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Influence of Basal Media Variants on the Accumulation of Phytochemicals in Leaves of Moringa Oleifera Lam. In Vitro

[1]Okeke, C.M., [2]Chigor, C. B. and [2]Okafor, U. C.

[1]Natural Science Unit, School of General Studies;
[2]Department of Plant Science and Biotechnology, University of Nigeria Nsukka
Corresponding Author's email: chinenye.okeke@unn.edu.ng

Abstract
Moringa oleifera Lam. is renowned for its medicinal and nutritional values due to the presence of various phytochemicals and nutrients which are beneficial to man. This study compared the influence of three standard plant tissue culture basal media, namely: Murashige and Skoog medium, Gamborg B5 medium and White medium, on the accumulation of some secondary metabolites in the leaves of *Moringa oleifera*. The explants were pre-sterilized and the sterile seeds were transferred to the culture tubes containing the different basal media and kept in a growth room for four weeks. The leaves from the treatments were harvested and air dried for the phytochemical studies. Agar and water served as the control. The experimental design was Completely Randomized Design (CRD). Data were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's New Multiple Range Test (DNMRT) at ($P \leqslant 0.05$). All the treatments supported the production of phytochemicals; however there were significant differences among the treatments in some of the parameters studied. B5 medium best enhanced the production of tannin (0.038 ± 0.00 %), alkaloids (25.00 ± 0.11 %), glycoside (4.75 ± 0.25 %) and carotenoids (80.00 ± 0.00 %), while the White medium enhanced the production of flavonoid (22.00 ± 2.00 %) when compared to other treatments ($P \leqslant 0.05$). This study provides useful information that could be exploited for the production of phytochemicals from *M. oleifera* seeds *in vitro*.

Keywords: Moringa oleifera, Medicinal, Phytochemicals, Tissue culture, Basal media, Secondary metabolites

Introduction

Plants contribute a lot to both traditional and modern medicine. The medicinal value of plants comes from the phytochemicals (secondary metabolites) they contain, however, there are variations in the concentration of phytochemicals in different plant sources with the levels of many compounds being quite low and absent in some others (Jamshidi-Kia *et al*., 2018). *Moringa oleifera* is well known for its medicinal and nutritional values as all parts of the plant are consumed. Nutritive and non-nutritive phytochemicals have been reported in this plant (Ijeomah *et al*., 2012). Some of the secondary metabolites that have been identified in the leaves of *Moringa oleifera* include flavonoids, alkaloids, anthraquinones, saponins, steroids, terpenoids, glycosides, anthocyanins, tannins and carotenoids (Nweze and Nwafor, 2014; Shanmugavel *et al*., 2018), however, climatic conditions, soil composition and other environmental factors are reported to influence the synthesis of these metabolites (Adamu *et al*., 2016). Researchers have reported on successful elicitation of secondary metabolites *in vivo* and *in vitro* (Halder *et al*., 2019).

There are several factors that can be optimized *in vitro* to improve growth and the production of secondary metabolites in plants. Reduced levels of total nitrogen improved the production of anthraquinones in *Morinda citrifolia* and anthocyanins in *Vitis* species (Shilpa *et al*., 2010). Cui *et al*. (2010) also reported that *Hypericum perforatum* showed higher accumulation of flavonoid when the ratio of ammonium to nitrate was adjusted to 5:25 in MS medium. Reduction of nitrate increased the total flavonoid content of *Matricaria chamomilla* plants (Kovacik *et al*. 2014). Some of the effects of mineral salts on the accumulation of secondary metabolites reported in *in vivo* studies include that of calcium and potassium supplementation in *Cicer avietinum* which increased the total phenols and flavonoids (Ahmad *et al*., 2016) and in *Piper belle* L. where the absence of nitrogen with full sunlight increased the accumulation of total flavonoids (Muttaleb *et al*., 2018).

