Effects of *Moringa* (*Moringa Oleifera*) Leaf Extract on Growth, Yield and Yield Components of Snap Beans (*Phaseolus vulgaris*)

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Authors’ contributions

This work was carried out by author VEE, who designed the study, performed the statistical analysis, interpretation and discussion of data, literature searches, wrote, edited and proof read the final manuscript.

ABSTRACT

Two field experiments were carried out to evaluate the effects of *Moringa* leaf extract on the growth, yield and yield components of snap bean. The results showed that *Moringa* leaf extract applied at 11, 20, 33 and 50% concentration to snap bean plants at 10 days after emergence significantly (*P* ≤ 0.05) increased vegetative growth, leaf chlorophyll content, plant dry matter (shoot and root), yield components and fresh pod yield. The snap bean response to increasing *Moringa* extract concentration was quadratic with respect to plant height, leaf area, leaf number, leaf chlorophyll content, shoot dry matter and root dry matter. However, *Moringa* leaf extract significantly (*P* ≤ 0.05) reduced shoot and root water contents. Due to the *Moringa* extracts-induced increase in vegetative growth, leaf chlorophyll content, yield components and yield of snap beans, it was concluded that *Moringa* leaf extract could be used to enhance the growth and development of snap beans.

Keywords: *Moringa oleifera*; leaf extract; snap bean; natural growth and yield enhancer; pod quality.
1. INTRODUCTION

The snap bean, probably is the most important grain legume in Africa, grown on an estimated 3.7 million hectares/year and provides food and income to at least 100 million people in Eastern, Central and Southern Africa [1,2]. Per capita consumption in this region is perhaps the highest in the world exceeding 50 kg in the Eastern and Southern Africa reaching 66 kg in the densely populated districts [3]. Much of the production is at elevations from 1000 m to 1800 m above sea level. The Eastern African highlands account for 32% of bean production in Africa, with about 518 000 hectares in areas up to 1500 m above sea level.

Grain legumes are important foodstuffs in all tropical and sub-tropical countries where they are second in importance to cereals, as a source of protein [2]. Beans have often been described as ‘meat of the poor’ and in Eastern and Southern Africa they are recognized as the second most important source of calories [2]. The proteins in legumes contain relatively more of the essential amino acids not supplied by cereals in which the content of lysine and tryptophan are relatively small. Since animal protein is more expensive than plant protein, legumes are important in less developed countries for the supply of relatively inexpensive proteins for rural and urban populations. However, grain yield of commercial crops of common beans is less than 1.0 tons/ha in most developing countries [2]. In order to meet the rising food demand, emphasis has to be put on improving yields per hectare. This can be achieved through plant breeding [4], biotechnology and application of plant growth regulators to positively modify plant growth to economic advantage [2].

Plant growth regulators can be used to modify photosynthesis efficiency and assimilate partitioning in bean growth [2]. Extracts from fresh Moringa leaves could be used to produce an effective plant growth enhancer, increasing yield by 25-30% for nearly any crop [5]. The active growth enhancing substances in Moringa leaf extract are reported to be zeatin, dihydrozeatin and isopentyladenine which are natural (endogenous) cytokinins [5,6]. The moringa leaf is also composed of proteins, minerals, vitamins, essential amino acids, glucosinolates, isothiocyanates and phenolics [7,8,9,10]. Cytokinins stimulate cell division, growing cell tissues, delay the processes of senescence and aging in many plant tissues and, promote nutrient partitioning and uptake [2,6,11]. The use of Moringa leaf extracts as a possible plant growth enhancer or natural plant growth regulator has the potential to increase agricultural production because most subsistence farmers in Africa practice low input agriculture. A number of factors such as naturally-occurring toxicants (glycoalkaloids in potatoes), natural contaminants (mycotoxins and bacterial toxins), heavy metals (cadmium, arsenic, lead and mercury), environmental pollutants, pesticide residues and microbial contamination threaten the safety of fruits and vegetables. The use of Moringa leaf extract as a possible plant growth enhancer can provide a relatively environmentally safe, easily accessible and affordable means of increasing crop yields to meet the increasing demand of food. The use of natural plant growth enhancers could improve the situation for small scale and commercial farmers. Therefore, the objective of this study was to evaluate the effects of Moringa (Moringa oleifera Lam) leaf extract on growth, yield and yield components of snap bean (Phaseolus vulgaris L.).

