} decreases the carotenoid, and further addition of $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ increases the carotenoid content. Changes in carotenoid contents of the plant at $0.005 \text{ mol dm}^{-3}$ acetyl acetone were insignificant ($p > 0.05$).

Changes in Proline



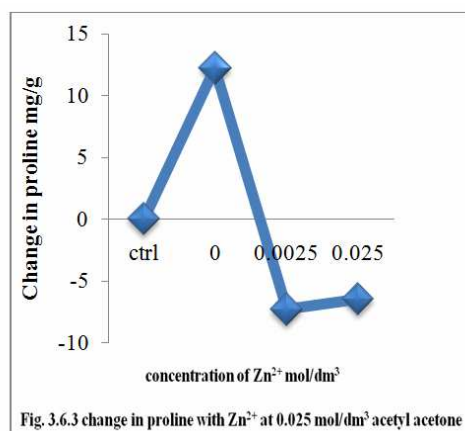
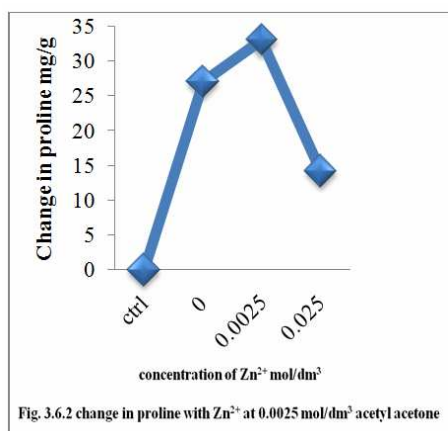
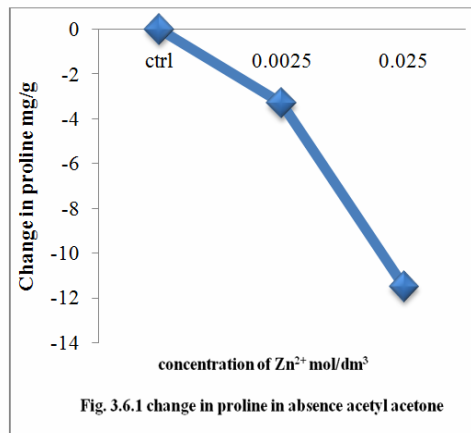
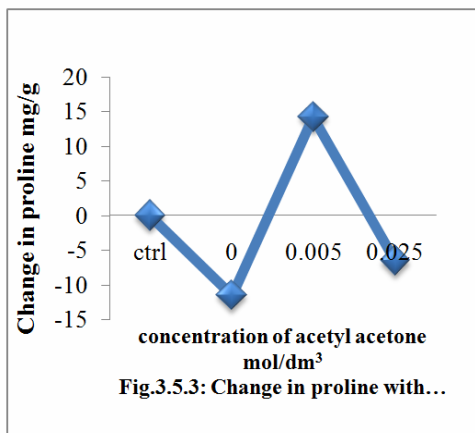


Table 3.1E. ANOVA output for changes in proline.

Parameter	Source	DF	SS	MS	F	P-value
Δ prln	Δ Zn ²⁺ + 0.000AA	1	0.065209	0.065209	534.9257	0.001864
	Δ Zn ²⁺ + 0.005AA	1	0.110078	0.110078	8.897387	0.040624
	Δ Zn ²⁺ + 0.025AA	1	0.096307	0.096307	1.744987	0.25701
	Δ AA + 0.000 Zn ²⁺	1	0.355782	0.355782	0.743044	0.479536

	ΔAA + 0.0025 Zn^{2+}	1	0.163779	0.163779	56.9637	0.001651
	ΔAA + 0.025 Zn^{2+}	1	0.203821	0.203821	22.99585	0.008677

Abbreviations: ΔAA = change in concentration of acetyl acetone, Δprln = change in proline, ΔZn^{2+} = change in concentration of zinc

Table 3.1E shows the changes in proline.

