LEVELS OF FLUORIDE IN NIGER SEED (GUIZOTIA ABYSSINICA) CULTIVATED IN ETHIOPIA AND ERITREA

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SUMMARY: The levels of fluoride in niger seed (Guizotia abyssinica) cultivated in ten areas in Ethiopia and Eritrea were determined. Eight samples were from production sites in Ethiopia and two samples were from the Ethiopian Gene Bank from seed grown in the Wellega region of Ethiopia (12 accessions) and in Eritrea (10 accessions). Alkali fusion was used to extract the fluoride which was then measured with a fluoride ion selective electrode. The range of fluoride concentration found in the niger seeds was 3.42–7.35 µg/g. The daily intake of niger seeds in Ethiopia was calculated to be 1.8 g/day, containing 6–13 µg F/day (0.006–0.013 mg F/day), a level well below that likely to result in the adverse effects of dental or skeletal fluorosis.

Keywords: Ethiopia; Fluoride; Food; Guizotia abyssinica; Niger seed; Oil seed.

INTRODUCTION

The fluoride ion (F) is not an essential element for human growth and development, including the development of healthy teeth and bones.1 While the evidence that topical F has a protective effect against dental caries is considered by some to be strong, the scientific evidence that the systemic application of F via drinking water is beneficial is less convincing and, in high dose, F can cause both dental and skeletal fluorosis.1-3 Many water sources in Ethiopia contain F at elevated concentrations of up to 33 mg/L.4 The public health problem of fluorosis is prevalent in the high water F levels found in the Rift Valley region of Ethiopia, which is characterized by relatively high volcanic activity.4-6 According to estimates of the Ethiopian Ministry of Water Resources, more than 11 million people in the Ethiopian Rift Valley, including in the states of Afar, Amhara, Oromia, and SNNPR, rely on drinking water contaminated by F.7-8

Although virtually all foodstuffs contain at least some traces of F, the dietary intakes of F, in water, beverages, and foods, vary widely according to the various sources of exposure.9-18 All vegetation contains some F, which is absorbed from the soil and water.19 Together with exposure to F from atmospheric pollution, dietary F exposure from the consumption of vegetables, grains and other staples, local salt, and drinks (especially tea), may lead to the development of dental and skeletal fluorosis.9,20,21

Food grown in areas where soils have high amounts of F, or where phosphate fertilizers are used, may have higher levels of F.22,23 In addition to food sources, the total exposure to F depends also on the contributions from other sources, including drinking water, water-based beverages, food supplements, and the use of F-containing toothpaste.1

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Niger (Guizotia abyssinica (L. f.) Cass., Compositae) is an oilseed crop cultivated in Ethiopia and India. It constitutes about 50% of the Ethiopian and 3% of the Indian oilseed production. It is also a minor oil crop in Kenya, Uganda, Sudan, Malawi, and other African countries. In Ethiopia, it is cultivated on waterlogged soils, where most crops and all other oilseeds fail to grow, and contributes a great deal to soil conservation and land rehabilitation.

The niger plant is consumed by sheep but not by cattle, to which only niger silage can be fed. Niger is also used as a green manure for increasing the soil organic matter. Besides its culinary use, niger seed oil is used in the manufacture of soap and paints, and as a lubricant and illuminant. The protein-rich meal which remains after the extraction of the oil is used as feed, manure, and fuel. In addition to its oil, the crop offers an important source of seed protein which contributes significantly to the human dietary protein intake.

The niger seed contains up to 40% edible semidrying oil, 20.9% carbohydrate, and 27.8% protein. The niger meal remaining after the extraction of the oil contains approximately 30% protein and 23% crude fiber. Other than being very important for their nutritional value, oilseeds have considerable importance for industrial and pharmaceutical purposes.

There is no report in the literature about the F content of the niger seeds consumed in Ethiopia and other countries. The objective of this study was to determine the concentration of F in niger seeds grown in Ethiopian and Eritrea.

**MATERIAL AND METHODS**

*Chemicals and reagents:* All the chemicals and reagents used were of analytical grade. Sodium fluoride (99%, NaF, BDH Analar, England) was used to prepare a F stock standard solution and for the spiking experiment. Glacial acetic acid (100%, Sigma-Aldrich, Germany), sodium chloride (Oxford, India), sodium citrate (Research-Lab Fine Chem. Industries Mumbai, India) and EDTA (Scharlau, European Union) were used to prepare a total ionic strength adjustment buffer (TISAB) solution. Sodium hydroxide (Scharlau, European Union) solution was used to adjust the pH of the TISAB solution and the fusion cake in the total fluoride determination. HCl (37%, Sigma-Aldrich, Germany) was also used for the neutralization of the fusion cake in the total fluoride determination. Deionized water (chemically pure with a conductivity of 1.5 µs/cm) was used throughout.

