AJFAND ONLINE
Volume 10 No. 5
May 2010
ISSN 1684 5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

MICRO-ELEMENT CONTENTS IN ROSELLE (*HIBISCUS SABDARIFFA* L.) AT DIFFERENT GROWTH STAGES

Atta S*[1], Diallo AB[2], Bakasso Y[3], Sarr B[1], Saadou M[3] and RH Glew[4]

Sanoussi Atta

*Corresponding author email: s.atta@agrhymet.ne

[1]Centre Régional AGRHYMET, BP 11011 Niamey, Niger

[2]Direction de la Météorologie Nationale, BP 218 Niamey, Niger

[3]Département de Biologie, Faculté des Sciences, Université Abdou Moumouni, BP 10662 Niamey, Niger

[4]Departement of Biochemistry and Molecular Biology, School of Medicine, University of New Mexico, Albuquerque, New Mexico 87131, U.S.A.

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

ABSTRACT

In the western Sahel, leaves of Roselle (*Hibiscus sabdariffa*) have considerable economic importance because of their nutritional and medical uses. These plant organs are used to supplement nutrients provided by cereals such as millet and sorghum. However, there is a lack of information on the nutrient composition of these plant organs of Roselle at different growth stages. Therefore, the experiment was carried out under rainfall conditions during the 2006 rainy season (from July to September) at the experimental station of the Agrhymet Regional Centre in Niamey (Niger). The content of the micronutrients Fe, Mn, Cu and Zn in leaves of three ecotypes of Roselle (A3, A7 and A9) at three growth stages, vegetative (stage I), flowering (stage II), and mature (stage III) was determined. The experimental design was a randomized complete block with four replicates and one variable (ecotype). Results indicated that at stage I, ecotype A3 had higher Fe content in leaves. In addition, A3 had also the highest Zn content in leaves at stage I. For all three ecotypes, Fe and Zn content in the leaves decreased significantly ($p<0.05$) from stage I to stage II, then remained constant until stage III. For Fe, the decrease between stage I and II was 37% for A3 and 50%, respectively for A7 and A9. The corresponding decrease of Zn content was 30% for A7 and 50%, respectively, for A3 and A9. The Mn content in the leaves of Roselle was similar for the three ecotypes at stage I, thereafter increased continuously during plant growth. From stage I to II, the increase was about 90%, 70% and 50%, respectively for A9, A7 and A3. From stage II to III, the increase in Mn content in the leaves was significantly ($p<0.05$) higher for A3 and A7, respectively 180% and 80%. At stages I and II, the highest Cu content was recorded for A3 and the lowest one for A7. During the whole cycle of plant growth, the Cu content in the leaves was relatively constant for A9. In contrast, Cu content in the leaves decreased for the remaining ecotypes. Therefore the vegetative stage corresponding to 25 days after sowing is the recommended optimal harvest time of Roselle to maximise on the nutrients.

Keys words: Roselle, micronutrient, leaves, stage, Niger

INTRODUCTION

In the Sahel, Roselle (*Hibiscus sabdariffa* L.), is a vegetable with considerable economic importance because of its leaves, seeds and calyces that are used for nutritional and medicinal uses. The leaves are consumed as a green vegetable and prepared like spinach [1]. In Niger, they are also used as an ingredient in sauces and therefore serve as a nutrient complement in cereals such as sorghum or millet. The leaves of *Hibiscus sabdariffa* were found to contain useful amount of micronutrients such as iron, manganese, copper and zinc [2, 3].

Iron and zinc are two of the micronutrients that are most often deficient in the diet of children in developing countries and women of reproductive age especially at risk for such deficiencies [4]. Iron deficiency is the primary cause of nutritional anemia worldwide [5]. In children, zinc deficiency leads to poor growth, impaired immunity, and increased morbidity from common infectious diseases and increased mortality [6, 7, 8].

The regular consumption of copper and manganese may prevent it deficiency adverse effects on the central nervous systems and skeletal anomalies among children [9].

However, several authors have reported that the micronutrient contents in leafy vegetable vary in plant organs such as leaves [10, 2, 3, 11]. This variation could be attributed mainly to the stage of plant growth. Therefore, the iron and manganese content of leaves increased while that of zinc and copper decreased between 15 to 30 days in some leafy vegetables [12]. Since Roselle leaves are consumed at different stages of maturity, it is important to have information about their mineral content at different stages of the growth of the plant. Moreover, the database of Roselle nutrient and chemical composition is incomplete and fragmentary [13]. The objective of this study was to investigate the microelement (Cu, Fe, Mn and Zn) contents in leaves of three Roselle ecotypes from Niger, at different stages of plant growth. This information will be important for nutrition educators as leaves are consumed at different stages of Roselle growth.

