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Calcium amendment based on the shells of *Amiantis umbonella*: Elaboration, characterization and its use in planta in the cultivation of *Vicia faba* L.

Fatouma MOHAMED ABDOUL-LATIF[1]*, Mohamed ELMI OBSIEH[2], Ali MERITO ALI[1], Abdirahman ELMI[1], Houda MOHAMED[1]

[1] *Medicinal Research Institute, Center for Studies and Research of Djibouti, IRM-CERD, Haramous, Djibouti City P.O. Box 486, Djibouti.*
[2] *Directorate of Ecology and Nature Protection, Ministry of the Environment and Sustainable Development, BP 1413, Djibouti city, Djibouti.*

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ABSTRACT
This study aims to formulate calcium amendments from the shells of *Amiantis umbonella* and to evaluate their effectiveness in the cultivation of *Vicia faba* L. The physicochemical properties of these amendments were analyzed using advanced techniques such as scanning electron microscopy coupled with X-ray diffraction (SEM/EDX), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The growth parameters of the plant (length of the aerial part and yield) as well as its nutritional characteristics (chlorophyll content, proteins, lipids and sugars) were rigorously monitored. The results indicate that the calcium amendments developed can be used at concentrations between 5% and 10% while maintaining optimal yield without altering nutritional quality. This study highlights the potential of calcium amendments from *Amiantis umbonella* shells to improve the cultivation of *Vicia faba* L., thus paving the way for more sustainable and environmentally friendly agricultural practices.

1. Introduction:

Calcium, as an essential nutrient, plays a vital role in plant defense against various stresses, including reducing heavy metal toxicity and pathogen attack **[1-2]**. It exerts a significant influence on the growth, anatomy, morphology and chemical composition of plants, giving them a certain resistance to diseases and pests **[3-4]**. In addition, calcium amendments, such as calcium carbonate ($CaCO_3$), calcium sulfate ($CaSO_4$) or calcium oxide (CaO)/hydroxide [$Ca(OH)_2$], present notable advantages in improving the stability soil aggregates, reducing clay dispersion and limiting soil erosion **[5-7]**.
These amendments also provide microbiological benefits by promoting interactions between calcium and fungi, particularly ectomycorrhizae (ECM) **[8]**. They increase the availability of calcium in the soil, thus promoting better colonization of tree roots by ECM fungi. Additionally, increased soil pH associated with calcium amendments can alter the composition of ECM fungal communities, which can positively influence fungal biodiversity and protect trees against soil pathogens **[9]**.
Furthermore, calcium amendments offer an effective solution in the management of heavy metals in crops **[10]**. They reduce the harmful effects of these metals on photosynthetic pigments, thus preserving the health and vitality of plants exposed to these toxic substances **[11-12]**. Additionally, they mitigate oxidative damage caused by the overproduction of reactive oxygen species (ROS) under metal stress, thereby enhancing the ability of plants to withstand adverse environmental conditions **[13]**. By promoting the activity of antioxidant enzymes, calcium strengthens the plant defense system against

(*) Corresponding author:
Tel.: + 253 77032781
E-mail address: fatoumaabdoulatif@gmail.com

oxidative stress. Finally, by limiting the accumulation of heavy metals in the edible parts of crops, calcium amendments help reduce the risks to human health associated with the consumption of contaminated food products **[14]**.
As part of this work, the objective is to develop calcium amendments based on the shells of *Amiantis umbonella* and to evaluate their use in planta in the cultivation of *Vicia faba* L. The physicochemical characterizations will be carried out by electron microscopy scanning coupled with X-ray diffraction (SEM/EDX), X-ray diffraction and Fourier transform infrared spectroscopy (FTIR). The growth parameters of the faba bean (aerial part length and production yield), as well as the physicochemical parameters (hlorophyll content, protein content, lipids content and sugar content), will be meticulously monitored. This review aims to evaluate the impact of calcium amendments on improving overall crop performance.

2. Materials and methods:

2.1. Calcium amendment preparation:

Amiantis umbonella shells were collected from the sea coast in the Obock region, Djibouti (11°58'00.1"N; 43°17'42.5"E). After collection, the shells underwent a meticulous cleaning process with distilled water to remove any impurities such as sand and other unwanted residue adhering to their external surface. Subsequently, they were left to dry in the open air to allow all residual moisture to evaporate. Once completely dry, the shells were ground using a mining jaw crusher until a fine consistency was obtained. The material thus obtained was then sieved through a 400 µM sieve to guarantee a homogeneous and uniform particle size which presents the final calcium amendment.