Figure 3.5.1 shows the changes in proline, with concentration of acetyl acetone in the absence of Zn^{2+} . The change in plant proline for all treatments including the control was determined by comparing with the corresponding control value. Addition of $0.005 \text{ mol dm}^{-3}$ acetyl acetone increases the proline, further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone causes decrease of proline content. Changes in proline contents of the plant due to the absence of Zn^{2+} were significant ($p < 0.05$).

Figure 3.5.2 shows the changes in proline, with concentration of acetyl acetone at $0.0025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$. At $0.000 \text{ mol dm}^{-3}$ acetyl acetone the proline decrease, addition of $0.005 \text{ mol dm}^{-3}$ acetyl acetone the proline increase, further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone, the proline content decreases. Changes in proline contents of the plant at $0.0025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ were significant ($p < 0.05$).

Figure 3.5.3 shows the changes in proline, with concentration of acetyl acetone at 0.025 Zn^{2+} . At $0.000 \text{ mol dm}^{-3}$ of acetyl acetone the proline decreases. Addition of $0.005 \text{ mol dm}^{-3}$ increases the proline content, further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone decreases the proline. Changes in proline contents of the plant at $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ were insignificantly ($p > 0.05$).

Figure 3.6.1 shows the changes in proline with concentration of Zn^{2+} in the absence of acetyl acetone. At $0.000 \text{ mol dm}^{-3}$ of acetyl acetone the proline decreases. Addition of $0.0025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ decreases the proline, further addition of $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ decreases the proline. Changes in proline contents of the plant due to the absence of acetyl acetone were insignificant ($p > 0.05$).

Figure 3.6.2 shows the changes in proline, with concentration of Zn^{2+} at 0.005 mol dm^{-3} of acetyl acetone. At 0.000 mol dm^{-3} and 0.0025 mol dm^{-3} Zn^{2+} , the proline content increases, further addition of 0.025 mol dm^{-3} Zn^{2+} the proline content decreases drastically. Changes in proline contents of the plant due to the absence of acetyl acetone were significant ($p < 0.05$).

Figure 3.6.3 shows the changes in proline, with concentration of Zn^{2+} at 0.025 mol dm^{-3} of acetyl acetone. At 0.000 mol dm^{-3} Zn^{2+} the proline content increases, addition of 0.025 mol dm^{-3} Zn^{2+} decreases the proline, further addition of 0.025 mol dm^{-3} Zn^{2+} increases in proline, Changes in proline contents of the plant at 0.005 mol dm^{-3} acetyl acetone were significant ($p < 0.05$).

Changes in Root and Shoot Zinc

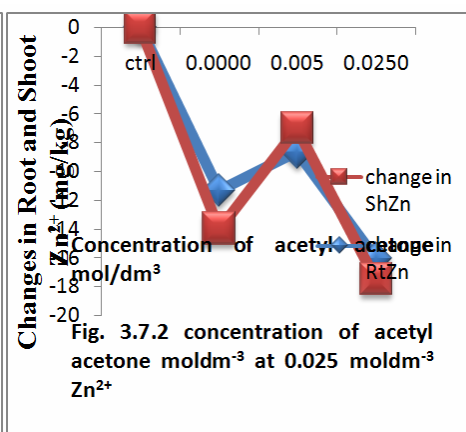
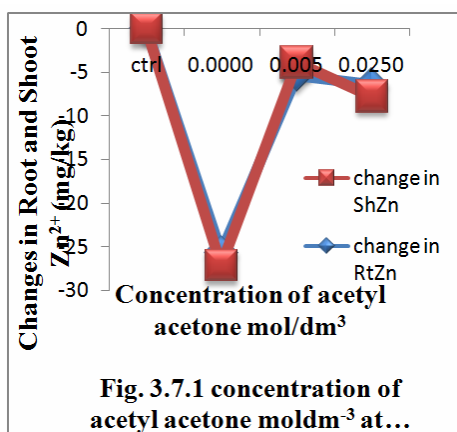


Table 3.1F. ANOVA output for shoot and root zinc.