MATERIALS AND METHODS

Growing conditions

The experiment was conducted under natural rainfall conditions during the 2006 rainy season at the experimental station at the Agrhymet Regional Centre in Niamey, Niger (latitude 13° 29' N and longitude 2° 10' E, and altitude 222 m). The daily temperature varied from 20.3 to 27.4°C for the minimum and 28.8 to 37.6°C for the maximum during the growing season. The variation of air humidity was from 29.4 to 69.5% for the minimum and 74.7 to 97% for the maximum. The incoming radiation varied from 4,94 to 25,97MJ.m-2.j-1. The accumulated rainfall was 395 mm. However, a dry spell occurred during plant growth which particularly affected the maturation stage. The pH of soil at the experimental site was near neutral (7.4), with approximately 0.20 % of C, 0.162% of total N and 0.0479% of P [14].

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

Plant material

Three Roselle ecotypes (A3, A7 and A9) were field tested. These ecotypes were collected in the south sahelian zone of Niger (Fig. 1) by Professor Saadou Mahamane and Dr Bakasso Yakoubou [15]. Ecotypes A3 and A9 were collected in the area around the town of Dosso, and A7 in the area around the town of Maradi. Ecotypes A3 and A7 belong to the botanical type called "Waré" in the Hausa dialect which is cultivated mainly for its developed calyx. The colour of the calyx is dark red for A3 and black for A7. Ecotype A9 has a rose-colored calyx and belongs to the botanical type "Yakua", which has a medium sized calyx and is cultivated mainly for its leaves and seeds.

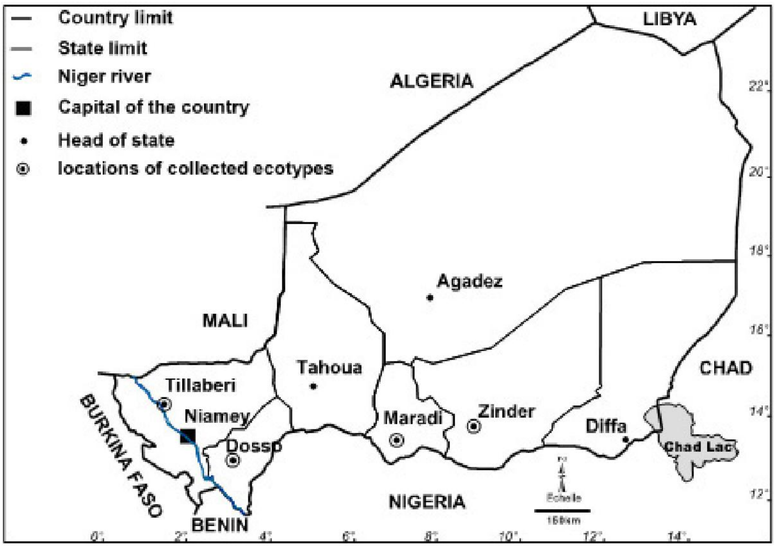

Figure 1: Map of the Republic of Niger and locations where ecotypes of Roselle were collected

Experimental design

The experimental design consisted of a complete randomized block design with four replications. The distance between two consecutive blocks was 2 m. The plot was 10 m long and 6 m wide. All plots consisted of 6 rows of plants with 1 m apart between rows and 1 m between plants within rows. The four central rows were intended for plant sampling.

Soil preparation and sowing

The soil of the experimental site was sandy with low moisture storage capacity and very high nutrient leaching. Therefore, 10 tons/ha of organic matter was applied in order to improve the water storage capacity of the soil moisture. Before sowing, composite fertilizer NPK (15-15-15) was applied at level of 100 kg N.ha^{-1}. The fertilizer was incorporated into the soil using a plow. Ten seeds per hole were hand sown on July 14, 2006. After 22 days, the crop was thinned to two plants. The experimental plots were weeded with the aid of a hoe, five times to assure good conditions for plant growth.

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

Plant sampling

Plants were sampled at three stages of growth to determine the variation of their nutrient composition:

- Stage I: 25 days after sowing (DAS) corresponding to vegetative growth stage;
- Stage II: 75 DAS (flowering stage);
- Stage III: 115 DAS (maturity stage).

For a given stage, five plants were randomly selected from each plot. The plants were then separated into different organs (leaves, stems, calyces and seeds) which were sun-dried.

Chemical analyses [16]

The leaves of three replicates were oven-dried (65ºC overnight) and finely milled using the multi-beads shocker (Yasui Kikai, model MB500E).

Prior to analysis, the samples were digested for the determination of Cu, Fe, Mn and Zn. The larger part of organic matter was oxidized by H_2O_2 at a relatively low temperature (100 to 110ºC during 2 hours). After decomposition of the excess of H_2O_2 and evaporation of water, the digestion was completed by concentrated sulfuric acid at elevated temperature (330ºC during 2 hours) under the influence of Se as a catalyst.

To the nearest 0.100 g, 0.3 g of the sample was weighed into a digestion tube. The quantitative determination of Cu, Fe, Mn and Zn in the extract was done by atomic absorption spectrometry using the Atomic Absorption Spectrometer, model Perkin-Elmer AAnalyst 400 (Shelton, USA). The samples were nebulized into an air-acetylene flame where they were vaporized. The compounds were atomized. Thereafter, the atoms thus formed measured at a wavelength of 324.7 nm for Cu, 248.3 nm for Fe, 285.2 nm for Mn and 213.9 nm for Zn.

