

Nutritional Composition of Phyto-extracts for Moringa, Comfrey, *Bidens Pilosa* and Algae as Potential Bio-stimulants in Vegetable Production

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Abstract: Various plant extracts have the potential to enhance growth and reproduction in vegetable crops but their nutritional composition may differ. The current study was aimed at analyzing the nutritional composition of leaf extracts for *Moringa oleifera* (moringa), *Chlorophyta* (algae), *Symphytum officinale* (Comfrey), *Bidens pilosa* (black jack) to prospectively increase their use as biostimulants in horticulture. Fresh plant leaves were collected at vegetative stage prior to flowering and processed for specific laboratory analytic techniques of major and trace nutrient elements. Analysis of variance results revealed that algae had significantly higher nitrogen and calcium levels of 8.13 and 18.69% respectively close to the levels of some commercially available fertilizers. Comfrey and blackjack had appreciably levels of potash of 4.39 and 3.78% respectively. Magnesium levels were very high in blackjack (60.54%) ahead of all the assessed plant extracts. All the tested plant materials had generally low levels of phosphorus. The leaf extracts of the four assessed plants are variable in their composition of major and trace elements but are in the range of nutritional requirements of most vegetable crops. Algae has the potential to be exploited as a biostimulant due to its observed high levels of nitrogen, calcium and trace elements close to the levels of some commercially available inorganic fertilizers.

Keywords: *Chlorophyta*, blackjack, leaf extracts, *Symphytum officinale*, trace elements

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1. Introduction

Agricultural bio stimulants are biologically produced additives that are used in agricultural production to improve vigor and productivity of crop plants (Dipak Kumar and Alope, 2020). Biostimulants are used to enhance plant tolerance to harsh abiotic factors, nutrient use efficiency, crop quality and yield (Rai et al., 2021; Teklić et al., 2021; El Sheikha et al., 2022; Raza et al., 2024). Five categories of bio stimulants include microbial, organic acids, peptides or free amino acids found from animal and plant residues, plant extracts and plant or animal agro-industrial by-products (Van Oosten et al., 2017; Rouphael and Colla, 2020; Rai et al., 2021). They possess various abilities to enhance germination, seedling vigor, flowering, fruiting and leaf quality and crop output under abiotic stresses such as nutrient deficiencies, toxicity, heat and drought (Bulgari et al., 2015; Teklić et al., 2021; Gómez et al., 2024). More importantly they provide major and trace elements that form key growth and reproductive factors in crops such as vegetables. However, there is vast deficit in empirical evidence on the precise nutritional composition of potential plant extracts to enable their full exploitation as bio stimulants in crop production. A limited number of farmers in different parts of the world use plant extracts to stimulate growth and productivity of crops depending on their availability in their localities (Kisvarga et al., 2022). There are several plants commonly found in tropical regions that hold high potential for use as bio-stimulants in vegetable production. These include *Moringa oleifera* (moringa), *Chlorophyta* (algae), *Symphytum officinale* (Comfrey), *Bidens pilosa* (black jack) extracts among the notable ones (Fiorentino et al., 2018; Zulfiqar et al., 2020; Rai et al., 2021; Lau et al., 2022; Sun et al., 2024).

Moringa is a nutrient-rich tree found in many tropical and subtropical areas whose leaf extracts, pods, seeds and twigs have been used by farmers as a bio-organic fertilizer for a variety of crops (Boopathi and Raveendran, 2021). Moringa leaf extracts have shown to improve seed germination, plant growth, development, and yield, as well as the effectiveness of nutrient consumption, leaf and fruit quality in vegetables and tolerance to all stresses (Bulgari et al., 2019). *Bidens pilosa* is a common herb that grows in most arable and non-arable ecologies that has been known as a nuisance weed in cropping areas and has recently been discovered to have some medicinal and agrochemical value (Kato-Noguchi and Kurniadie, 2024). Comfrey is a perennial herb of European and Asian origin used as a foliar spray, direct mulch, or powder to enhance soil nutrition (Salehi et al., 2019). It is utilized as green manure and a rich source of bioactive chemicals that promote plant growth and development. Algae is a class of micro and macro aquatic organisms that produce extracts that serve as growth enhancers (Zuzunaga-Rosas et al., 2024). A well proclaimed source of plant nutrition in organic farming that contains complex combinations of physiologically active chemicals (Van Oosten et al., 2017). In addition, it is understood to have high levels of major and minor mineral elements (Soliman and Hamed, 2019; Del Buono, 2021).