2.2. Physicochemical analysis:

The calcium amendment sample was subjected to a series of analytical techniques to study its morphology and composition. First, a scanning electron microscope (SEM) coupled with energy dispersive X-ray (EDX) microanalysis was used to examine the structure and elemental composition of the sample **[15]**. This method, carried out with an FEI Type Quattro S model, allows detailed observation of the sample surface as well as the identification of the elements present using X-rays. Then, X-ray diffraction was carried out to determine the crystalline phases present in the sample **[16]**. This analysis was carried out using CCD-Apex equipment from Bruker, using an X-ray generator with a wavelength of 1.549 Å, operating at 40 kV and 40 mA. This technique makes it possible to identify the crystal structures present in the sample, thus providing information on its composition and stability. In addition to these analyses, Fourier transform infrared spectroscopy (FTIR) was used to confirm the previous results. This technique, carried out in transmission mode using a Bruker Alpha spectrometer, allows the identification of chemical bonds present in the sample, thus providing additional validation of the data obtained by SEM-EDX and X-ray diffraction **[17]**.

2.3. In planta tests:

As part of the *in planta* experiments aimed at evaluating the effectiveness of calcium amendments derived from shells in the cultivation of faba beans (*Vicia faba* L.), strict protocols were put in place. The faba bean seeds were pre-sterilized by exposing them to a 1.5% (v/v) sodium hypochlorite solution for 15 minutes, followed by a period of imbibition in distilled water at room temperature. for 24 hours. These treated seeds were then sown in 20 cm diameter pots filled with 1 kg of commercial peat substrate rich in organic matter. The pots were treated with calcium amendments made from shells, at concentrations ranging from 5% to 20%. Untreated pots were used as controls for comparisons. All experiments were carried out in triplicate to ensure the reliability of the results. Growth conditions were kept uniform for each experimental group, including ensuring constant irrigation at room temperature and normal atmospheric pressure. After a period of 3 months, a further evaluation of morphological parameters such as aerial part length and production yield will be carried out **[18]**. At the same time, the chlorophyll content of fresh samples will be measured, serving as a basis for determining the protein, sugar and lipid contents after drying at 40°C for 2 weeks.

2.4. Production quality assessment:

The analysis of the production quality of faba beans requires the application of specific methods to evaluate the different components, whether plant biomass or fruit organs. The content pigment such us chlorophylls a and b in faba bean plants is carried out using a colorimetric method that uses specific wavelengths, including 645 nm and 662 nm **[19]**. To determine the protein content present in faba bean fruits, the method of Bradford is used, thus allowing a precise assessment of their protein content **[20]**. The sugar content is carried out using a colorimetric method using phenol-sulfuric acid, thus providing a reliable measurement of the sugar level in faba bean fruits **[21]**. Furthermore, the lipid content is evaluated using the Soxhlet method, which consists of extracting lipids from faba bean fruits using organic solvents such as ether or hexane. Following this extraction, the organic solvents are evaporated, leaving residual lipids which are then weighed to determine their quantity in the faba bean fruits **[22]**.

2.5. Statistical Analysis:

The statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, IL). Variations in significance were assessed using the ANOVA test, and group differences were examined using the Tukey post-hoc test. Statistical significance was established when probability values were below 0.05 **[23]**.

3. Results and discussion:

After the preparation of the calcium amendment from *Amiantis umbonella* shells, a series of physicochemical analyzes was undertaken to provide detailed information on the composition and morphology of the sample. These analyzes included scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM/EDX), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR).

Figure 1 shows a scanning electron microscopy (SEM) image of the external surface of the sample, at a scale of 5 µm. An irregular, compact and not very porous structure of the sample is observed. Furthermore, the elemental analysis (Table 1) revealed a high concentration of calcium, representing 42.2% of the total elements detected. Other elements present include silicon, chloride, magnesium, sodium, phosphorus and potassium, although their presence is less significant. In addition, a well-defined proportion of organic matter was identified, with specifically determined levels of carbon (28.1%) and oxygen (20.1%).