Statistical analyses

Statistical analysis was performed using the GenStat software version 7.0. Tests for significant difference between means were made using the procedure of analysis of variance (ANOVA) and the Student Newman Keuls test at 0.05 probability level. A critical comparison was made to investigate the significant difference among the three growth stages.

RESULTS

Iron content. At the vegetative and flowering stages, ecotype A3 had the highest Fe content in leaves. A7 and A9 had similar content at vegetative stage, namely 330 µg of Fe/g d.w (Figure 2). At flowering stage, A9 had the lowest iron content in leaves. During plant growth, iron content in leaves decreased significant ($p<0.05$) for the three ecotypes. However, the magnitude of decrease differed among ecotypes and

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

between the two periods of growth (from stage I to II and from stage II to III). The highest decrease was recorded between stage I and II. During this period, iron content in leaves decreased significantly ($p<0.05$), by 50% for A9 and 40%, respectively for A3 and A7. From stage II to III, the decrease was similar for A3 and A9. However, Fe content in leaves remained constant for A7 during this period. At maturity stage, A7 and A9 had similar iron content in leaves, namely, 190 µg/g d.w.

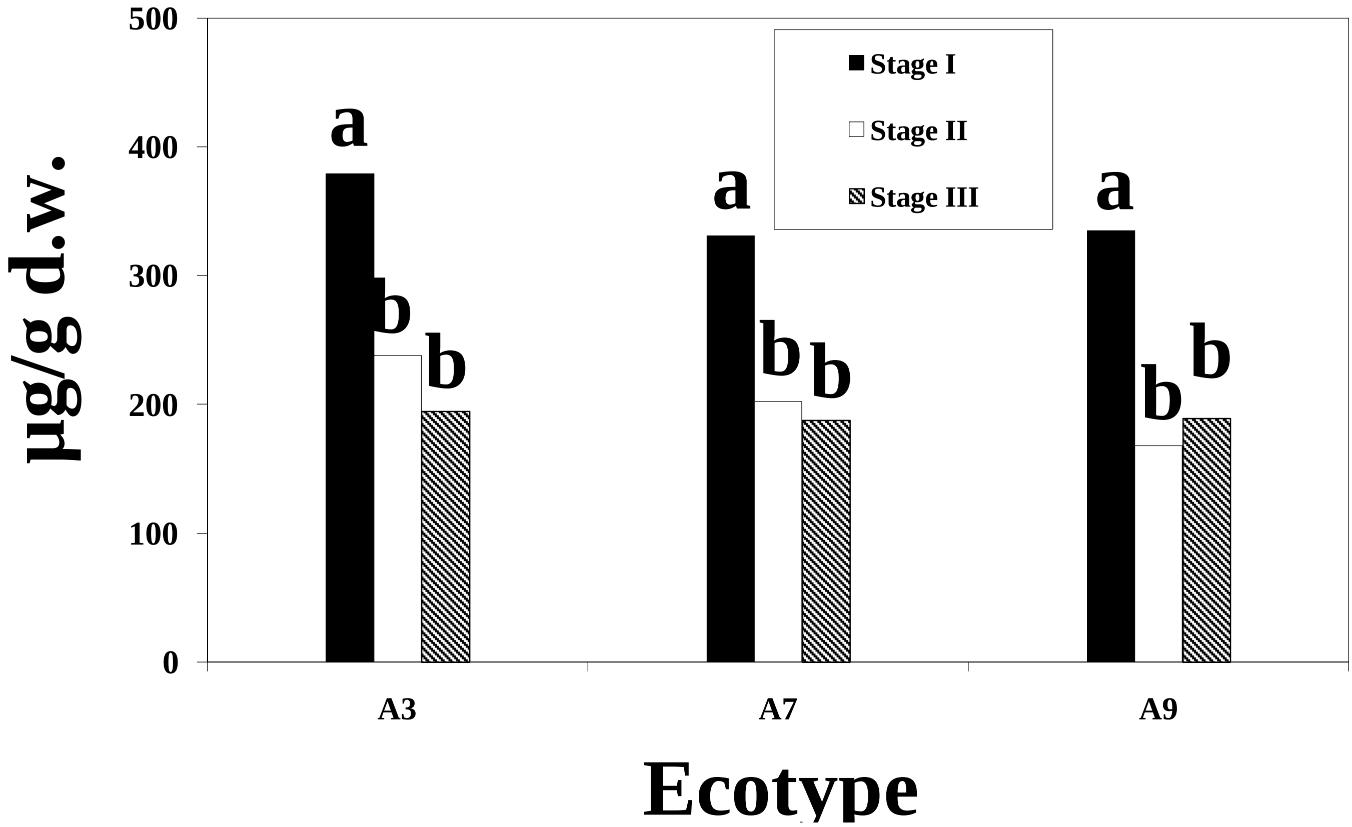

Figure 2: Fe content of leaves at different growth stages of Roselle
(stage I= 25 days after sowing; stage II= 75 DAS; stage III = 115 DAS)

Manganese content. At vegetative stage, the Mn content in leaves was similar for the three ecotypes (Figure 3). From the vegetative (stage I) to mature stage (stage III), a significant ($p<0.05$) increase in the Mn content was observed in leaves of all ecotypes: from the vegetative stage to flowering (stage II), the increase was about 90%, 70% and 50%, respectively for A9, A7 and A3. From stage II to III, the increase in Mn content in the leaves was significantly ($p<0.05$) greater for ecotypes A3 and A7 compared to those of previous period of growth, 180% and 80%, respectively for A3 and A7. For A9, the increase was about 50%.