Several studies have been documented on medicinal importance of moringa worldwide, but few reports exist on how to improve the phytochemical content of the plant. This study determined the influence of three basal

media on the accumulation of phytochemicals in the leaves of *Moringa oleifera.*

Materials and Methods

The research work was carried out in the Plant Tissue Culture Laboratory, University of Nigeria, Nsukka, Enugu State. Fruits (pods containing the seeds) of *Moringa oleifera* were collected from a domesticated tree at the University of Nigeria, Nsukka (UNN). The seeds of *Moringa oleifera* were dehulled and used as explants. A viability test was carried out by testing some dehulled seeds using tetrazolium chloride which change in colour of cotyledon from colourless to red colour was an indication of viability (ISTA, 1999). The viability was further tested by planting some dehulled seeds in the field. Only the viable seeds were used for the study. The dehulled seeds (explants) of *Moringa oleifera* were carefully selected and pre-sterilized by a quick dip for few seconds in 70% ethanol. This was followed by sterilization which was done by submerging the explants into 10% sodium hypochlorite (NaOCl) for twenty-five minutes; the sterilant of choice was the commercial bleach of Jik brand. After twenty-five minutes, the explants were rinsed three times in sterile distilled water to wash off the sterilant from the explants. The sterile seeds were transferred to the culture tubes containing the different basal media (sixty test tubes per each treatment), corked, labelled, placed in the culture racks and kept in a growth room (all done under aseptic conditions). The experiment was laid out in a Completely Randomized Design (CRD), with three treatments; Murashige and Skoog basal medium, Gamborg B5 basal medium and White basal medium and each treatment was replicated three times. Agar and water served as the control media. The culture period lasted for four weeks.

Phytochemical constituent determination

The leaves from individual treatments were harvested separately and air dried for the phytochemical studies. Phytochemical constituents of flavonoids, alkaloids, anthraquinones, saponins, steroids, terpenoids, glycosides, tannins and carotenoids were determined using the AOAC (2010) standard method while the phytochemical content of anthocyanin was determined using Harborne (1998) standard method.

Analysis

The data was subjected to Analysis of Variance (ANOVA) using SPSS version 25 and significant differences in means were separated using Duncan's New Multiple Range Test (DNMRT) at $P \leqslant 0.05$.

Results and Discussion

The influence of three basal media (MS, B5 and White basal media) on the accumulation of phytochemicals in leaves of seedlings of *Moringa oleifera* was determined after 28 days. The result of this study showed that flavonoids in leaves of *M. oleifera* from White basal medium are significantly higher ($P \leqslant 0.05$) than those of MS, B5 and Control treatment (Table 1). Flavonoids in leaves of *M. oleifera* from MS and B5 basal media are statistically similar ($P > 0.05$) but differed significantly from that of the Control. Anthocyanin contents in leaves of *M. oleifera* from B5, White basal medium and Control did not differ significantly ($P > 0.05$). The lowest concentration of anthocyanin was obtained in MS medium ($P \leqslant 0.05$). Tannin contents of *M. oleifera* leaves were highest in B5 followed by MS, Control and White basal medium in decreasing order. The tannin contents for the different basal media and the control differed significantly ($P \leqslant 0.05$). Alkaloid contents in the leaves of *M. oleifera* were highest for those from B5 medium and lowest in Control ($P \leqslant 0.05$). Alkaloid contents of B5 medium differed significantly ($P \leqslant 0.05$) from those of other treatments. Gamborg B5 basal medium showed a highly significant ($P \leqslant 0.05$) difference in glycosides content when compared to other basal media (MS, White) and Control. Glycoside contents for MS and White basal media were similar ($P > 0.05$) but differed from that of the Control ($P \leqslant 0.05$).

Results presented in Table 2 showed that anthraquinone content was highest in the control and differed significantly ($P \leqslant 0.05$) from those of MS, B5 and White basal media. Anthraquinone contents in the B5 basal medium were significantly higher ($P \leqslant 0.05$). There was a significant variation in terpenoid, carotenoid and steroid contents among the treatments. Saponin was statistically similar in all the treatments investigated. Terpenoids was highest in the control and lowest in MS medium ($P \leqslant 0.05$). Terpenoids were similar for B5 and White basal media ($P > 0.05$). Carotenoid contents were highest in B5 medium and differed ($P \leqslant 0.05$) from other treatments. Carotenoid content was similar for MS, White and Control. Steroid contents were highest for the Control and lowest for MS and B5 ($P \leqslant 0.05$). Steroid contents were highest for Control and lowest for MS and B5 basal media ($P \leqslant 0.05$). Steroid content for White differed ($P \leqslant 0.05$) from those of Control, MS and B5; MS and B5 recorded similar steroid contents.

