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Evaluation of Nutritional Value of *Capsicum annum*(Pepper) in Response to *Moringa oleifera* Leaf Extract, *Cocos nucifera* (Coconut) Water and N-(2-Chloro-4-Pyridyl)-N-Phenylurea (Cppu) Application

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Abstract

So many experiments have been conducted to determine the effect of foliar spray of hormones extracted from natural sources on growth, yield and quality of horticultural plants. These sources are not only organic but are also cost effective, easily adapted, and environmentally friendly. Moringa leaves extract and coconut water contain physiologically-active compounds and mineral elements among which is plant hormone of the cytokinins group. N-(2-chloro-4-pyriyl)-N-phenyl urea (CPPU) is a synthetic cytokinin with high activity in enhancing plant growth. A study was conducted at International Institute of Tropical Agriculture (IITA), Kano station, Nigeria to investigate the effect of Moringa oleifera leaf extract(MLE), Cocos nucifera (coconut) water (CW) and CPPU on the nutritional value of Capsicumannum (pepper). Single treatments comprising 3%v/v, 4%v/v and 5%v/v MLE, 10% v/v, 15%v/v and 20%v/v CW and 10 parts per million (ppm) CPPU were applied as foliar spray in four doses at three-weeks interval, starting from one week after transplanting (1WAT). The experiment was carried out for twelve weeks from transplanting day. Results obtained showed that treatment with 5%MLE resulted in greater vitamin A content. The calcium (Ca), sodium (Na) and manganese (Mn) levels were also higher in fruits of plants treated with 5% MLE. The levels of magnesium (Mg) and iron (Fe) were found to be higher in 10ppm CPPU whereas potassium (K) and zinc (Zn) levels were higher in 10% and 15% CW respectively. The study therefore suggests that single treatment of 5% MLE could increase the nutritive value of pepper.

Keywords: *Capsicum annum*, *Moringa* leaf extract, *Cocos nucifera* water,N-(2-chloro-4-pyridyl)-N-phenyl urea, nutritional value

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Introduction

Capsicum species popularly known as pepper is the world's second most important vegetable crop after tomato (Asawalam *et al.*,2007). It is an herbaceous annual or short-lived perennial that belongs to the family Solanaceae (Raemaekers,2001). Five different species were domesticated; *Capsicum annuum, C.frutescens, C.baccatum, C. chinese* and *C.pubescens* .Among these species, *C.annuum* is the most widely spread and most important. The common red pepper is classified as *Capsicum annum* ("Capsicum",2008). *Capsicum annuum*, however, is used to refer not only to red or bell peppers, but also to wax peppers, cayenne peppers, chilli peppers, and jalapeno peppers and therefore divided into two categories: sweet (ormild) peppers and hot (orchilli) peppers (Cantrill,2008). This study is going to focus on the red (mild) pepper only.

It is an important vegetable spice crop from several perspectives. Peppers are a valued spice throughout the world because they offer an exceptional range of flavors and pungency ranging from home prepared foods to world food and pharmaceutical industries (Margen, 1992; Badia *et al.,* 2017). About 20% of average vegetable consumption in Nigeria accounts for pepper. They are used extensively in food flavoring in the daily diet of Nigerians irrespective of their socio-economic status (Alegbejo,2002). Peppers are excellent sources of many essential nutrients, especially vitamins A and C (Terekhina,2009). They contain high amount of antioxidant and anti-inflammatory phytonutrients which include capsaicinoids, flavonoids, carotenoids, vitamins and minerals (Cantrill, 2008; Badia *et al.,* 2017). One hundred grams (100g) of the edible part of sweet pepper (approximately 87% of the total weight of the fruit) contain 92g water, 1.3g protein, 10.3g carbohydrate (including 1.4g cellulose), 12 mg calcium (Ca), 0.9mg iron(Fe), 10mg magnesium (Mg), 20mg phosphorus(P), 175mg potassium (K), 3mg sodium(Na), 0.1mg zinc(Zn), 0.1mg copper (Cu), 0.1mg manganese (Mn), 1.8 mg carotene, 0.07mg thiamine, 0.08 mg riboflavin, 0.8 mg niacin, 0.2 vitamin B_6, 3.7 µg vitamin A, 7.4 µg vitamin K and 103mg vitamin C. The energy value is 108kJ (or 26kcal)/100g. (Raemaekers, 2001; "CondéNast", 2012).