The nutritive value of plant extracts that can be used as bio stimulants is not statistic. Highly significant variations in nutritional composition of plant extracts have been reported (Gómez et al., 2024). A concoction of factors, including growing environment, agro-ecological region, season, species, and organ of the plants used can influence the chemical makeup of active chemicals in the original plant raw material, exacerbating the fundamental problem in bio-stimulant production and use (Ngoroyemoto, 2020). The limited knowledge on their nutritional composition that should inform their precise application rates and suitable crops often leads to either under-or over-application of key nutrients to the detriment of the crops (Bulgari et al., 2015). Thus effectiveness of various novelty bio-agrochemicals has been difficult to assess (Halpern et al., 2015). This has stalled the improvement and commercialization of potential bio stimulants that would offer cheap and sustainable organic farming.

Leafy vegetables require high levels of fertilizer at the same time are sensitive to certain nutrients such as nitrates (Di Mola et al., 2019). The continuous use of synthetic chemicals and inorganic fertilizers is of global concern hence the recent interest in bio stimulants in the mainstream research which improve growth without compromising quality in vegetables (Bhupenchandra et al., 2020). Thus, research is obligatory to ascertain nutrient and mineral composition of selected bio-

stimulants in order to make recommendations to farmers on precise quantities for use and application in enhancing soil amendment for specific crop growth and productivity. Knowledge on mineral composition of bio-stimulants will assist determination of their recommended dosage. This will circumvent over application which may result in phyto-toxicity yet on the other hand under application could result in nutrients deficiencies in sensitive crops especially leafy vegetables. However, leafy vegetables tend to respond well to plant based bio stimulants if provided in precise quantities (Bulgari et al., 2015). The different nutritional requirements of crops necessitate the need to evaluate the nutritional composition of candidate plant extracts for use as bio stimulants for specific crops.

The purpose of the current study was to determine the nutritional composition of leaf extracts for four selected high potential plants namely *Moringa oleifera* (moringa), *Chlorophyta* (algae), *Symphytum officinale* (Comfrey), *Bidens pilosa* (black jack) with a vision to intensify their use as bio-stimulants to improve plant growth, development, and yield specifically in leaf vegetable production. The study will act as baseline for further research on the efficacy of the assessed plant extracts on growth and yield of specific vegetable crops that are known to respond well to bio stimulants but are also sensitive to nutritional compositions of conventional soil amendments.

2. Materials and Methods

The study involved collection of samples of the four selected plants using a bio stimulant followed by processing of foliar extracts for the laboratory procedures in testing of their chemical composition.

2.1. Description of the location where plant leaf samples were collected

Plant materials were all collected from the Esigodini College Farm located on -20.29495 latitude and 28.93681 longitude. The altitude is 1.18 meters above sea level. The average annual temperature is 30.6°C in summer and can drop to as low as 9°C in winter. The area has 73.48 rainy days with an average rainfall of 58.07 mm accumulating a total of 632 mm per annum. The dominant soils are deep red to dark clay loams with good water-holding capacity with moderate fertility. The soils crack under extremely dry conditions. The samples used are *Bidens pilosa*, *Chlorophyta*, *Symphytum officinale* and *Moringa oleifera*.

2.2. Collection of the plant material

Fresh leaves of plants were collected, that is at most five leaves from the apex for moringa and blackjack, leaving the two newly developing leaves. For the comfrey plant, ten leaves were collected from the growing point excluding the two newly developed leaves. Collection of the plant materials was done manually during the summer season towards the end of November 2022 during the early morning hours and were kept in separate khaki labeled envelopes. The envelopes were stored at room temperature of 25°C for a day awaiting the laboratory procedure.

2.3. Laboratory procedures for preparing samples

The foliar analysis was conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) laboratory in Matobo District, Matabeleland South Province in Zimbabwe in November 2022. Two samples of 500 g for each plant material were used for the laboratory tests. Testing of plant materials was done at vegetative stage prior to flowering where similar plant parts were taken separately at the same growth stage to avoid differences in nutrient concentration. The plant samples were dried in dried at a temperature of 70°C for 24 hours and then ground into powder and put into a digester so as to determine the total nitrogen and phosphorus. The dried ground plant samples were treated with reagents. Dried biomass was used since it allowed accurate nutrient assessment as it eliminates variability caused by moisture content if it were in wet biomass.

2.4. Experimental design

The prepared samples were analyzed following a completely randomized design with two replicates for each plant extract. The design is suitable for homogenous conditions under which the samples were prepared.

2.5. Analytic techniques of nutrient composition

The specific analytic techniques of the major and trace nutrient elements of interest are detailed as follows:

2.5.1. Nitrogen

The Kjeldahl distillation method was used to quantify total nitrogen (Bradstreet, 1954. Atomic absorption spectrophotometer (AAS) was used to test the mixture, which contained calcium, magnesium, potassium, sodium, zinc, iron, manganese, and copper. The digest solution was diluted appropriately to allow precise measurement of each analyte.