The nutrient composition of culture media influenced the secondary metabolite production just as reported by Shilpa *et al*. (2010). Tannins, alkaloids, glycosides, anthraquinones and carotenoids accumulated more in leaves of seedlings obtained from the B5 basal medium while flavonoids and steroids accumulated more in the White basal medium. The level of inorganic nutrients in B5 basal medium are lower than in MS basal medium but higher than the White basal medium. BS basal medium has a higher concentration of nitrate and potassium and lower concentration of ammonia while MS basal medium has a very high concentration of nitrate, potassium and ammonia and White basal medium has a lower salt concentration. It has been reported that nitrate and phosphate concentrations play important roles in secondary metabolite production (Jin-Kwon, 2003; Radušienė *et*. *al*., 2019). The different concentrations of nitrate in the treatments may have contributed to the accumulation of the phytochemicals in the leaves of the seedlings.

Conclusion

The results of this study suggest that B5 medium enhanced the production of phytochemicals in the

leaves of *M. oleifera.* Therefore, with the increase in human population and depletion in human resources, *M. oleifera* can offer good alternative to propagation of plants through conventional methods, in food shortage and production of useful phytochemicals.

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Table I: Mean variation in the flavonoid, anthocyanin, tannin, alkaloid and glycoside contents of the leaves of *Moringa oleifera* seedlings from the different basal media (%)

Treatments	Flavonoids	Anthocyanins	Tannins	Alkaloids	Glycosides
MS	10.00 ± 0.00^{c}	0.10 ± 0.00^{b}	0.030 ± 0.00^{b}	18.50 ± 3.50^{a}	0.07 ± 0.34^{c}
B5	9.75 ± 0.25^{c}	0.24 ± 0.04^{a}	0.038 ± 0.00^{a}	25.00 ± 0.11^{a}	4.75 ± 0.25^{a}
WHITE	22.00 ± 2.00^{a}	0.35 ± 0.05^{a}	0.011 ± 0.00^{d}	13.50 ± 3.50^{b}	0.15 ± 0.05^{c}
CONTROL	16.00 ± 1.00^{b}	0.29 ± 0.01^{a}	0.023 ± 0.00^{c}	10.50 ± 1.50^{b}	2.20 ± 0.20^{b}

Values represent means ± standard error. Mean Values with different alphabet in each column are significantly different from each other by DNMRT (P⩽0.05)

Table 2: Mean variation in the anthraquinone, saponin, terpenoid, carotenoid and steroid contents of the leaves of *Moringa oleifera* seedlings from the different basal media.

Treatments	Anthraquinones	Saponins	Terpenoids	Carotenoids	Steroids
MS	0.20 ± 0.00^{c}	0.67 ± 0.13^{a}	9.65 ± 0.35^{c}	50.87 ± 5.87^{b}	7.50 ± 2.50^{c}
B5	0.33 ± 0.01^{b}	0.72 ± 0.02^{a}	15.00 ± 0.00^{b}	80.00 ± 0.00^{a}	7.50 ± 0.50^{c}
WHITE	0.25 ± 0.00^{c}	0.65 ± 0.00^{a}	16.50 ± 1.50^{b}	56.00 ± 0.00^{b}	13.50 ± 1.50^{b}
CONTROL	0.42 ± 0.02^{a}	0.80 ± 0.20^{a}	26.50 ± 1.50^{a}	46.27 ± 3.72^{b}	20.0 ± 0.00^{a}

Values represent means ± standard error. Mean Values with different alphabet in each column are significantly different from each other by DNMRT ($P \leqslant 0.05$)