2. MATERIALS AND METHODS

2.1 Experimental Site

Two field experiments were carried out at The Botswana College of Agriculture Content Farm in Sebele (24° 34’S, 25° 54’E, 994 m above sea level) from December 2009 to March 2010 and January 2011 to April 2011, growing seasons. The climate is semi-arid, characterized by a cold winter and hot summer. Minimum temperature during winter can be as low as 0°C and as high as 40°C in summer. The mean annual rainfall is between 500 and 550 mm between October and May. The soils are shallow, ferruginous tropical soils, mainly consisting of medium to coarse grain sands and loam with a low water holding capacity and subject to crusting after heavy rains. The soils have low mineral nitrogen, organic matter and are deficient in phosphorous [12].

Moringa seeds were planted in 270 pots and were later transplanted when seedlings were eight weeks old. The plants were taken care of until ready for use (3 months after transplanting). Young leaves were harvested and juice extracted using a blender. The extract was made by homogenizing young Moringa shoots with 80% ethanol (1 kilogram of fresh Moringa shoots per 1 liter of ethanol). The extract was filtered and then
diluted with distilled water at various ratios of 1:1, 1:2, 1:4 and 1:8.

2.2 Experimental Design

The experimental design was a randomized complete block design with three replications. The treatments were *Moringa* leaf extract dilutions at 0 (control), 1:1 (50%), 1:2 (33%), 1:4 (20%) and 1:8 (11%). The experimental plots measured 5 m x 5m. The snap bean plants were directly sprayed with *Moringa* leaf extract dilutions to run-off using a pressurized knap sack sprayer. The snap bean plants were sprayed with *Moringa* leaf extracts 10 days after emergence.

2.3 Crop Husbandry

One snap bean seed was planted per hole at a depth of 2.5 cm with a spacing of 30 cm between rows and 15 cm between plants. The fertilizer 2:3:2 containing 63 g nitrogen, 93 g phosphorous and 63 g potassium/kg, respectively, was applied at a rate of 400 kg/hectare in two splits. The first fertilizer application was done at planting and the second fertilizer application done at the onset of flowering. Weeds were controlled by hand hoeing between rows and between plants.

2.4 Dependent Variables Determined

The dependent variables that were determined included: plant height, number of leaves per plant, leaf area, leaf total chlorophyll content, leaf chlorophyll a and b contents, pod dry matter and water content, pod length, shoot and root dry matter and pod yield per hectare. Plant height was determined by measuring the height of 10 randomly selected plants/replication at the onset of flowering using a 1 meter steel ruler. The number of leaves/plant were determined by counting the number of leaves of 10 randomly selected plants/replication. The fresh and dry weight of snap bean plants was determined by destructive harvesting of 10 plants/replication at the onset of flowering. The plants were watered thoroughly 24 hours earlier before being carefully dug to ensure that at least 99% of the roots were included. The shoots and root plant samples were separated and put in already weighed brown paper bags. The shoot, root and pod samples were weighed using a Mettler PM 400 digital balance. The shoots and roots was oven dried at 66°C to constant weight. The water content of shoots and roots was determined by subtracting dry weight from corresponding fresh weights. Pod length was determined by measuring the length of pods from 10 tagged plants using a 30 cm ruler. Leaf area was determined using 10 plants/replication using a leaf area meter (CI 202).

