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Effects of Acetyl Acetone on Growth and Zinc Uptake by Drumstick (*Moringa Oleifera*) Seedlings Replanted in Hydroponic Solutions

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ABSTRACT

The aim of this work was to investigate the effects of acetyl acetone on Zinc (Zn) uptake and growth of Drumstick (*Moringa oleifera*) plants. Thirty seedlings were raised in hydroponic solution containing varied concentrations of Zn and acetyl acetone. The results showed a significant ($p>0.05$) change in weights of the plants when concentrations of Zn and acetyl acetone were added. Plants grown in treated hydroponics died before control plants. Zn uptake and accumulation were dose dependent with respect to addition of both Zn and acetyl acetone. The translocation factor decreased highly insignificantly ($P>0.05$) at lower concentration of Zn and significant at higher concentration in treated plants compared to control. The chlorophyll, carotenoid and proline content were severely decreased after addition of Zn and acetyl acetone, inducing toxicity symptoms. Thus, acetyl acetone did not play any significant role in alleviating Zn-induced toxicity.

Keywords : Acetyl acetone, *Moringa oleifera,* Zn, Hydroponic, Proline, Zn

I. INTRODUCTION

Zinc is one of the most common elements in the Earth's crust. It is found in the air, soil, and water and is present in all foods. It acts as a plant nutrient and plays an important role in many metabolic processes and it is a co-factor of numerous enzymes. It is however toxic at elevated concentrations. The first symptom of Zn toxicity is a general chlorosis of the younger leaves [1, 2, 3]. It has been reported that the plants exhibiting Zn toxicity have smaller leaves than control plants [3].

Acetyl acetone is an organic compound that exists in two tautomeric forms, which interconvert rapidly and are treated as a single compound in most applications. Although the compound is formally named as the diketone, pentane-2, 4-dione, the enol tautomer forms a substantial component of the material and is actually the favoured form in many solvents. It is a colourless liquid, a precursor acetylacetonate (acac), a common bidentate ligand. It is also a building block for the synthesis of heterocyclic compounds. Enzyme acetylacetonedioxygenase cleaves the carbon-carbon bond of acetyl acetone, producing acetate and 2-oxopronal. The enzyme is Fe(II)-dependent, but has been proven to bind to zinc as well [4].

Drumstick (*Moringa oleifera*) is the most widely cultivated species of a *monogeneric* family, the *Moringaceae* [5]. The tree originated from the Indian subcontinent and has become naturalized in the tropical and subtropical areas of the world [6]. While it grows best in dry sandy or loamy soil that is slightly alkaline [7]. It is adaptable to various soil conditions from 4.5 to 8.0 pH, but does not tolerate water logging, freezing or frost conditions [8].There is no data indicating the role of acetyl acetone in enhancing Zn uptake and reducing Zn-induced toxicity. For this reason, this study was designed to

examine the effect of acetyl acetone addition on Zn uptake and the growth of *Moringa oleifera* plants.

II. METHODS AND MATERIAL

2.1 Plant Material

Thirty seedlings of Drumstick (*Moringa oleifera*) were obtained from a garden of Dr. Gunter's Newmann Dental Center Zaria Road Kano State on Monday, 3rd August, 2015 at 10:00am. The seedlings were identified by Baha'uddeen Said Adam of the Department of Plant Science, Bayero University, Kano. The seedlings were then uprooted from their planting bags, washed thoroughly with tap water and then rinsed with deionized water.