At stage II, A3 and A9 had higher and similar Mn content in leaves. At stage III, the Mn content in the leaves was significantly ($p<0.05$) higher for A3 (1500 µg of Mn/g d.w.) compared to those of A9 and A7 (respectively 800 µg of Mn/g d.w. and 780 µg of Mn/g d.w.).

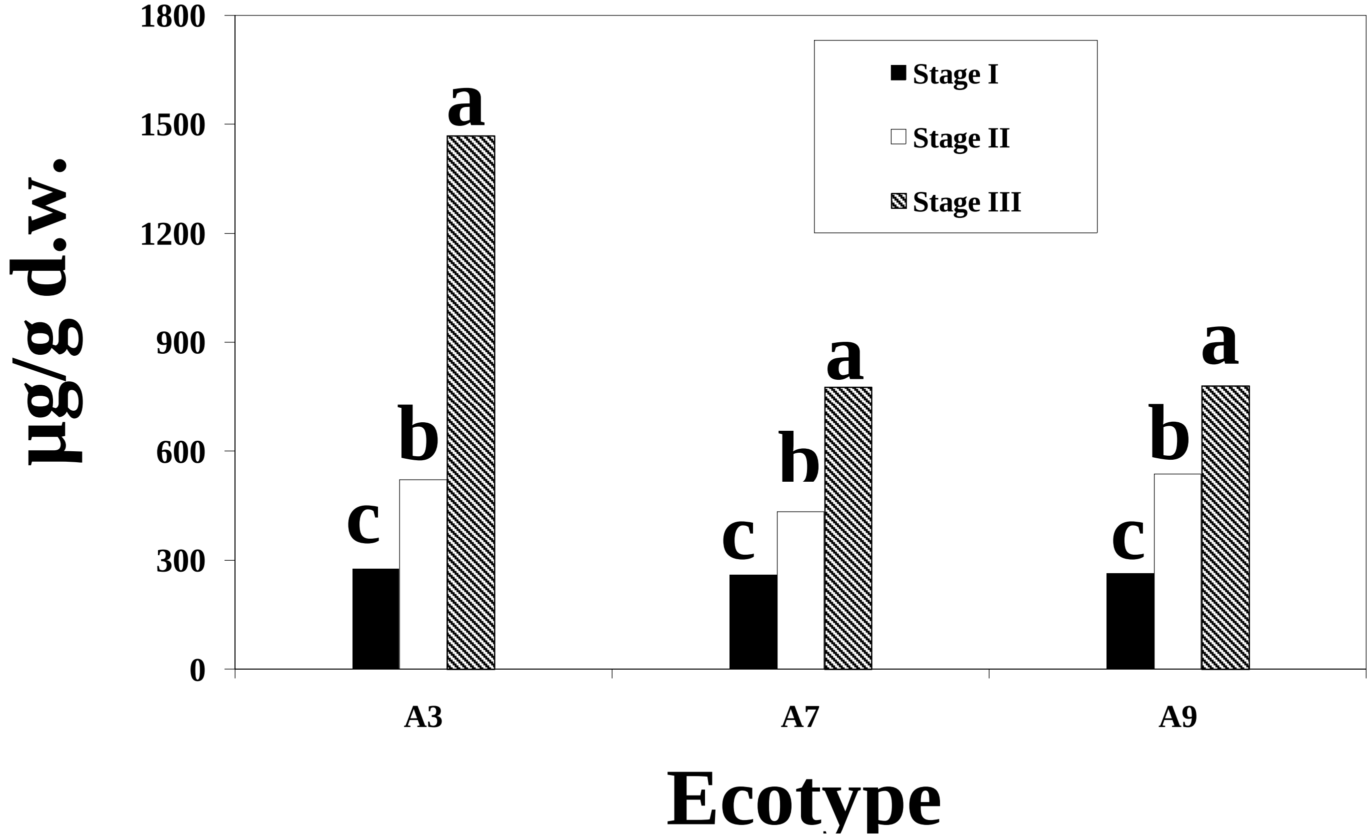

Figure 3: Mn content of leaves at different growth stages of Roselle

Copper content. There was a great variation among the ecotypes in the Cu content in the leaves at all stages of plant growth (Figure 4). At stages I and II, the highest Cu content was recorded for A3 and the lowest one for A7. A9 was intermediate. The Cu content in leaves was relatively constant during the whole cycle of plant growth for A7 and A9. In contrast, it has significantly ($p<0.05$) decreased from stage II to III for A3, about 50 %. At stage III, the highest Cu content in leaves was recorded for A9 and the lowest one for A7.