Parameter	Source	DF	SS	MS	F	P-value
$\Delta RtZn$ and $\Delta ShZn$	ΔAA + 0.0025 Zn^{2+}	2	300.3774	150.1887	3.351164	0.105391
	ΔAA + 0.0250 Zn^{2+}	2	269.9856	134.9928	21.28785	0.001885

Abbreviations: ΔAA = change in concentration of acetyl acetone, $\Delta RtZn$ = changes in root zinc, ΔZn^{2+} = change in concentration of zinc, $\Delta ShZn$ = changes in Shoot zinc

Table 3.1F shows the changes in root and shoot zinc.

Figure 3.7.1 shows the changes in root ($\Delta RtZn$) and shoots ($\Delta ShZn$) Zinc in Cucumber (*Cucumis sativus*) seedlings replanted in various hydroponic mixture. These changes were determined by subtracting the corresponding control values from the values of individual treatments. For $0.0025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ with change in concentration of acetyl acetone mol dm^{-3} , $\Delta ShZn$ uptake was slightly lower than that of $\Delta RtZn$ at 0.000 acetyl acetone. When the concentration of acetyl acetone was increased to $0.005 \text{ mol dm}^{-3}$, the root and shoot Zn decreased. Further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone slightly decreases the Zinc uptake of the shoot, and that of root maintained. Changes in root and shoot of the plant at $0.0025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ were insignificant ($p > 0.05$).

Figure 3.7.2 shows the changes in root ($\Delta RtZn$) and shoots ($\Delta ShZn$) Zinc in Cucumber (*Cucumis sativus*) seedlings replanted in various hydroponic mixtures. These changes were determined by subtracting the corresponding control values from the values of individual treatments. For $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ with change in concentration of acetyl acetone mol dm^{-3} , $\Delta ShZn$ uptake was lower than that of $\Delta RtZn$ at $0.000 \text{ mol dm}^{-3}$ acetyl acetone. When the concentration of acetyl acetone was increased to $0.005 \text{ mol dm}^{-3}$, the root and shoot Zn decreased. Further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone of the zinc uptake of but the root and that of shoot increases. Changes in root and shoot of the plant at $0.025 \text{ mol dm}^{-3} \text{ Zn}^{2+}$ were significant ($p < 0.05$).

Changes in Translocation Factor

Table 3.1G. ANOVA output for change in translocation factor.

Parameter	Source	DF	SS	MS	F	P-value
ΔTF	ΔAA + 0.0025 Zn^{2+}	1	0.018194	0.018194	0.137627	0.729464
	ΔAA + 0.0250 Zn^{2+}	1	0.000314	0.000314	0.014404	0.910258

Abbreviations: ΔAA = change in concentration of acetyl acetone, ΔTF = Change in translocation factor, ΔZn^{2+} = change in concentration of zinc

Table 3.1G shows the changes in translocation factor.

Figure 3.8.1 shows the changes in Zn^{2+} translocation factor in Cucumber (*Cucumis sativus*) seedlings replanted in various hydroponic mixtures. Zinc translocation factor (TF) is defined as ratio of shoot to root concentration of zinc (Adriano [1]).

The changes in translocation factor (ΔTF) were determined by subtracting the corresponding control values from the values of individual treatments. For $0.0025 \text{ mol dm}^{-3} Zn^{2+}$ with $0.000 \text{ mol dm}^{-3}$ acetyl acetone change in translocation factor increased. When the concentration of acetyl acetone was increased to $0.005 \text{ mol dm}^{-3}$, the translocation factor decreased, further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone causes the decrease of the translocation factor. These changes in translocation factor at $0.0025 \text{ mol dm}^{-3}$ were insignificant ($p > 0.05$).

Figure 3.8.2 shows the changes in Zn^{2+} translocation factor in Cucumber (*Cucumis sativus*) seedlings replanted in various hydroponic mixtures. For $0.0025 \text{ mol dm}^{-3} Zn^{2+}$ with $0.000 \text{ mol dm}^{-3}$ acetyl acetone change in translocation factor decreased. When the concentration of acetyl acetone was increased to $0.005 \text{ mol dm}^{-3}$, the change in translocation factor increases, further addition of $0.025 \text{ mol dm}^{-3}$ acetyl acetone caused a serious increase of the translocation factor. These changes in translocation factor at $0.025 \text{ mol dm}^{-3}$ were insignificant ($p > 0.05$).