The response of plant or plant parts to growth regulators varies due to fluctuations in endogenous hormonal level of the plant and the manner in which the natural growth regulators interact with the applied growth regulator (Jain, 2009). Application of cytokinins to intact plants results in so many different effects. They particularly stimulate protein synthesis and participate in cell cycle control (George *et al.*,2008). According to Fuglie (2009), the leaves of horse radish tree (*Moringa oleifera*) seem to contain a plant hormone from the cytokinins group in addition to other growth enhancing compounds like ascorbates, phenolic and minerals like Ca, K and Fe that makes it an excellent crop growth enhancer. Mehboob (2011) reported an increased yield by *M. oleifera* leaf extract priming in maize which was attributed to enhanced seedling emergence, chlorophyll content and cell membrane permeability. *Cocosnucifera* (coconut) water is a rich supplement that naturally contains several physiologically-active substances amongst which have been found the natural cytokininzeatin, and 1,3-diphenyl urea (George *et al.*, 2008). The cytokinins found in coconut water support cell division, and thus promote rapid growth (Jean *et al.*, 2009). CPPU is a substituted diphenyl urea found to have a high cytokinin activity. Karanov *et al.*, (1992) observed that the exogenous application of CPPU resulted in an increase in chlorophyll and protein contents in maize seedlings.

Various substances of organic origin are increasingly being used in their crude form to determine their bio-stimulating effects on plant growth and development. Biostimulants offer a potentially novel approach for the regulation and/or modification of physiological

processes in plants to stimulate growth, to mitigate stress induced limitations, and to increase yield. (Yakhin *et al*., 2017). They also reduce the need for fertilizers since they act as a hormonal and nutritional increment (Russo and Berlyn, 1990; Du Jardin, 2015).

Moringa leaf extract and coconut water are among the organic substances that get recognition by researchers as biostimulants. They are also cost effective, easily adopted and environmentally friendly. So many researches have been done on these substances on their effects on physiological characters and productivity of several plants, yet their influences on the nutritional value of treated plants is inadequate. Although there are reports on the influence of coconut water on some plants, information on the effect of *M.oleifera* leaf extract on the nutritional value of most plants in Nigeria is scarce. This is because most researches put more emphasis on the growth and yield perspective. Therefore, it is of paramount importance to look at the nutritional composition of the plants treated with these substances in order to ascertain their effects on the consumer health. This study was therefore carried out to evaluate the effect of foliar spray of *M.oleifera* leaf extract, coconut water and CPPU on the nutritional value of pepper fruits.

MATERIALS AND METHODS

The experiment was conducted at International Institute of Tropical Agriculture (IITA) Kano Station (latitude 11°30' 00" N, longitude 8°30' 00" E and of 476m altitude).Soil sample for the experiment was collected at the study area. The soil was used to fill 24 plastic pots (i.e. for 8 treatments and 3 replications per treatment) of 24cm diameter and 26 cm depth to three-quarter to allow room for watering. The seedlings were transplanted into the pots at the rate of one plant per pot. The pots were arranged in completely randomized design.

Preparation of extracts and treatments

The *Moringa oleifera* leaf extract was prepared by crushing 10kg of fresh *M.oleifera* leaf in one litre (1L) of water and filtered out as described by Muhamman *et al*.(2009). The liquid extract obtained was diluted with distilled water to arrive at the following concentrations(v/v): 3% (3 ml MLE /97 ml water), 4% (4 ml of MLE/96 ml water) and 5% (5 ml of MLE/95 mlwater).

The coconut water (CW) obtained from the endosperm of the coconut fruit was diluted with water to arrive at the following concentrations (v/v): 10% (10 ml CW/90 ml of water), 15% (15 ml CW/85 ml of water) and 20% (20 ml CW/80ml of water).
There were eight treatments including the control viz.: 3%, 4% and 5% *M.oleifera* leaf extract (MLE), 10%, 15% and 20% coconut water (CW), 10ppm CPPU ($10mgL^{-1}$w/v) and the control. Foliar spray of treatments was applied at the rate of 25ml per plant using the method of Muhamman *et al.* (2009), and was applied four times at three-weeks interval i.e. one week after transplanting (1WAT), 4WAT, 7WAT and 10 WAT. The experiment was carried out for twelve weeks from transplanting day.

Vitamins content determination

The fruits of the treated and the control plants were collected at full ripening, dried in an oven at $80^{O}C$ for 12 hours and finely ground. The powders were used for the vitamins A and C content determination. These were carried out according to the method described by Harold *et al.*(1981). Five cubic metres (5 cm^3) Diethyl ether containing butyl hydroxytoluene (100µg/cm^3) was added to 1 g of the powdered sample, hand-shaken until thorough mixing was achieved, and kept overnight in a refrigerator. The tube was again hand-shaken the following day, and allowed to stand for a few minutes in a refrigerator.