*Instrumentation:* A fluoride ion selective electrode (Orion Model, EA 940 Expandable Ion Analyzer, USA) was used to assay the F content of the niger seed. A drying oven (Digitheat, J.P. Selecta, Spain) was used for evaporation and the drying of the niger seed samples. A muffle furnace (Audiotronics, Wagtech International Ltd., U.K.) was used for the fusion and ashing of the niger seed samples.

*Description of the sampling areas:* The niger seeds were collected from the major niger seed production areas in different parts of Ethiopia and, for comparison, from one area in Eritrea. The sites were also selected to reflect differences in various parameters including rainfall, altitude, soil fertility and
climatic conditions. The samples were collected from Jimma (South Western Ethiopia, Oromia region), Debre Markos (North West Addis Ababa, Amhara region), Kombolcha (North-Central Ethiopia, Amhara region), Mekelle (North Ethiopia, Tigray region), Gondar (North Ethiopia, Amhara region), Debre Birhan (North Ethiopia, Amhara region), Awi Zone (North West Ethiopia, Amhara region), and Alamata (South west Amhara region) and Wellega (Oromia region). The sample from Wellega, was formed by mixing together seeds from 12 different sites held at the Ethiopian Gene Bank. The Eritrean sample was also made by mixing 10 accessions from Eritrea held at the Ethiopian Gene Bank. Apart from the Wellega and Eritrea samples, the samples were collected from the production area where they were grown in 2012.

**Sample collection:** Except for the Wellega and Eritrea samples, 0.5 kg niger seed samples were collected from three different places for each production area site, mixed together in a polyethylene bag, labeled, and brought to the laboratory for further pretreatments. For the Wellega and Eritrea samples, 3 g samples (one full pack) from each of 12 and 10 accessions, respectively, were collected from the Ethiopian Gene Bank, mixed in a polyethylene bag, labeled, and brought to the laboratory for further pretreatments.

**Sample pretreatment:** The niger seed samples collected from the 10 sampling areas were sieved through a polyethylene sieve to remove large debris, stones, and pebbles. The sieved samples were washed twice with tap water and rinsed with deionized water three times to remove the soil from the seeds and then dried in sunlight. The dried samples were ground with a blender and sieved with a 1.4 mm polyethylene sieve.

**Fluoride determination in niger seed:** The total F content in the niger seed was measured using alkali fusion by the method of Malde et al. The standard solutions (0.5, 1, 5, 10, and 20 mg F/L) for calibration were prepared from the stock F solution (1000 mg/L). The standard solution (5 mL) was mixed with an equal amount with TISAB in a 50 mL plastic beaker. A similar procedure was used for the sample solution. The mixture was stirred thoroughly and measured with the fluoride ion selective electrode for the calibration and the determination. All measurements of the samples were made in triplicate and six blank samples were used.

**Method validation for fluoride determination:** The method validation of the fluoride analysis was established by a spiking experiment. The spiked sample was prepared by adding a known concentration of the standard fluoride solution to 0.5 g of niger seeds from three sample sites: Kombolcha, Debre Markos, and Mekelle. The amounts of the 20 mg/L standard F solution added to the 0.5 g seed samples were: 35, 70, and 140 µL for the Kombolcha site; 45, 90, and 180 µL for the Debre Markos site; and 40, 85, and 170 µL for the Mekelle sample site. The spiked and non-spiked (blanks during the recovery test) samples were ashed under similar conditions in triplicate.
RESULTS AND DISCUSSION

**Recovery results:** The percentage recoveries for the F in niger seeds were found to be in an acceptable range of 93–107% (Table 1). This verifies that the optimized digestion procedures and instruments used were valid for F determinations in niger seeds.

**Distribution patterns of fluoride in the niger seed samples:** The F levels in the niger seeds lay in the range 3.42–7.35 µg/g. The sample from Debre Markos had the highest F content with the content being progressively lower in the samples from Awi Zone, Eritrea, Mekelle, Kombolcha, Gondar, Debre Birhan, Alamata, Wellega, and Jimma (Table 2). This variation may be due to the presence of different concentrations of F in the soil and water, the pH of the soil, and the accumulation of Al in the soil in which the niger seeds were grown.