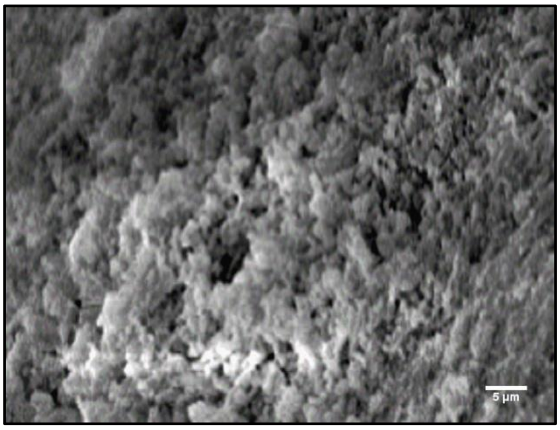

Figure 1. SEM of calcium amendment.

Table 1. Elements present on the surface of the shell sample.

Element	C	O	Ca	Si	Cl	Mg	Na	P	K
Weight (%)	28.1	20.1	42.2	3.4	2.3	1.6	1.2	0.8	0.3

Figure 2 presents the XRD diffraction spectrum of the sample, highlighting the characteristics of the prepared amendments. Very intense peaks corresponding to calcite ($CaCO_3$) are observed, with reflections characteristic of calcite corresponding to the hkl planes: (012), (104), (006), (110) and (113) **[24-25]**. Additionally, relatively weak peaks are observed, indicating the presence of aragonite (MgCO3), characterized by reflections at the (212) and (330) planes **[26-27]**. These observations highlight the crystalline composition of the sample, demonstrating the significant presence of calcite as well as the lesser presence of aragonite.

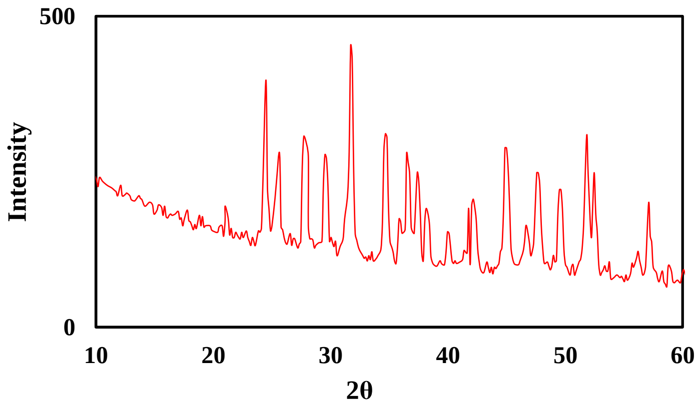

Figure 2. XRD of calcium amendment.

The FTIR spectrum of the calcium amendment sample is shown in the range of 400 to 4000 cm^{-1}, as shown in Figure 3. This spectrum reveals a characteristic band at 1475 cm^{-1}, associated with the vibrations of the C-O group of carbonates **[28]**. Additionally, a distinct band at 865 cm^{-1} is observed, attributed to specific vibrations of calcite **[29]**. The bands located around 745 and 705 cm^{-1} are also discernible, corresponding respectively to the vibrations of aragonite **[30]**. Furthermore, the group of bands between 1000 and 1100 cm^{-1} is the result of internal vibrations of the Si-O group present in the quartz **[31]**. These observations highlight the distinct spectroscopic characteristics of the calcium amendment, allowing precise identification of its components. This FTIR analysis provides complementary information to previous analyses, which is essential to confirm the chemical composition of the calcium amendment. This confirmation facilitates the understanding of its structure and properties, which is helpful for its effective use in subsequent agricultural applications.