2.5 Leaf Chlorophyll Determination

Ten leaves from five plants were sampled and two discs per leaf were cut using 60 mm cork borer. The 20 discs were extracted in 4 ml of 0.1N HCL in methanol at 25°C in the dark for 24 hours. Absorbance of extracts was measured using a UV visible spectrophotometer (UV – 160IPC) at wavelength 645 nm, 653 nm and 663 nm. Total chlorophyll, chlorophyll a and chlorophyll b were estimated using the formulae given below:

\[
\text{Chlorophyll a (mg/cm}^2\text{)} = 12.7 A_{663} - 2.069 A_{645} \\
\text{Chlorophyll b (mg/cm}^2\text{)} = 22.9 A_{645} - 4.68 A_{663} \\
\text{Total chlorophyll (mg/cm}^2\text{)} = 24.88 A_{653} \ [13]
\]

2.6 Data Analysis

Data collected was subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 2013) package. Due to the similarity of the data for the two trials, data was pooled during analysis [14]. Treatment means were separated using the Least Significant Difference (LSD) at P=0.05. Appropriate regression models were used to examine the nature of the response surface of snap bean plants to increasing concentration of *Moringa* leaf extracts.

3. RESULTS

Snap bean plants treated with *Moringa* leaf extract significantly (P ≤ 0.001) increased plant height compared to control plants (Fig. 1). The response of snap bean plants to increasing *Moringa* leaf extract concentration was quadratic (Fig. 1) with 11% extract giving the tallest plants.

*Moringa* leaf extract applied at 11, 20, 33 and 50% to snap bean plants significantly (P ≤ 0.05) increased the leaf area and number/plant than control plants (Figs. 2 and 3). The response of snap bean plants to increasing *Moringa* leaf extract concentration to both leaf area and leaf number was quadratic (Figs. 2 and 3).
y = -0.020x^2 + 1.226x + 17.56
\[ R^2 = 0.752 \]

Fig. 1. Effect of *Moringa* leaf extract on snap bean plant height. The bars are LSD bars

\[ y = -0.2028x^2 + 9.8339x + 407.47 \]
\[ R^2 = 0.85 \]

Fig. 2. Effect of *Moringa* leaf extract on snap bean leaf area the bars are LSD bars

\[ y = -0.017x^2 + 1.094x + 23.57 \]
\[ R^2 = 0.701 \]

Fig. 3. Effect of *Moringa* leaf extract on leaf number of snap beans. The bars are LSD bars
*Moringa* leaf extract applied at 11, 20, 33 and 50% to snap bean plants significantly (*P* ≤ 0.05) increased total leaf chlorophyll content and chlorophyll a and b contents compared to control plants sprayed with distilled water (Fig. 4). The highest leaf chlorophyll content (total and chlorophyll a and b) was obtained on snap bean plants sprayed with 20% *Moringa* leaf extract, beyond which leaf chlorophyll content decreased (Fig. 4).

Application of *Moringa* leaf extract at 11, 20, 33 or 50% to snap bean plants had no significant (*P* ≤ 0.05) effect on shoot and root dry matter and water contents compared to control plants (Figs. 5 and 6). However, the *Moringa* leaf extract tended to have a non-significant increase and decrease in both shoot and root dry matter and water contents, respectively (Figs. 5 and 6).

Fig. 4. Effect of *Moringa* leaf extract on leaf chlorophyll content of snap beans. The bars are LSD bars

Fig. 5. Effect of *Moringa* leaf extract on snap bean shoot dry matter and water content. The bars are LSD bars
Snap bean plants sprayed with *Moringa* leaf extract at 11, 20, 33 and 50% significantly (P = 0.01) increased the pod length compared to control plants sprayed with distilled water (Fig. 7). The response of snap bean plants to increasing *Moringa* leaf extract concentration with respect to pod length was quadratic with 20% extract giving the best response to pod length (Fig. 7).

Application of *Moringa* leaf extract at 11, 20, 33 or 50% to snap bean plants had no significant (P ≤ 0.05) effect on pod dry matter and water contents compared to control plants (Fig. 8).

Snap bean plants sprayed with *Moringa* leaf extract at 11, 20, 33 and 50% significantly (P=0.01) increased the fresh pod yield compared to control plants sprayed with distilled water (Fig. 9). The response of snap bean plants to increasing *Moringa* leaf extract concentration with respect to fresh pod yield was quadratic with 20% extract giving the best response to fresh pod yield (Fig. 9).