**Table 1.** Recovery test results of fluoride ion (F) determination in the niger seed samples

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Concentration of F in unspiked sample (µg/g)</th>
<th>Amount of F added (µg/g)</th>
<th>Concentration of F in spiked sample (µg/g)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kombolcha</td>
<td>5.55</td>
<td>1.4</td>
<td>6.91±1.0</td>
<td>97±2</td>
</tr>
<tr>
<td></td>
<td>5.55</td>
<td>2.8</td>
<td>8.30±0.4</td>
<td>98±1</td>
</tr>
<tr>
<td></td>
<td>5.55</td>
<td>5.5</td>
<td>11.2±1.4</td>
<td>103±2</td>
</tr>
<tr>
<td>Debre Markos</td>
<td>7.35</td>
<td>1.8</td>
<td>9.02±0.9</td>
<td>93±2</td>
</tr>
<tr>
<td></td>
<td>7.35</td>
<td>3.7</td>
<td>10.8±1.4</td>
<td>93±2</td>
</tr>
<tr>
<td></td>
<td>7.35</td>
<td>7.3</td>
<td>14.5±2.1</td>
<td>98±2</td>
</tr>
<tr>
<td>Mekelle</td>
<td>6.72</td>
<td>1.7</td>
<td>8.29±2.1</td>
<td>100±4</td>
</tr>
<tr>
<td></td>
<td>6.72</td>
<td>3.4</td>
<td>10.2±3.2</td>
<td>103±5</td>
</tr>
<tr>
<td></td>
<td>6.72</td>
<td>6.7</td>
<td>13.9±3.9</td>
<td>107±5</td>
</tr>
</tbody>
</table>

Comparing the F levels from the 10 regions with ANOVA, *p=0.05.

**Table 2.** Fluoride ion (F) concentration (mean±SD, µg F/g dry weight) in the niger seed samples (n=9 for each sample site comprising measurement in triplicate and 6 blanks)

<table>
<thead>
<tr>
<th>Sample sites</th>
<th>F concentration (µg/g)</th>
<th>Sample sites</th>
<th>F concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kombolcha</td>
<td>5.55±0.25*</td>
<td>Jimma</td>
<td>3.42±0.04*</td>
</tr>
<tr>
<td>Gondar</td>
<td>4.99±0.12*</td>
<td>Wellega</td>
<td>4.12±0.09*</td>
</tr>
<tr>
<td>Debre Birhan</td>
<td>4.82±0.17*</td>
<td>Alamata</td>
<td>4.42±0.07*</td>
</tr>
<tr>
<td>Awi Zone</td>
<td>7.09±0.15*</td>
<td>Mekelle</td>
<td>6.72±0.318*</td>
</tr>
<tr>
<td>Debre Markos</td>
<td>7.35±0.42*</td>
<td>Eritrea</td>
<td>6.75±0.15*</td>
</tr>
</tbody>
</table>

Comparing the F levels from the 10 regions with ANOVA, *p=0.05.
Comparison of the fluoride levels found in the niger seeds with the values recommended in the literature for the prevention of dental fluorosis: The assessment of dietary F intake is important for assessing the risk of dental and skeletal fluorosis developing. Topical F application, rather than systemic F ingestion, is considered to have a cariostatic role in dental health. Despite the Institute of Medicine, USA, specifying Adequate Intakes (AI) for F of 0.01 mg/kg/day for infants through 6 months, 0.05 mg/kg/day beyond 6 months of age, and 3 mg/day and 4 mg/day for adult women and men, respectively, to prevent dental caries, F is not an essential element for human growth and development. The Upper Limits (UL) of the Institute of Medicine of 0.10 mg/kg/day in children less than 8 years and 10 mg/day for those older than 8 years are recommended for prevention of dental fluorosis. Similar levels have been endorsed by the American Dental Association and the American Dietetic Association.

According to Duke, 100,000–200,000 tons of niger seeds were produced annually in Ethiopia with 50–60% of the seeds being used for oil production in factories or by traditional methods. The United States Department of Commerce reported that, in 2003, Ethiopia exported 18,290 tons of niger seeds to the USA. In Ethiopia, approximately 49,210 tons of niger seeds were used annually for normal household activities. The niger seed in Ethiopia and Eritrea contained 3.42 to 7.35 µg/g fluoride. The daily intake of niger seed in Ethiopia was calculated to be 1.8 g/day with a F content of approximately 6–13 µg (0.006–0.013 mg), a figure much lower than the values given by the Institute of Medicine as being likely to lead to dental fluorosis.

Analysis of variance (ANOVA): Using analysis of variance (ANOVA), significant differences (p=0.05) were found to be present in the F levels in the samples from the ten sites. The differences may be due to geographical factors including rain fall and the F levels in the soil and water.

CONCLUSION

In this study the levels of F were determined in the niger seed which is produced in Ethiopia and Eritrea and found in the range of 3.42–7.35 µg/g. The daily intake of niger seeds in Ethiopia was calculated to be 1.8 g/day, containing 6–13 µg F/day (0.006–0.013 mg F/day), a level well below that likely to result in the adverse effects of dental or skeletal fluorosis

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