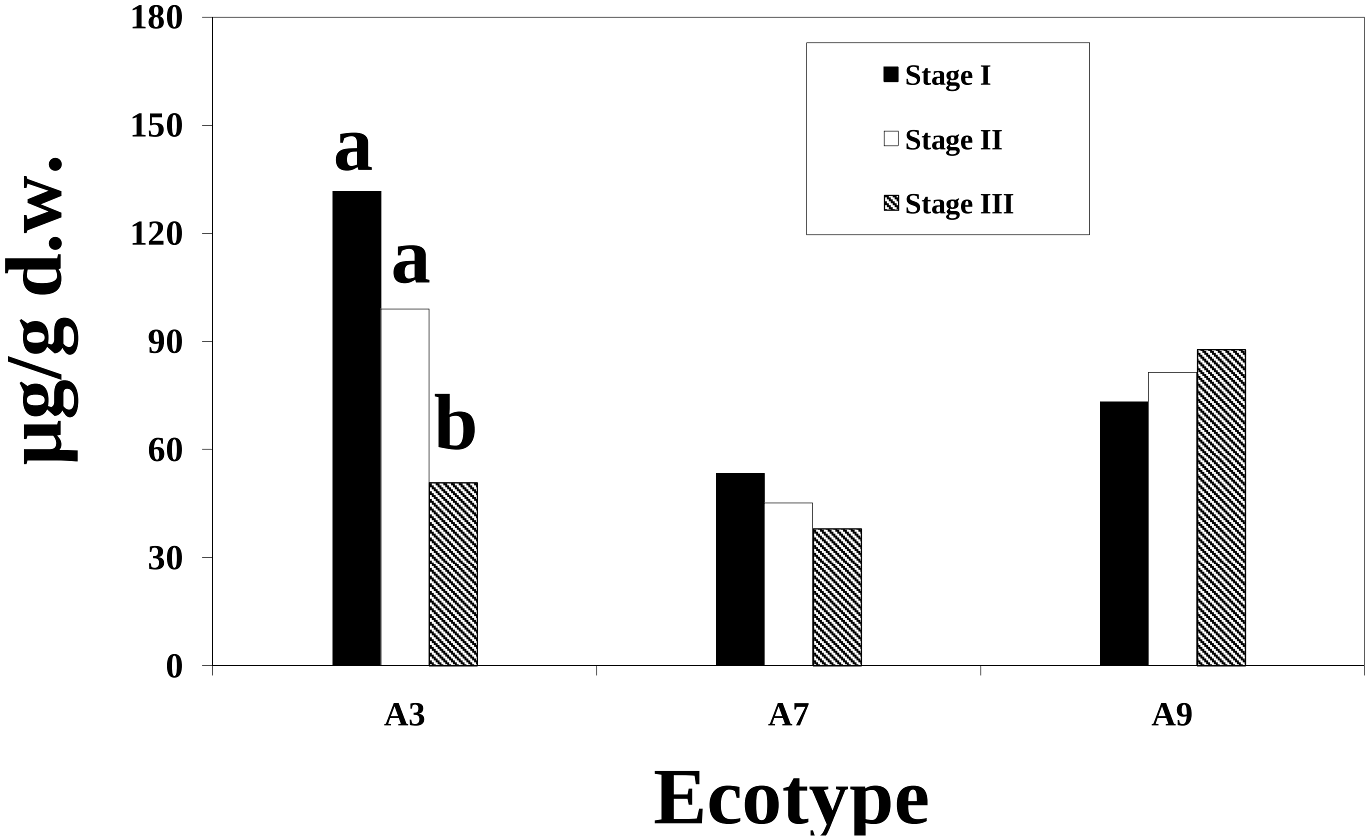

Figure 4: Cu content of leaves at different growth stages of Roselle

Zinc content. A3 had the highest Zn content in leaves at stage I (Figure 5). The remains ecotypes had lower and similar Zn content. The Zn content of the leaves decreased significantly ($p<0.05$) from stage I to II for the three ecotypes. During this period, the decrease was similar for A3 and A9, around 50%. A7 recorded lower decrease (30%). At stage II, the Zn content in leaves was relatively similar for the three ecotypes, about 30 µg of Zn/g d.w. and remained relatively steady until stage III.