Vitamin A content determination

Vitamin A content was determined by taking an aliquot($0.2cm^3$) of the supernatant, and dried under nitrogen gas at room temperature. The extract was dissolved in $2cm^3$ hexane, dried, and re-dissolved in chloroform. This was reacted with trifluoroacetic (TFA) reagent in equal volume (1:1). The resulting transient blue colour absorbance was measured at 620nm using chloroform as blank. This indicated a nucleophilic attack of the functional group of vitamin A by trifluoroacetic (TFA)acid.

Vitamin C content determination

Vitamin C was determined by pipetting 5ml of the sample solution as indicated above into a boiling tube, and1ml of glacial acetic acid was added followed by 1ml of chloroform. The resulting mixture was titrated with the dye 2,6-dichlorophenolindophenol solution to a permanent pink colour, and this indicated that ascorbic acid (vitamin C) was oxidized by the dye to dehydroascorbic acid. The titration was repeated with 5ml of water for the blank(B) and 5ml ascorbic acid standard solution(S). The concentration was calculated as mg/g:

Mineral elements content determination

The content of phosphorus(P), potassium(K), magnesium(Mg), calcium(Ca), iron(Fe), zinc(Zn), manganese(Mn), copper (Cu) and sodium(Na) were determined from the pepper fruits. Dry ashing method as described by Udo *et al* (2009) was adopted as the digestion procedure. The contents of K, Mg, Ca, Fe, Zn, Cu, Mn and Na were determined by atomic absorption spectroscopy. The P content was analysed by vanado-molybdate (yellow) method all as described by Udo *et al.* (2009).

Dry ashing procedure

The fruits were dried at approximately 80^oC for 12 hours and finely ground. One gram (1g) of the ground sample was placed into a porcelain crucible ashed in a muffle furnace at 475-500^oC for 2 - 4 hours. The ash was cooled and dissolved in 5ml of 20% (2M) HCl, warming it to effect complete solution of the residue. The solution was filtered through an acid washed filter paper into a 50ml volumetric flask.

Vanado-molybdate (yellow) method of determination of phosphorus

Five millilitre (5ml) of sample solution (from dry ashing) was pipetted into a 50ml volumetric flask and 30ml distilled water added. Within 5 minutes, 10ml of vanado-molybdate reagent was added and diluted to volume. It was mixed and allowed to stand for 10 minutes. Phosphorus was determined from a standard curve made from the standards. For the curve, 0, 2, 4, 6, and 10ml aliquots of the 25µmP/ml standard solution was taken in a series of 50ml volumetric flasks and developed the colour.

Experimental design and statistical analysis

A completely randomized experimental design was used. The data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's Multiple Range Test (DMRT) using SAS Version 9.3 at $P \leq 0.05$.

Results

Effect of treatments on vitamins content

The vitamin A content of the pepper fruits (Table1) was found to be highest in treatment with 5% MLE (69.00µg/L). This was followed by 3% MLE, 4%MLE and 10% CW (44.75,41.99 and 41.26µg/L respectively). Of all the treatments and the control, 20% CW had the least content of vitamin A (12.69 µg/L).

Vitamin C (mg/L) content of the fruits was statistically insignificant(Table1). The control recorded the highest content of vitamin C (126.60 mg/L) while treatment with 4% MLE had the least vitamin C content (81.24 mg/L).

Table1: Effect of *M.oleifera* leaf extract ,coconut water and CPPU on vitamins A and C content of *Capsicum annum*

	Vitamin	
Treatment	**Vitamin A (µg/L)**	**VitaminC(mg/L)**
3%MLE	44.75ab	108.03
4%MLE	41.99ab	81.24
5%MLE	69.00a	101.25
10%CW	41.26ab	93.33
15%CW	24.30b	114.58
20%CW	12.69b	122.20
10ppmCPPU	21.86b	116.99
Control	39.18ab	126.60
S.E±	10.91	13.97

Means followed with the same letter(s) in each column are not significantly different using DMRT at ≤ 0.05

Effect of treatments on mineral elements content

The results of the effect of the treatments on some mineral elements of the pepper fruit are presented in Table 2. Statistically, significant effect ($p \leq 0.05$) was observed in all the mineral elements except copper (Cu). Treatment with 5% MLE (237.5mg/kg) ranked highest for calcium(Ca) followed by 10% CW which was at par with 15% CW (175mg/kg) while 3% MLE had the lowest content (108.33mg/kg). Treatment with 10% CW had the highest content of potassium(K) followed by 5% MLE and 3% MLE (737.5,666.67 and 570.83 mg/kg respectively), and 20% CW had the lowest content (425 mg/kg). For magnesium (Mg), treatment with 10ppm CPPU resulted in greater content (183.33mg/kg), followed by 15% CW (150mg/kg) and 5% MLE which was at par with the control (133.33mg/kg). Treatment with 5% MLE was found to have the highest content of sodium (Na) followed by 10% CW and 15% CW (591.4,569.89 and 526.88 mg/kg respectively), whereas 4% MLE had the lowest content (451.61mg/kg). Phosphorus (P) content was, however, found to be highest in the control (387.88mg/kg) followed by treatments with 15%CW and 10ppm CPPU (385.60 and 287.88 mg/kg respectively) while 3% MLE had the lowest content (87.12mg/kg).