2.2 Preparation of Hydroponic Solutions

2.2.1 Control.

The control contained 2.56×10^{-6} moldm^{-3} KNO_3, 5.0×10^{-4} moldm^{-3} $MgSO_4.H_2O$, 1.03×10^{-3} moldm^{-3} $FeCl_3.6H_2O$, 2.50×10^{-6} moldm^{-3} KI, 2.31×10^{-3} moldm^{-3} $MnSO_4.H_2O$, 2.27×10^{-3} moldm^{-3} H_3BO_3, 3.57×10^{-4} moldm^{-3} $Ca(NO_3)_2.4H_2O$, 5.0×10^{-4} moldm^{-3} $Na_2H_2P_2O_7$ and 0.10 moldm^{-3} HNO3 500 cm^3 of the control was prepared by pipetting 1.28 cm^3 of 0.10 moldm^{-3} KNO_3, 5.15 cm^3 of 0.10 moldm^{-3} $FeCl_3.6H_2O$, 11.35 cm^3 of 0.10 moldm^{-3} H3BO3, 5.00 cm^3 of 0.05 moldm^{-3} $MgSO_4.H_2O$, 3.57 cm^3 of 0.05 moldm^{-3} $Ca(NO_3)_2.4H_2O$, 5.00 cm^3 of 0.05 moldm^{-3} $Na_2H_2P_2O_7$, 0.17 cm^3 of 0.0075 moldm^{-3} KI, 23.10 cm^3 of 0.05 moldm^{-3} $MnSO_4.H_2O$ and 5.00 cm^3 of 0.10 moldm^{-3} HNO_3 into a 500 cm^3 volumetric flask The volume was made to mark with deionized water.

2.2.2 Hydroponics Containing 0.0025 moldm^{-3} and 0.025 moldm^{-3}

A 500 cm^3 of a hydroponic containing 0.0025 moldm^{-3} Zn(NO3)2 was prepared by pipetting 5 cm^3 of 0.25 moldm^{-3} $Zn(NO_3)_2$ 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 moldm^{-3} zinc nitrate was prepared by pipetting 50 cm^3 of 0.25 moldm^{-3} $Zn(NO_3)_2$ into a 500 cm^3 volumetric flask. The volume was made to mark with deionized water after adding the other components.

2.2.3 Hydroponics Containing 0.0025 moldm^{-3} $Zn(NO_3)_2$ and 0.005 moldm^{-3} Acetyl acetone.

A 500 cm^3 of a hydroponic containing 0.0025 moldm^{-3} $Zn(NO_3)_2$ and 0.005 moldm^{-3} acetyl acetone was prepared by pipetting 5 cm^3 of 0.25 moldm^{-3} $Zn(NO_3)_2$ and 10 cm^3 of 0.25 moldm^{-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 hydroponic mixtures containing different concentrations of $Zn(NO_3)_2$ and acetyl acetone were prepared by adding appropriate volumes of reagents and diluting to 500 cm^{-3} with deionised water.

2.3 Replanting of Drumstick (*Moringa Oleifera*) Seedlings

Pre-treated Drumstick (*Moringa oleifera*) seedlings were separately replanted in 500 cm^3 of hydropics containing 0.0000, 0.0025, 0.025 moldm^{-3} $Zn(NO_3)_2$ with 0.000, 0.005, 0.250 moldm^{-3} acetyl acetone respectively in clean 750 cm^3 table water plastic bottles on 5th August, 2015 around 10:00 am. Each treatment was replicated three times. The replanted seedlings were kept in the screen house of Biological Science Department, Bayero University Kano.

2.4 Harvesting of Seedlings

The seedlings were harvested separately. Seedlings in the control were harvested on Tuesday 16th, August

2015 by 1.20pm. However, seedlings in the hydroponics containing 0.0025 moldm^{-3} and 0.025 moldm^{-3} $Zn(NO_3)_2$ were only harvested on 11th , 12th and 13th August. The harvested seedlings were washed with tap water and rinsed thoroughly with deionized water and dried.

2.5 Analysis of Zinc

Two grams of each vegetable was weighed in different crucibles. One millilitre 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 4800C for 3 hours, after which they cooled to room temperature. The cooled samples were dissolved in 5ml of 30% HCl and then filtered using Whatman filter papers. The filtrates were individually poured into 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.

2.6 Chlorophyll and Carotenoid Analysis

Chlorophyll estimation of leaves of treated and control plants was done according to the method of Arnon (1949). Two hundred milligrams (200 mg) of fresh leaf tissues of each sample was homogenized using chilled acetone in a test tubes. The homogenate was centrifuged 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 became 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 for chlorophyll a and chlorophyll b respectively.