2.5.2. Phosphorus

Total phosphorus was analyzed from the digest solution using the vanadate molybdate reagent and quantification done colorimetrically at 470 nm wavelength (Garg, 2012).

2.5.3 Potassium

0.5 g of finely ground plant materials were shaken with 40 ml of 0.5 N HCl for 5 minutes. The extract was filtered and read in an AAS.

2.5.4 Trace elements

A modified Kjeldahl wet digestion method was specifically adapted to determine calcium, magnesium, sodium, zinc, copper, iron and manganese by incorporating salicylic acid and selenium as catalysts (Haynes, 1980). Total were determined. This was done for each sample that included moringa, comfrey, blackjack and algae.

2.6. Statistical analysis

Data on nutritional composition was subjected to one-way analysis of variance (ANOVA) in GenStat 14th edition to determine significant differences in the means of plant extracts. Data were also subjected to normality and homogeneity of variance tests prior to ANOVA. Where significant differences were detected, the means were separated using the Bonferroni's test at 5% level of significance.

3. Results

The F-test results of the macro and micronutrients of the phyto-extracts revealed that algae had a significantly higher nitrogen levels of 8.13% followed by moringa at 3.68%. Comfrey and blackjack had low nitrogen levels of 3.22% and 2.89% respectively (Table 1). All the tested plant materials had generally low levels of phosphorus with blackjack having the highest at 0.53% and algae at 0.03%. Comfrey and blackjack had appreciably high levels of potash at 4.39 and 3.78 respectively. Algae had very low potash levels. Algae had statistically higher calcium levels of 18.69% while the rest of the assessed materials had low levels of calcium. Magnesium levels were very high in blackjack (60.54%) ahead of all the other plant materials with moringa (0.42%) containing the least.

Table 1. Variations in macro and micronutrient levels in phyto extracts

Plant extract	Means of macro and micro nutrient									
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Mn (µg/g)	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)
Algae	8.13 ^a	0.03 ^c	0.05 ^b	18.69 ^a	1.12 ^a	0.13 ^a	106.89 ^a	8.26 ^b	1159.3 ^a	265.42 ^a
Black Jack	2.89 ^c	0.53 ^a	3.78 ^a	0.95 ^c	0.54 ^b	0.02 ^b	96.81 ^a	15.29 ^b	717.6 ^{ab}	41.21 ^b
Comfrey	3.22 ^{bc}	0.45 ^a	4.39 ^a	1.2 ^c	0.52 ^b	0.01 ^b	96.81 ^a	29.90 ^a	637.7 ^{ab}	30.69 ^b
Moringa	3.68 ^b	0.33 ^b	1.55 ^{ab}	1.96 ^b	0.42 ^b	0.06 ^b	90.6 ^a	13.63 ^b	167.8 ^b	18.88 ^b

Overall mean	4.48	0.337	2.44	5.7	0.65	0.054	97.8	16.77	671.	89.0
Fpr	<0.001	<0.001	0.005	<0.001	0.007	0.003	0.31	<0.001	0.007	<0.001
LSD	0.26	0.06	1.63	0.34	0.28	0.04	20.51	4.717	349.4	46.41
%CV	2.1	6.7	24.1	2.1	15.5	23.7	7.6	10.1	18.8	18.8

Means superscripted with different letters were significantly different at $p < 0.05$

Algae had the highest levels of Zinc at 265.42 $\mu\text{g/g}$ far ahead of all other plant materials with moringa containing the least quantity of 18.88 $\mu\text{g/g}$. On the same note, iron levels were very high in algae and lowest in moringa with 1159.29 $\mu\text{g/g}$ and 167.79 $\mu\text{g/g}$ respectively. Manganese was also high in algae (106.89 $\mu\text{g/g}$) and lowest in moringa (90.60 $\mu\text{g/g}$). Copper levels were high in comfrey (29.90 $\mu\text{g/g}$) and algae (8.28 $\mu\text{g/g}$) contained the lowest levels.

4. Discussion

The high nitrogen levels observed in algae in the current study are close to the levels of some commercially available nitrogenous fertilizers (Rai et al., 2021). The observed nitrogen levels are in line with the research findings by Baweja et al., (2016) and Miranda et al., (2024) who also observed that nitrogen content was high in the algae. Chabili et al., (2024) also advanced that algae fresh extracts contain nitrogen levels that can be sustainably used in crop production. It is a cheap source that can be obtained from stored water and waste water to provide nutrients for plant growth and stimulate some biological activities within the soil for nutrient releases (Gonçalves et al., 2023). The major setback in its use is its antagonistic effects that emanate from the toxic pyrrolizidine alkaloids which can interfere with the beneficiary properties of other plant compounds. It can further inhibit seed germination and growth in some plant species (Trifan et al, 2021; Asimakis et al., 2022). This calls for prior establishment of its effects in intended crops.