AJFAND ONLINE
Volume 10 No. 5
May 2010
ISSN 1684 5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

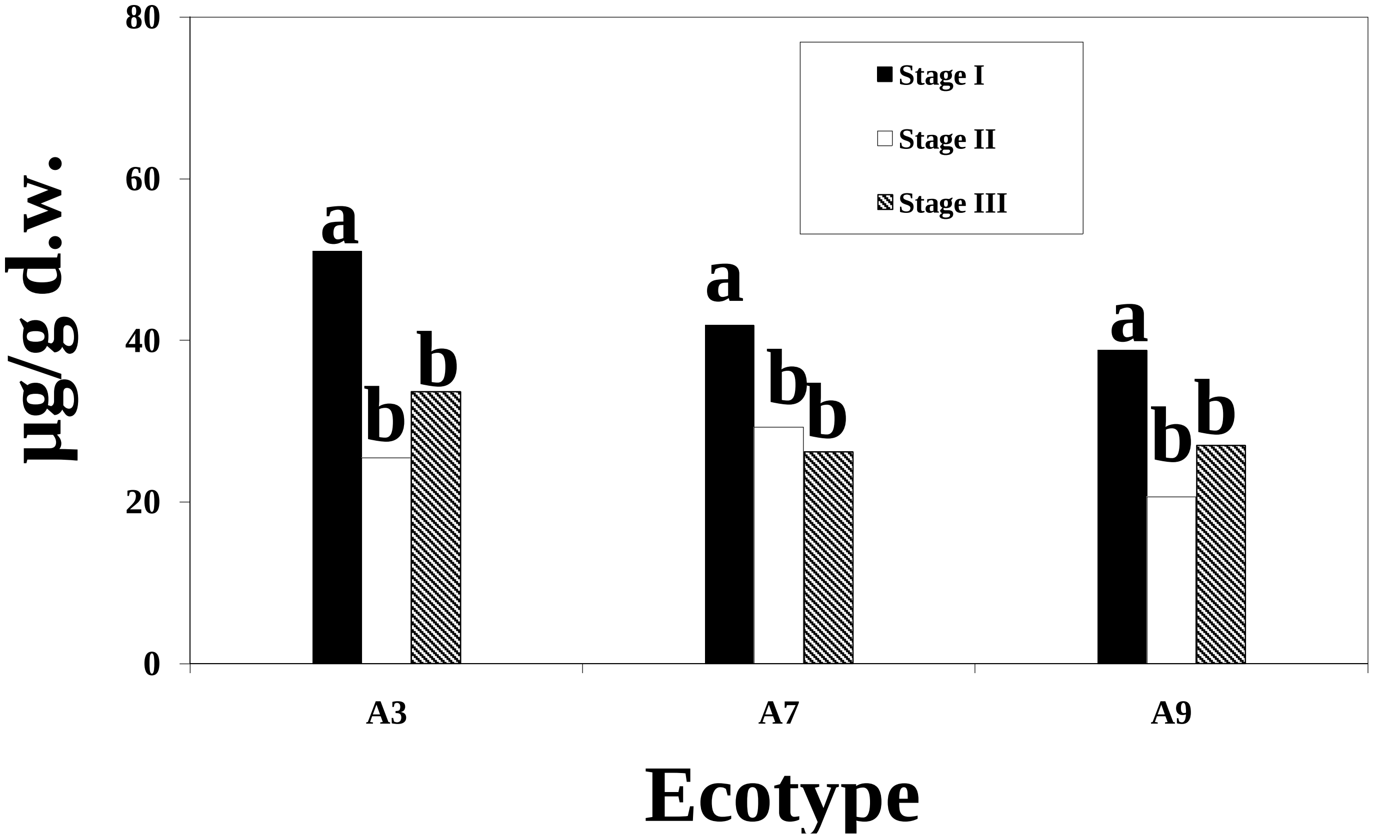

Figure 5: Zn content of leaves at different growth stages of Roselle

DISCUSSION

In all ecotypes, the zinc content of the leaves was higher at the vegetative stage of plant development and than decreased during a subsequent plant growth. This result is in agreement with those of other researchers who found a decrease in the zinc content of the leaves of *Hibiscus sabdariffa* and *Hibiscus cannabinus* and also in other similar leafy vegetables such as *Amaranthus blitum*, *Amaranthy gongecus and Spineces olerecea* between 15 and 45 days after sowing [12]. A progressive decrease in zinc content from three months to one year of age in leaves of *Chekurmenis* was also found [17]. The leaves' zinc content we found was higher than those reported for *H. sabdariffa*, respectively 16.9 μg/g d.w and less than 5 μg/g d.w [3, 18]. But is lower than those reported in another research, which was 72.9 μg/g d.w [2]. The mineral content of plants can be significantly influenced by variety, location, and environmental conditions [19, 20]. In terms of comparison with similar leafy plants, the leaves' zinc content recorded here was higher than values reported for *Adansonia digitata* (17.5 μg/g d.w), *Leptadenia hatata* (18.9 μg/g d.w), *Moringa oleifera* (22.7 μg/g d.w) and *Marua crassifolia* [2, 21, 22]; however, our results agree well with those reported for *Brassica oleracea* and *Cerathotheca sesamoides* [3, 23].

Zinc plays an important role in human nutrition. Zinc deficiency results in retarded growth and delayed sexual maturation because of its role in nucleic acid metabolism and protein synthesis [24]. Therefore, sufficient intake of minerals and trace elements such as iron, zinc and calcium are important for ensuring optimal health, growth, and development of infants and young children [25, 26].