2.7 Proline Analysis

The plant material was homogenized in 3% aqueous sulfosalicylic acid (0.01g /0.5 ml) and the residue was removed by centrifugation at 3000 rpm for 10 minutes. A 1 ml of homogenized tissue was reacted with 1ml acid-ninhydrin and 1ml glacial acetic acid in a test tube for 1 hour at 1000 ℃ and the reaction was terminated in an ice bath. The reaction mixture was extracted with 2ml toluene, mixed vigorously and left at room temperature for 30 minutes until the two phases separated. The chromophore-containing toluene (1 ml, upper phase) was warmed to room temperature and its optical activity measured at 520 nm using toluene as blank. The proline concentration is determined from a standard curve using D-proline.

2.8 Data Analysis

The data were analyzed through one-way analysis of variance (ANOVA) to determine the effect of treatments.

III. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effects of Zinc and Acetyl acetone on the Change in Weight of *Moringa oleifera* Plants

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 7.913±0.959 g. Values of ΔWP for 0.0025 and 0.025 moldm^{-3} Zn^{2+} were -10.846±1.475 g and -8.490±0.645 g 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 moldm^{-3}, the values of ΔWP were -10.846±1.475, -3.513±0.888 and -9.533±2.107 g, for 0.000, 0.005 and 0.025 moldm^{-3} acetyl acetone respectively. The corresponding values of ΔWP for 0.025 moldm^{-3} were -8.490±0.645, -11.640±1.028 and -8.250±1.721 for 0.000, 0.005 and 0.025 moldm^{-3} acetyl acetone respectively. However, the correlation of acetyl acetone with ΔWP was insignificant (p>0.05).

Table 1: Effects of Zn and Acetyl acetone on Change in Weight (ΔWP)

Treatments		ΔWP
Zinc	Acetyl acetone	
0.0000	0.0000	-7.913±0.959
0.0025	0.0000	-10.846±1.475
0.0250	0.0000	-8.490±0.645
0.0000	0.0050	-10.030±2.047
0.0000	0.0250	-5.476±1.601
0.0025	0.0050	-3.513±0.888
0.0025	0.0250	-9.533±2.107
0.0250	0.0050	-11.640±1.028
0.0250	0.0250	-8.250±1.721

3.1.2 Effects of Zn and Acetyl acetone on the Roots and Shoots Uptakes of *Moringa oleifera* Plants

Fig. 1 shows the changes in root (ΔRtZn) and shoot (ΔShZn) Zn concentrations on Drumstick (*Moringa oleifera*) seedlings replanted in various hydroponic mixtures. These changes were determined by subtracting the corresponding control values from the values of individual treatments. For 0.0025 moldm^{-3} Zn^{2+} with change in concentration of acetyl acetone moldm^{-3}, ΔShZn uptake was higher than that of ΔRtZn at 0.000 acetyl acetone. When the concentration of acetyl acetone was increased to 0.005 moldm^{-3}, the root and shoot Zn decreased. Further addition of 0.025 moldm^{-3} acetyl acetone maintained the Zinc uptake of the shoot, but slightly increased root uptake. Changes in root and shoot uptake at 0.0025 moldm^{-3} Zn^{2+} were insignificant (P>0.05).

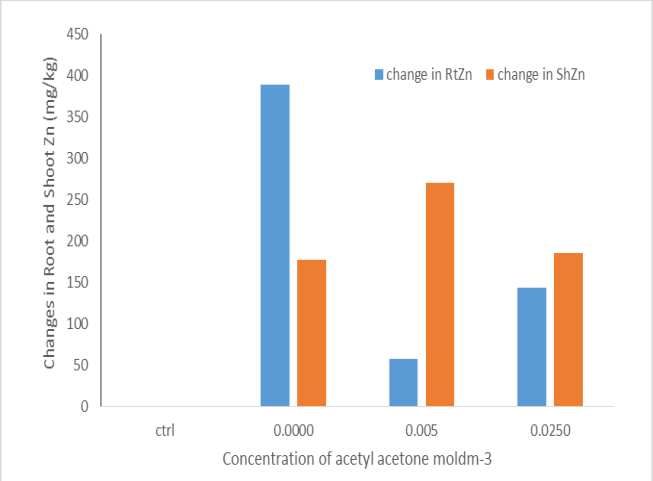

Figure 1: Effects of Zn and Acetyl acetone on the Root and Shoot Uptakes of *Moringa oleifera* Plants