In the current study, moringa leaf extracts were found to contain higher nitrogen levels after algae. This is in line with the studies carried out by Abohassan and Abusuwar (2018) who discovered that as a leguminous plant, moringa contains significant levels of nitrogen that can be used to improve plant growth and yield. Moringa leaf extracts have been attested to improve physiological and yield attributes of most vegetable crops (Sileshi et al., 2024). Like most bio stimulants, moringa plant extracts and algae can also be used to stimulate biological activities in the soil like biological nitrogen fixation (Niewiadomska et al., 2020). Phytotoxic effects of moringa limit its use as a bio stimulant as exhibited in the study by Vyas and Sharma, (2021) who established that its leaf extracts inhibited more than 50% tomato seed germination plus also impaired root and shoot growth in seedlings. Therefore, it can be use at later of growth stages of the crop. In a study by Abdelkhali et al., (2024), where moringa leaf extracts were applied with microorganisms in sweet potatoes led to the reduction in the accumulation of cadmium in roots by 55.6% and 31.4% in leaves.

Blackjack was found to contain nitrogen levels enough to qualify its use in promoting plant growth and development. Kato-Noguchi and Kurniadie, (2024) concluded that due to its chemical properties, blackjack can also be used in crop protection to suppress different weeds and crop pests like the root-knot nematode. It is noteworthy that excess application of leaf extracts of *Bidens Pilosa* in some plants might inhibit enzymes responsible for metabolizing other plant compounds, thus resulting in reduced growth in other plants (Khanh et al., 2009). Comfrey was found to contain significant potassium levels that can suffice plant development were determined in the current study. The above and below ground parts of Comfrey were reported to contain high levels of potash and moderate levels of nitrogen ideal for promoting the growth and development of leafy vegetables like cabbage that are heavy nitrogen feeders (Howard et al., 2018; Salehi et al., 2019; Tamakhina et al.,

2019; Trifan et al., 2021). It, therefore, qualifies for use as a bio fertilizer to promote the metabolic activities in vegetable crops.

Blackjack also had appreciably high levels of potash while algae proved to be low in potash. In a similar study high levels of potassium in *Bidens pilosa* was reported by Wei et al., (2017). Algae had very high calcium which is quite comparable to some soil pH-adjusting supplements (Nair and kale, 2022). These findings concur with the findings by Battacharyya et al., (2015) and Mamede et al., (2024). It can therefore be tested for its potential as a liming material. Earlier studies by Baweja et al., (2016) indicated that microalgae and sea weeds extracts contained high levels of alkane compounds. All the tested bio-stimulants have generally low levels of phosphorus when compared to the other two macronutrients that is nitrogen and potassium though the observed phosphorus levels are in line with some of the commercially available bio-stimulants (Kisvarga et al., 2022). Where there are varying deficiencies of different nutrients extracts that complement each other in terms of the needed nutrients should be identified.

The observed zinc, manganese, iron, and copper levels for all the bio-stimulants especially in algae are within the levels provided by most synthetic bio stimulants (Bulgari et al., 2019; Niewiadomska et al., 2020; El Sheikha et al., 2022). Comfrey has been discovered to contain considerable amounts of trace elements like iron, zinc, and sodium (Sun et al., 2024). The other studies by Ahmed et al., (2018) and Zulfiqar et al., (2020) concluded that trace elements like iron and zinc are found in moringa leaf extracts. It is noteworthy that chemical composition of plant extracts tend to vary according to seasons and locations which is a factor of climatic and edaphic factors which makes it very difficult to make conclusive results on this matter (Prinsloo and Nogemane, 2018, Lamidi et al., 2017; and Ukwueze et al., 2019).

5. Conclusions and recommendations

It can therefore be concluded that algae proved to be highly nutritive in most essential macro and micronutrients except for phosphorus. It is recommended for heavy nitrogen feeders such as cabbages. Where phosphorus is limiting or for crops that need high levels of phosphorus blackjack and Comfrey can be good complements. In addition, algae are highly recommended for use as a buffer in ameliorating soil acidity due to their high calcium levels. All the assessed plant extracts are good sources of trace elements which include zinc, copper, manganese, and iron within the range required by the most vegetable plants. It is also recommended that other extraction methods such as water and organic solvents be considered in future research for yields and efficacy differences.

Author contributions

Conceptualization (BD and EN), acquisition of research materials (BD and E.N) data collection (BD), data analysis (EN and BD), writing of original draft (BD and EN). All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

Data availability

The data sets will be availed on request.

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