For all ecotypes, the highest iron content in leaves was observed at the vegetative stage (25 DAS), which corresponds to the primary utilisation of Roselle leaves for sauces in most areas in Niger. The variation in the iron content in the leaves was higher between different stages of growth than among ecotypes. For all ecotypes, the iron content of the leaves decreased significantly from stage I (25 DAS) to II (75 DAS) then remained relatively constant from stage II to stage III (115 DAS). This result is in agreement with those reported by other colleagues [12] who showed that the iron content decreases in leaves of *Spineces olerecea*, *H. sabdariffa* and *H. cannabinus* from 30 days after sowing to 45 days after sowing. The range of iron content in the leaves recorded in this study at vegetative stage (300 to 400 µg/g d.w) agrees with those reported in another research which was 334 µg/g d.w. [3]. However, other authors reported higher values [17; 2]. These differences could be attributed to varieties, stages of development, or conditions of plant growth.

The copper content varied greatly among ecotypes at each stage of plant growth. Ecotype A3 had the highest Cu content at the vegetative and flowering stages and A3, the lowest content at all stages of plant growth. The copper content of the leaves recorded in this study was much higher than that reported for Roselle by other investigators and which ranged between 5.4 µg/g d.w and 13.5 µg/g d.w [2, 3, 11, 17]. It is also higher than values obtained in leaves for other similar leafy plants such as *H. canabius* (6.38 µg/g d.w), *C. samoides* (10.43 µg/g d.w), *M. oleifera* (8.71 µg/g d.w), *A. digitata* (11.6 µg/g d.w) and *B. oleracea* (2.27 µg/g d.w) [11, 22]. The copper content in the leaves of ecotype A9 was relatively constant at all stages of plant growth. But for A3 and A7, it tended to decrease, from flowering to mature stage. In *H. sabdariffa* and *H. cannabinus*, the copper content decreased from 15 DAS to 30 DAS, then increased from 30 DAS to 45 DAS [12]. The same variation was also recorded by these authors for other leafy plant such as *Amaranthy gongecus*; however, for *Amaranthus blitum* leaves, the decrease in copper content was maintened until 45 DAS [12]. To some extent, these results were stand in contrast to those of other colleagues who found a decrease in the copper content from three months to four months and an increase from four months to one year of age in leaves of *Chekurmenis* [16].

Considering all of the ecotypes, the variation of manganese content in leaves was slight at stage I but higher at stage II (from 430 µg/g d.w for A7 to 630 µg/g d.w for A9) and stage III (from 780 µg/g d.w to 1310 µg/g d.w). These values are higher than those reported by other investigators for *H. sabdariffa* or other similar leafy plants [21, 17]. However, lower values were also reported for *H. sabdariffa* [3, 11, 17]. In *C. sesamoides*, the Mn content in leaves was 380 µg/g d.w [2]. This result is close to the value recorded at flowering stage in our study. However, other investigators reported

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net

Volume 10 No. 5
May 2010
ISSN 1684 5374
AJFAND ONLINE
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

much lower Mn content in the leaves of others plants including *A. digitata* [20, 27], *M. crassifolia* [20], *L. hastata* [2], and *M. oleifera* [17]. During the growth of *H. sabdariffa*, we observed a progressive and significant increase in the Mn content from the vegetative to the mature stage in all ecotypes: the increase between these two stages was about 4-fold for A3 and 3-fold, respectively, for A7 and A9. Other authors have also observed a continuous increase in the Mn content from the initial to the final stage of growth of *H. sabdariffa* and *Portulaca olerecea* [12]. An increase in the Mn content between 3 months and 1 year of age in leaves of *Chekurmenis* was also reported [16]. Thus, it appears that Mn accumulates in leaves as age increases.

CONCLUSION

The results indicated that the Cu, Fe and Zn content in the leaves of the Roselle were higher at the vegetative stage while Mn content was lower.

The data contained in the present report provide further evidence of the potential nutritional value of Roselle for populations of western Sahel. Therefore the vegetative stage corresponding to 25 days after sowing is the recommended optimal harvest time of Roselle to maximise on the nutrients.

Published by African Scholarly Science Communications Trust
Josem Trust Place, Bunyala Road, Upper Hill, Nairobi
P.O. Box 29086-00625 Tel: +254-20-2351785 Fax: +254-20-4444030, Nairobi, KENYA
Email: oniango@iconnect.co.ke OR info@ajfand.net www.ajfand.net
African Scholarly Science Communications Trust

AJFAND ONLINE
Volume 10 No. 5
May 2010
ISSN 1684 5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

REFERENCES

1. **Small T and EG Rhoden** Production and nitrogen uptake of Roselle. *Hort. Sci.* 1991; **26:** 738.

2. **Sena LP, Vanderjagt DJ, Rivera C, Tsin ATC, Muhamadu I, Mahamadou O, Millson M, Pastuszyn A and RH Glew** Analysis of nutritional components of eight famine foods of the republic of Niger. *Plant Foods Hum. Nutr.* 1998; **52:** 17-30.

3. **Smith GC, Clegg MS, Keen CL and LE Grivetti** Mineral values of selected plant foods common to the southern Burkina Faso and to Niamey, Niger, West Africa. *Intern. J. Food Sci. Nutr.* 1996; **47:** 41- 53.