3.1.3 Effects of Zn and Acetyl acetone on the Roots and Shoots Uptakes of *Moringa oleifera* Plants

Fig. 2 shows the changes in Zn^{2+} translocation factor in Drumstick (*Moringa oleifera*) seedlings replanted in various hydroponic mixtures. Zinc translocation factor (TF) is defined as ratio of shoot to root concentration of zinc [9].The changes in translocation factor (ΔTF) were determined by subtracting the corresponding control values from the values of individual treatments. For 0.0025 moldm^{-3} Zn^{2+} with 0.000 moldm^{-3}, acetyl acetone change in translocation factor increased slightly. When the concentration of acetyl acetone was increased to 0.005 moldm^{-3} the translocation factor increased, further addition of 0.025 moldm^{-3} acetyl acetone caused a serious decrease of the translocation factor. These changes in translocation factor at 0.0025 moldm^{-3} were insignificant (p>0.05).

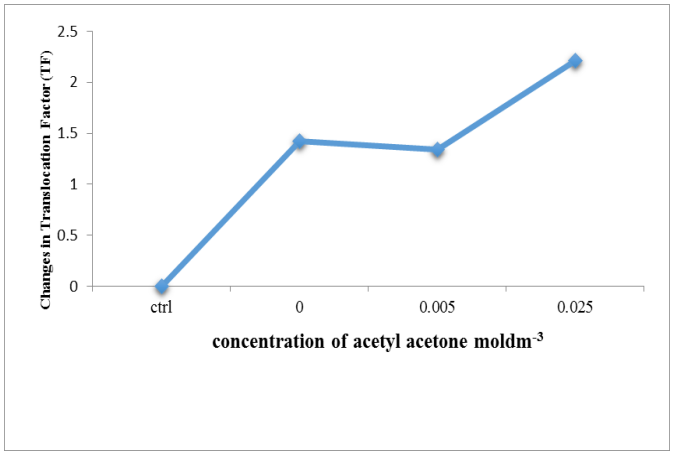

Figure 2: Effects of Zn and Acetyl acetone Translocation factor

Fig. 3 shows the changes in chlorophyll (a) and chlorophyll (b).Concentration of Zn^{2+} was varied from 0.000, 0.0025 and 0.025 moldm^{-3} at 0.0050 moldm^{-3} acetyl acetone. At 0.000 moldm^{-3} Zn^{2+}, there was increase in the chlorophyll (a) and subsequent decrease in the chlorophyll (b). Addition of 0.0025 moldm^{-3} caused increase in the chlorophyll (a) and (b). Further addition of Zn^{2+} to 0.025 moldm^{-3} decreased the content of chlorophyll (a) and (b). Changes in chlorophyll (a) and chlorophyll (b) contents of the plant at 0.0050 moldm^{-3} of acetyl acetone were significant ($p<0.05$).