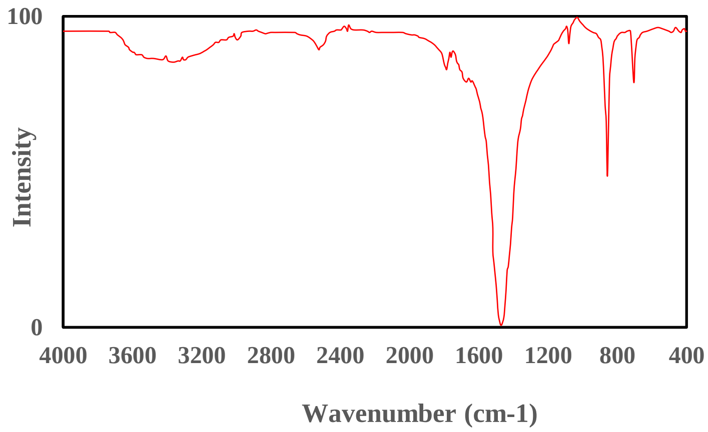

Figure 3. FTIR of calcium amendment.

For in planta experiments, the designed calcium amendment was applied at different percentages during faba bean cultivation. Table 2 presents the performance in terms of fruit yield and length of the aerial part of the beans grown as a function of the rate of calcium amendment applied after a period of 3 months. The results reveal a slight increase in fruit yield and the length of the aerial part with the increase in the amendment rate, compared to the untreated control group, particularly at the percentages of 5% and 10%. These observations indicate a potential for growth improvement, suggesting that the use of calcium amendments could favorably influence the growth and development of faba beans **[31]**. These results demonstrate a possible beneficial effect of the application of calcium amendments in bean growing conditions, highlighting the importance of these amendments in optimizing agricultural performance and promoting sustainable farming practices.

Table 2. Fruit yield and length of the aerial part of cultivated faba beans.

Percentage of amendment	Control	5%	10%	15%	20%
Yield of fruit (g/cm^2)	309 ± 42	344 ± 51	358 ± 49	356 ± 52	349 ± 50
Aerial part length (cm)	88 ± 1	95 ± 1	98 ± 1	92 ± 1	92 ± 1

Table 3 shows the results of the nutritional quality of the products harvested during the cultivation of faba beans, in correlation with the percentages of amendment applied. The pigments, expressed as a percentage, serve as clues to the coloring of the aerial part and their potential nutritional value. We observe uniformity in the variations in pigment levels among the different groups, with values oscillating between 3.2% and 3.4%. The ANOVA statistical analysis confirmed the absence of significant impact of the calcium amendment on the concentration of pigments in the crops. Concerning proteins, carbohydrates and lipids, we also notice slight fluctuations between the various groups, without a clear upward or downward trend depending on the percentage of amendment. ANOVA examination of the discrepancies between the values revealed no significant differences, thus demonstrating that the calcium amendment did not noticeably influence these nutritional components of the harvested products.

Table 3. Nutritional quality of fruits obtained when growing beans.

Percentage of amendment	**Pigment (%)**	**Protein (%)**	**Sugar (%)**	**Lipid (%)**
Control	3.3 ± 0.5	8.4 ± 1.4	9.5 ± 1.6	0.8 ± 0.1
5%	3.4 ± 0.5	8.5 ± 1.5	9.6 ± 2.0	0.8 ± 0.1
10%	3.3 ± 0.5	8.3 ± 1.2	9.6 ± 1.5	0.8 ± 0.1
15%	3.2 ± 0.5	8.4 ± 1.5	9.5 ± 1.2	0.7 ± 0.1
20%	3.2 ± 0.5	8.4 ± 1.5	9.6 ± 1.5	0.8 ± 0.1

4. Conclusion:

This work introduced a new approach and a comprehensive methodology for the formulation and evaluation of calcium amendments from *Amiantis umbonella* shells. Detailed physicochemical analyzes were carried out to characterize in depth the composition and morphology of the sample. The results obtained highlight a predominance of calcite in the amendment, accompanied by low concentrations of other compounds such as aragonite, as well as the detection of additional elements such as phosphorus and potassium. The agronomic effectiveness of this amendment was evaluated through in planta experiments. The application of the calcium amendment induced a slight increase in fruit yield and the length of the aerial part of the faba beans, particularly at dosages of 5% and 10%. Regarding the nutritional quality of the harvested products, the variations observed in the levels of pigments, proteins, carbohydrates and lipids between the different groups were minimal and not significant. These results suggest that the amendment used has a positive effect on the crop without significantly altering its nutritional components. In conclusion, this study highlights the importance of calcium amendments in optimizing agronomic performance and supporting sustainable agricultural practices, particularly for other crops, notably leguminous plants.

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