4. **Gibson RS** Zinc nutrition in developing countries. *Nutr. Res. Rev.* 1994; **7:** 151-173.

5. **Awoyinka AF, Abegunde VO and SRA Adewusi** Nutrient content of young cassava leaves and assessment of their acceptance as a green vegetable in Nigeria. *Plant Foods for Human Nutrition* 1995; **47:**21-28.

6. **Brown KH, Peerso JM, Rivera J and LH Allen** Effect of supplementary zinc on the growth and serum zinc concentration of prepubertal children: a meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* 2002; **75:** 1062-1071.

7. **Zinc Investigators Collaborative Group** Prevention of diarrhoea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. *J. Pediatr.* 1999; **135:** 689-697.

8. **Sazawal S, Black RE, Jalla S, Mazumdar S, Sinha A and MK Bhan** Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: a double-blind, controlled trial. *Pediatr.* 1998; **102:** 1-5.

9. **Williams RB** Trace element and congenital abnormalities. *Proc. the Nutr Soc.* 1977; **36:**26-32.

10. **Tchiégang C and A Kitikil** Données ethno-nutritionnelles et caractéristiques physico-chimiques des légumes feuilles consommées dans la savane de l'Adamaoua (Cameroun). *Tropicult.* 2004; **22:** 11-18.

11. **Humphry CM, Clegg MS, Keen CL and LE Grivetti** Food diversity and drought survival. The Hausa example. *Intern. J. Food Sci. Nutr.* 1993; **44:** 1-16.

12. **Khader V and S Rama** Selected mineral content of common leafy vegetables consumed in India at different stages of maturity. *Plant Foods Hum. Nutr* 1998; **53:** 71-81.

13. **Cook JA, Vanderjagt DJ, Pastuszyn A, Mounkaila G, Glew RS, Millson M and RH Glew** Nutrient and chemical composition of 13 wild plant foods of Niger. *J. Food Comp. Anal.* 2000; **13:** 83-92.

14. **Ndiaye M** Interprétation des analyses chimiques des sols et recommandations. Document du Centre Régional AGRHYMET, 2002 : 8p.

15. **Bakasso Y, Saadou M, Zongo J-D and F Baidou-Forson** Analysis of agro-morphological variability of *Hibiscus sabdariffa* L. in Niger. *Ann. Sci Agron. Benin* 2008; **11:** 21-33.

16. **Houba VJG** Soil Analysis Procedures, Other Procedures. **In:** Van der Lee JJ and I Novozamsky (eds). Soil and Plant Analysis, part 5B, Syllabus. Wageningen Agricultural University Press, Wageningen. 1995: 100-125.

17. **Giri J, Bhuvaneswari W and D Rajeswari** Changes in the nutritive value of Chekurmenis at different stages of growth. *Indian J. Nutr. Diet.* 1984; **21:** 419-423.

18. **Freiberger CE, VanderJagt DJ, Pastuszyn A, Glew RS, Mounkaila G, Millson M and RH Glew** Nutrient content of the edible leaves of seven wild plants from Niger. *Plant Food Hum. Nutr.* 1998; **53:** 57-69.

19. **Barampama Z and RE Simard** Nutrient composition, protein quality and antinutritional factors of some varieties of dry beans (*Phaseolus vulgaris*) grown in Burundi. *Food Chem.* 1993; **47:**159-167.

20. **Rao PU** Nutrient composition and biological evaluation of mesta (*Hibiscus sabdariffa*) seeds. *Plant Foods Hum. Nutr.* 1996; **49:** 27-34.

21. **Cook J A, Vanderjagt D J, Pastuszyn A, Mounkaïla G, Glew RS and RH Glew** Nutrient content of two indigenous plant foods of the werstern Sahel : *Balanites aegyptiaca and Maerua crassifolia. J. F. Comp. anal.* 1998; **11:** 221-230.

22. **Barminas JT, Charles M and D Emmanuel** Mineral composition of non-conventional leafy vegetables. *Plant Foods Hum. Nutr.* 1998; **53:**29-36.

23. **Glew RS, VanderJagt DJ, Bosse R, Huang YS, Chuang LT and RH Glew** The nutrient content of three edible plants of the Republic of Niger. *J. Food Comp. Anal.* 2005; **18:** 15-27.

24. **Underwood EJ** Trace elements in human and animal nutrition, 3rd edition. Academic Press, New York, 1971:302pp.

25. **Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro RA, Eng RW and JR Boccio** The role of zinc in the growth and development of children. *Nutr*. 2002; **18:** 510-519.

26. **Untoro J, Karyadi E, Wibowo E, Erhardt J and R Gross** Multiple micronutrients supplements improve micronutrient status and anemia but not growth and morbidity of Indonesian infants: a randomized double blind, placebo-controlled trial. *J. Nutr.* 2005; **135:** 639-645.

27. **Glew RS, VanderJagt DJ, Bosse R, Huang YS, Chuang LT and RH Glew** The nutrient content of three edible plants of the Republic of Niger. *J. Food Comp. Anal.* 2005; **18:** 15-27.