Minerals (iron, calcium, and zinc contents) in leaves of in field-grown (Desi and Kabuli) chickpea varieties (Cicer arietinum L.) on dry matter basis

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Abstract
Micronutrient malnutrition, the hidden hunger, affects more than half of the world’s population, especially in Asia, Africa, and Latin America. Mineral deficiency is a major problem in both developing and developed countries, and much of this can be attributed to insufficient dietary intake. Over the past decades several measures, such as supplementation and food fortification, have helped to alleviate this problem. In this experiment the available mineral contents of iron, calcium and zinc in leaves of desii and kabuli chickpea did not vary significantly at different day intervals after sowing. However, higher content of these available minerals in leaves were observed at 45 days after sowing. Available iron content varied from 25.35 to 28.86 percent in leaves collected at varying time intervals after sowing in both the desi chickpea varieties and did not differ significantly among them. Similar trends were noticed for available calcium and zinc contents. Available iron, calcium, and zinc contents varied from 24.70 to 29.68 percent; 57.01 to 63.46 percent and 29.98 to 35.01 percent in desi and kabuli chickpea varieties. The increase in period of growth of leaves did not affect significantly the available iron, calcium and zinc contents in leaves of both the kabuli varieties.

Keywords: Mineral, iron, calcium, zinc

Introduction
Mineral deficiency is a major problem in both developing and developed countries, and much of this can be attributed to insufficient dietary intake. Over the past decades several measures, such as supplementation and food fortification, have helped to alleviate this problem. Malnutrition, as defined by the World Health Organization (WHO), is “the cellular disparity amid the supply of energy, nutrients and the body’s demand for them to ascertain maintenance, growth and specific functions” (Batool et al., 2013) [2]. It refers to both the insufficient and excessive intake of nutrients (both macro and micro) and as such covers not only food shortage but also obesity. Undernourishment can be classified categories: protein-energy malnutrition and micronutrient deficiency. As the names suggest, the former refers to inadequate calorie or protein intake while the latter to the lack of essential micronutrients such as vitamin A, iodine, zinc, and iron (Batool et al., 2013) [2]. Micronutrient malnutrition, the hidden hunger, affects more than half of the world’s population, especially in Asia, Africa, and Latin America. Populations in these regions depend mainly on cereal diets that are frequently deficient in iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg). Traditional methods of addressing micronutrient deficiency such as dietary supplementation, food fortification, and dietary diversification have had limited success in many regions and populations due to the lack of social and economic infrastructure (Welch and Graham, 2002) [1].

Material and Methods
Available minerals (Fe, Ca and Zn)

Available iron
Available iron in the sample was extracted according to the procedure of Rao and Prabhavathi (1978) [3].

Procedure
One gram sample was mixed with 25 ml pepsin HCl (0.5% pepsin in 0.1N HCl) in a conical flask. The pH of the mixture was adjusted to 1.35 with HCl and incubated at 37 °C for 90 min.
in a water bath-cum-shaker. After incubation, pH of the contents was adjusted to 7.5 with NaOH and again incubated at 37 °C in a water bath-cum-shaker for 90 min. Contents of the flasks were centrifuged at 3000 rpm for 45 min and the supernatant was filtered through Whatman #42 filter paper. The filtrate was oven dried, digested in the diacid mixture and proceeded for the determination of iron by atomic absorption spectrophotometric method.

**Available calcium and zinc**  
Available calcium and zinc were extracted by the method of Kim and Zemel (1986) [4].

**Reagents**  
0.1% Pepsin in 0.1 N HCl  
HCl  
NaHCO₃  
0.5% pancreatin in 5% bile

**Procedure**  
One gram of finely ground sample was taken in a conical flask and 3 ml distilled water was added to rehydrate it. To this 20 ml of pepsin solution (0.1% pepsin in 0.1N HCl) was added. The pH was adjusted to 1.5 with dilute HCl. The contents were incubated at 37°C in a shaker-cum-water bath for one hour. After one h, the pH contents were raised to 6.8 with sodium bicarbonate solution. Then 2.5 ml of a suspension containing 0.5 percent pancreatin in 5 percent bile was added and the contents were again incubated at 37 °C for one hour. Then the contents were taken out and total volume was made to 50 ml with distilled water. The contents were then immediately centrifuged at 5000 x g for 45 min at 5 °C. Supernatant was collected and re-centrifuged at 2500 x g for 45 min at 5 °C. The supernatant was collected; oven dried, digested in the diacid mixture and proceeded for the estimation of calcium and zinc by the atomic absorption spectrophotometric method.

**Results**  
**Available mineral content**  
The available mineral contents of iron, calcium and zinc in leaves of *desi* and *kabuli* chickpea did not vary significantly at different day intervals after sowing. However, higher content of these available minerals in leaves were observed at 45 days after sowing (Tables 1 and 2).

Available iron content varied from 25.35 to 28.86 percent in leaves collected at varying time intervals after sowing in both the *desi* chickpea varieties and did not differ significantly among them. Similar trends were noticed for available calcium and zinc contents. Available iron, calcium, and zinc contents varied from 24.70 to 29.68 percent and 29.98 to 35.01 percent in *desi* and *kabuli* chickpea varieties. The increase in period of growth of leaves did not affect significantly the available iron, calcium and zinc contents in leaves of both the *kabuli* varieties.

**Discussion**  
The highest available calcium content was observed in the leaves of *desi* chickpea variety C-235 (63.46%) at 45 days after sowing and lowest in the leaves of HK-2 (57.01) at 45 days after sowing. Statistically neither the stage of growth nor the variety affected the available calcium content in the leaves. However, the calcium content in chickpea leaves was found highest at 45 days after sowing. Available iron content in chickpea leaves increased with maturity. It was found highest in the leaves of HK-1 (29.68%) at 45 days after sowing and lowest in the leaves of HK-2 (24.70%) at 30 days after sowing. The available mineral content in leaves like calcium in C-235 (63.46% at 45 DAS), iron content in HK-1 (29.68%; 45 DAS) and zinc content in HK-2 (35.01%; 45 DAS) were found to be maximum.

**Acknowledgements**  
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**References**  
3. Rao BSN, Prabhavathi T. An In vitro method of

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**Table 1:** Available iron, calcium and zinc content (%) in leaves of *desi* chickpea varieties (on dry matter basis)

<table>
<thead>
<tr>
<th>Available minerals</th>
<th>HC-1 Days after sowing (DAS)</th>
<th>C-235 Days after sowing (DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Iron</td>
<td>25.35±1.46</td>
<td>26.93±1.56</td>
</tr>
<tr>
<td>Calcium</td>
<td>59.00±3.41</td>
<td>59.01±3.51</td>
</tr>
<tr>
<td>Zinc</td>
<td>33.00±0.29</td>
<td>34.06±0.32</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three independent determinations. The mean values in same row with similar superscripts did not differ significantly (p≤0.05).

**Table 2:** Available iron, calcium, and zinc contents (%) in leaves of *kabuli* chickpea varieties (on dry matter basis)

<table>
<thead>
<tr>
<th>Available minerals</th>
<th>HK-1 Days after sowing (DAS)</th>
<th>HK-2 Days after sowing (DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Iron</td>
<td>27.04±1.56</td>
<td>29.68±1.70</td>
</tr>
<tr>
<td>Calcium</td>
<td>60.01±3.46</td>
<td>58.01±3.35</td>
</tr>
<tr>
<td>Zinc</td>
<td>29.98±0.27</td>
<td>31.96±0.30</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three independent determinations. The mean values in same row with similar superscripts did not differ significantly (p≤0.05).