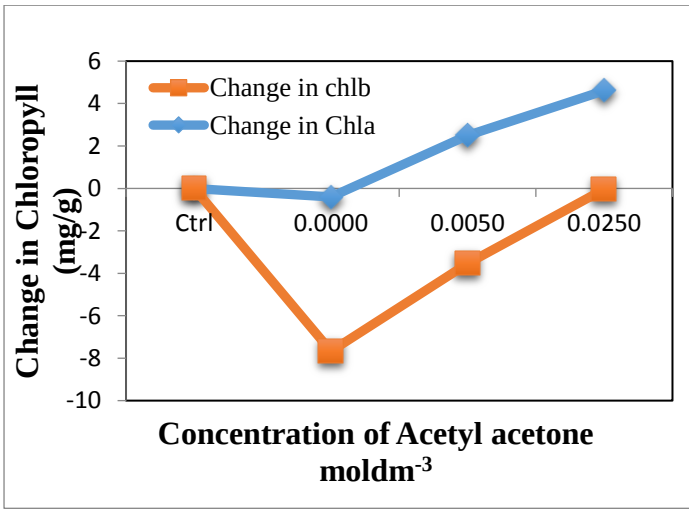

Figure 3 : Effects of Zn and Acetyl acetone Pigment Contents

3.1.4 Effects of Zinc and Acetyl acetone on Proline contents of *Moringa oleifera* Plants

Fig. 4 shows the changes in proline, with concentration of acetyl acetone at 0.025 Zn^{2+}. At 0.000 moldm^{-3} of acetyl acetone, no any change was observed. Addition of 0.005 moldm^{-3} decreased the proline content. Further addition of 0.025 moldm^{-3} acetyl acetone increased the proline. Changes in proline content of the plant at 0.025 moldm^{-3} Zn^{2+} were insignificantly ($p>0.05$).

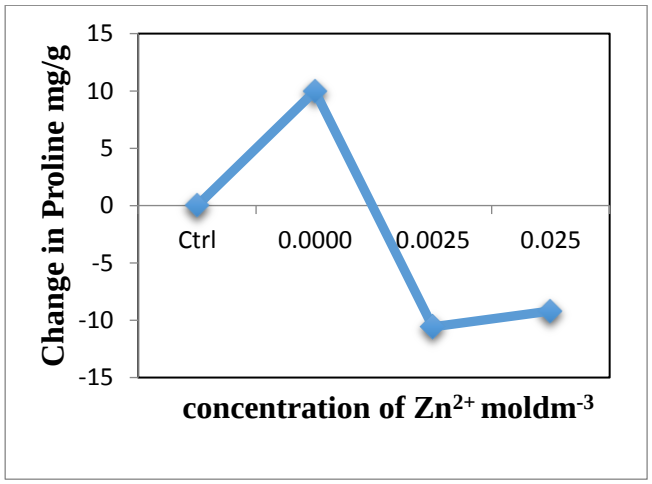

Figure 4: Effects of Zinc and Acetyl acetone Proline Contents

3.2 Discussion

Various abiotic stresses decrease the chlorophyll content in plants (Ahmad et al., 2007). 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 which showed decrease in chlorophyll content corroborated with the findings of (Bassi and Sarma, 1993) who found that Copper was more toxic than Zn in terms of chlorophyll inhibition in Moringa seedlings. The losses in chlorophyll content can

consequently lead to disruption of photosynthetic machinery. Further increase in zinc level significantly decreased the chlorophyll and carotenoid content. The increased chlorophyll and carotenoid content due to zinc at low-level acted as 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.

The first symptom of Zn toxicity is a general chlorosis of the younger leaves [2]. Increased concentration of Zn caused changes in plant growth parameter as reported [6] that excess zinc can be harmful and cause zinc toxicity. Zn^{2+} and 0.000, 0.005, 0.025 $moldm^{-3}$ acetyl acetone was added to various hydroponic mixtures in which *Moringa oleifera* seedlings were replanted. This affected the chlorophyll (a) and chlorophyll (b), crotenoid, proline, time of harvest, pH of the solutions, concentrations of Zn in root and shoot, translocation factor, weight of plant, volume of solution and 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 the excess zinc. The weights of plants harvested in all treatment were found to decrease. The bsorbed zinc resulted in reduction in growth rate of roots and change in branching pattern. Several workers have reported the inhibition of root growth at 1 cm to 1 cm Zn or at a soil Zn content of 10 µg/g. A considerable decrease in dry weights of plant parts is observed under Zn treatment [10].

Leaf fall was observed during the growth of seedlings. This could be due to the concentration of Zn^{2+} (0.000 – 0.025 $moldm^{-3}$) and acetyl acetone (0.000 – 0.250 $moldm^{-3}$). The toxic effects of heavy metals on plants depend largely on the metal concentrations in the nutrient solution [11]. As reported by Laurie *et al.*, [10], 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 [11].

Zinc translocation to root xylem occurs via simplast and apoplast [12], but high level of zinc have also been found in phloem, denoting that this metal is translocated both through xylem and phloem tissues [13].

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