Changes in pH of Hydroponics

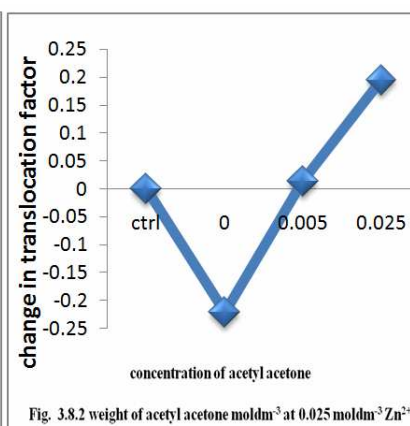
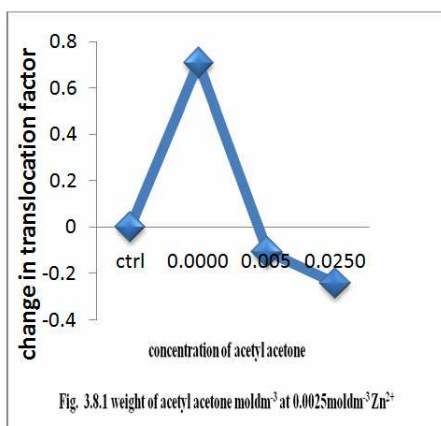


Table 3.1H. ANOVA Output for changes in hydroponic pH.

Parameter	Source	DF	SS	MS	F	P-value
Δ pHBP and Δ pHAH	Δ AA + 0.0025 Zn^{2+}	2	0.100716	0.050358	90.17713	3.34E-05
	Δ AA + 0.0250 Zn^{2+}	2	99.92982	49.96491	210.3034	2.78E-06

Abbreviations: Δ AA = change in concentration of acetyl acetone, Δ pHBP = change in pH before replanting, Δ Zn^{2+} = change in concentration of zinc, Δ pHAH = change in pH after harvest

Table 3.1H shows changes in hydroponic pH.

Figure 3.9.1 shows the changes in pH of hydroponic mixtures before planting (Δ pHBP) and after harvest (Δ pHAH). They were determined by subtracting the corresponding control values from the values of individual treatments. A negative value indicates that the pH of a treatment is less than the control pH. Changes in pH before planting and after harvest were zero for control. When the concentration of Zn^{2+} is 0.0025 mol dm⁻³, then the pH before planting increased with increase in the concentration of acetyl acetone from 0.000, 0.005 and 0.025 mol dm⁻³. When the concentration of Zn^{2+} is 0.0025 mol dm⁻³, the pH after harvest slightly decreased with increase in the concentration of acetyl acetone from 0.000 and 0.005 further addition of 0.025 mol dm⁻³ acetyl acetone increased the pH of but before planting and after harvest. These changes in pH before planting and after harvest at 0.0025 mol dm⁻³ are insignificant ($p > 0.05$).

Figure 3.9.2 shows the changes in pH of hydroponic mixtures before planting (Δ pHBP) and after harvest (Δ pHAH). When the concentration of Zn^{2+} is 0.0025 mol dm⁻³, the pH before planting and after harvest increased at 0.000 mol dm⁻³ of acetyl acetone, further addition of 0.005 mol dm⁻³ acetyl acetone caused a slightly decrease in pH before planting and after harvest, the slightly decrease was maintained after the addition of 0.025 mol dm⁻³ acetyl acetone. These changes in pH before planting and after harvest at 0.025 mol dm⁻³ are insignificant ($p > 0.05$).