![Fig. 6. Effect of *Moringa* leaf extract on root dry matter and water content of snap beans. The bars are LSD bars](image1)

![Fig. 7. Effect of *Moringa* leaf extract on pod length of snap beans. The bars are LSD bars](image2)
Fig. 8. Effect of *Moringa* leaf extract on snap bean pod dry matter and water content. The bars are LSD bars.

Fig. 9. Effect of *Moringa* leaf extract on fresh pod of snap beans. The bars are LSD bars.

### 4. DISCUSSION

The results from the study showed that *Moringa* leaf extract increased vegetative growth (plant height, leaf area and leaf number) of snap bean plants. The *Moringa* leaf extract induced increase in vegetative growth of snap beans was attributed to the role of cytokinins in promoting cell division and elongation. It has been reported that *Moringa* leaf extract contains zeatin, dihydrozeatin and isopentyladenine which are endogenous cytokinins [6]. Cytokinins have been reported to stimulate cell division and growing cell tissues [2,11,15]. Normal growth of stems and roots of legumes have been reported to require cytokinins [15]. A combination of benzyladenine and gibberellins (GA$_{4+7}$) at 5, 15 or 25 mg/L (Accel) has been reported to increase vegetative growth of snap bean plants [16]. The results from the current study showed that *Moringa* leaf extract increased the number of leaves of snap bean plants and the response was quadratic to increasing *Moringa* leaf extract concentration. This was attributed to the role of cytokinins in reducing the plastochron and/or increasing cell division of snap bean plants resulting in higher leaf number. The decrease in vegetative growth above 11% *Moringa* leaf extract concentration was attributed to toxicity of ethanol in the extract, suggesting that distilled water could be used as the solvent in *Moringa* leaf extraction process.
Moringa leaf extract increased pod length and pod yield of snap beans and the response was quadratic to increasing Moringa leaf extract concentration. The increase in pod length was explained by the role of cytokinins in promoting cell division at pod set. Gibberellins (GA_4+7) and benzyladenine (BA) have been shown to increase pod set and subsequently pod number per branch in leguminous crops [17,18,19]. Cytokinin treatments have been reported to enhance pod set in snap bean cultivars that fail to set pods in the summer [20]. Application of Moringa leaf extract to snap beans increased pod yield. The results of the current study are in agreement with the findings that the juice from fresh moringa leaves increased yield by 25-30% for nearly any crop [5]. Plants that were treated with Moringa leaf extracts produced more fruits that consequently resulted in higher yield [21]. The rate of supply and distribution of carbon assimilates to and within developing flowers and pods determines the numbers and sizes of the various yield components in legumes [20]. In the present study, application of Moringa leaf extract may have increased loading and unloading of assimilates across membrane boundaries of the vascular (phloem) tissues of snap bean plants, leading to increased dry mass accumulation and partitioning to the shoots, and increase in pod yield/ha due to the cytokinins in the extract. Cytokinins promote carbohydrate metabolism and create new source-sink relationships leading to increased dry matter accumulation in the snap bean [22,23]. The increase in pod yield, dry mass accumulation and dry mass partitioning to shoots induced by Moringa leaf extract was attributed to the effects of endogenous cytokinins (zeatin, dihydrozeatin and isopentyladenine) on assimilate mobilization and/or distribution. Cytokinins are also reported to increase pod set and pod number per branch in leguminous crops [2]. The increase in pod yield observed in the current study was also attributed to the increase in pod length, leaf area and number and leaf chlorophyll content which are yield components of snap bean plants. Increase in leaf area and number and leaf chlorophyll content might have increased net photosynthesis resulting in more photoassimilates in Moringa leaf extract applied snap bean plants, hence higher pod yield.

5. CONCLUSION

Due to the induced increase of vegetative growth, yield and yield components of snap bean plants by Moringa leaf extract, it was concluded that Moringa leaf extract has the potential to be used as a natural growth enhancer as a cheap source of plant growth hormones to modify the growth and development of snap beans plants. The researcher recommended that further studies on Moringa leaf extracts be investigated on other crops.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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