Discussion

Various abiotic stresses decrease the chlorophyll content in plants (Ahmad et al. [2]); It was shown that the plants treated with Zn exhibited inhibitory effect with respect to chlorophyll (a) chlorophyll (b) and carotenoid contents at high concentrations of Zn compared with controls. At low concentration of Zn the chlorophyll (a), chlorophyll (b) and carotenoid contents increased. Copper was more toxic than Zn in terms of chlorophyll inhibition. The present result showed decrease in chlorophyll content corroborated with the findings of (Bassi and Sharma [3]); who found that Copper was more toxic than Zn in terms of chlorophyll inhibition in cucumber seedlings. The losses in chlorophyll content can consequently lead to disruption of photosynthetic machinery. Further increase in zinc level significantly decreased the chlorophyll and corotenoid content. The increased chlorophyll and carotenoid content was obviously due to zinc at low-level act as a structural and catalytic components of proteins, enzymes and as cofactors for normal development of pigment biosynthesis. The excess zinc treatment brought about a marked depression in photosynthetic pigment in plants (Manivasagaperumal et al. [24]);

The first symptom of Zn toxicity is a general chlorosis of the younger leaves (Herren and Feller [13]); (Fontes and Cox [9]). Increased concentration of Zn causes changes in plant growth parameter as reported by (Fosmire [10]); that excess zinc can be harmful and cause zinc toxicity. Zn^{2+} and 0.000, 0.005, 0.025 mol dm⁻³ acetyl acetone to the various hydroponic mixtures in which *Cucumis sativus* seedlings were replanted affected the chlorophyll (a) and chlorophyll (b), carotenoid, proline, time of harvest, pH of the solutions, concentrations of Zn in root and shoot, translocation factor and weight of plant, volume of solution, Number of falling leaves. The plants were harvested when they died. Those planted in treated hydroponics died earlier than control seedlings. This could be due to that excess zinc. The weights of plants harvested in all treatment were found to decrease. According to (Wolt [43]), absorbed zinc resulted in reduction in growth rate of roots and change in branching pattern. Several workers have reported the inhibition of root grow that 1µM to 1cM Zn or at a soil Zn content of 10µgg¹. A considerable decrease in dry weights of plant parts is observed under Zn treatment (Richards et al. [34]).

Leaf fall was observed during the growth of seedlings. This could be due to the concentration of Zn^{2+} (0.000 – 0.025 mol dm⁻³) and acetyl acetone (0.000 – 0.250

mol dm⁻³). The toxic effects of heavy metals on plants depend largely on the metal concentrations in the nutrient solution (Rashid et al. [33]); As reported by (Laurie and Manthey [22]), acetyl acetone a chelating agent, forms a Zn-AA complex at pH 5.2 to pH 7.7. Increasing the concentration of acetyl acetone favours the formation of the complex.

Uptake of metals into plant roots is a complex process involving transfer of metals from the soil solution to the root surface and inside the root cells. Understanding of uptake processes is hampered by the complex nature of the rhizosphere, which is in continual dynamic change interacted upon by plant roots, the soil solution composing it and microorganisms living within the rhizosphere (Laurie and Manthey [22]);

Zinc translocation to root xylem occurs via symplast and apoplast (Brennan [4]); (Broadley et al. [5]); but high level of zinc have also been phloem, denoting that this metal is translocated both through xylem and phloem tissues (Pearson et al. [29]);

Conclusion

The addition of 0.000 to 0.025 mol dm⁻³ Zn²⁺ and 0.000 to 0.250 mol dm⁻³ acetyl acetone to various hydroponic solutions affected the growth of replanted Cucumber (*Cucumis sativus*) seedlings. Seedlings replanted in treated solutions died earlier than those replanted in control. The weights of harvested plants, volume of hydroponic solutions, chlorophyll (a), chlorophyll (b) and carotenoid decreased due to the toxicity of zinc. The rate and extent of translocation of zinc within the plants depended on the concentrations of Zn²⁺ and acetyl acetone in the hydroponic mixtures. The highest concentration of zinc taken up by the root over the control was about 400mg/kg. This was obtained from the seedlings replanted in hydroponic solution containing 0.0025 mol dm⁻³ Zn²⁺ and 0.000, 0.005 and 0.025 mol dm⁻³ acetyl acetone. The highest concentration of Zn²⁺ in the shoot was about 400mg/kg and that of root was about 150mg/kg. This was obtained from the seedling grown in hydroponic containing 0.025 mol dm⁻³ Zn²⁺ and 0.000, 0.005 and 0.025 mol dm⁻³ acetyl acetone.

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