Although not statistically significant, the highest content of copper(Cu) was recorded in plants treated with 3%MLE (22.55mg/kg) followed by 10% and 15% CW (20.59 and 19.61 mg/kg respectively) while the control and 5%MLE recorded the lowest (13.73mg/kg). Iron(Fe) content of plants treated with 10ppm CPPU and that of the control (12.50mg/kg) was found to be the highest followed by 20% CW (11.80mg/kg) and 15% CW had the lowest content (4.86mg/kg). Manganese(Mn) significantly increased in all the treatments as compared to the control. Plants treated with 5% MLE had the highest content of Mn, followed by 4% and 3% MLE (27.33, 23.33 and 18.67 mg/kg respectively). Treatment with 15% CW produced the highest content of zinc (Zn) followed by 5% and 3% MLE (12.96,

11.11 and 9.72 mg/kg respectively), and 10 ppm CPPU had the lowest content (7.41mg/kg).

Table 2: Effect of *M. oleifera* leaf extract, coconut water and 10ppm CPPU on some mineral elements content of *Capsicum annum*

Treatment	Mineral element (mg/kg)								
	C	K	M	N	P	C	F	M	Z
3%MLE	108.33b	570.83abc	116.67ab	500.00ab	87.12c	22.55	8.33abc	18.67bc	9.72ab
4%MLE	166.67ab	475.00c	100.00ab	451.61b	130.30c	17.65	5.56c	23.33ab	7.87b
5%MLE	237.50a	666.67ab	133.33ab	591.40a	275.76ab	13.73	6.94bc	27.33a	11.11ab
10%CW	175.00ab	737.50a	83.33b	569.89ab	166.67bc	20.59	6.94bc	15.33bc	8.33b
15%CW	175.00ab	529.17bc	150.00ab	526.88ab	385.60a	19.61	4.86c	13.33c	12.96a
20%CW	141.67b	425.00c	100.00ab	510.75ab	262.12b	15.69	11.8ab	12.00c	9.26ab
10ppm CPPU	116.67b	466.67c	183.33a	467.74ab	287.88ab	17.65	12.5a	14.00c	7.41b
Control	158.33ab	554.17bc	133.33ab	516.13ab	387.88a	13.73	12.5a	12.00c	7.87b
S.E	26.57	54.23	26.35	40.29	37.47	2.70	1.64	2.59	1.30

Means followed with the same letter(s) in each column are not significantly different using DMRT at P ≤0.05

Figure 1: An overview of the experimental plants at 10 WAT

Figure 2: Experimental plants showing 10%CW, 4%MLE, 20%CW and 15%CW respectively with arrows showing fruits at 10 WAT

Figure 3: Experimental plants showing control, 5%MLE, CPPU and 3%MLE respectively with arrows showing fruits at 10 WAT

Discussion

Peppers are important vegetable commodity highly prized for the flavour, colour, vitamin C and pungency they provide to the human diet (Hartmann *et al*., 1988). The members of the *Capsicum* genus are used as a raw material in the pharmaceutical industry due to their high antioxidant content and excellent source of flavonoids, carotenoids, vitamins A, C and E, (Badia *et al*., 2017). Vitamin A plays an important role in cell growth, vision, and the immune system (Vitamin, 2008). In the present study, vitamin A content of the pepper fruits showed significant effect where treatment with 5%MLE had the highest content of the vitamin, while 20%CW had the lowest content. Thanaa *et al.* (2017) reported that foliar application of 6% *Moringa* leaf extract can improve fruit quality of Hollywood plum. Use of *Moringa* leaf extract as a priming agent, and foliar spray in mitigating the salinity effect in wheat produced higher contents of total phenolic and ascorbic acid compared to the control (Azra *et al*., 2011). Abdel-Rahaman and Merwad (2017) reported a 45% increase in protein content in pea seeds after a foliar spray of 4% MLE on the plant. A foliar spray of 15% coconutwater resulted a greater increase in carbohydrate and vitamins A, B_6 and C contents of *Hibiscussabdariffa* (redsorrel) after a foliar spray of 15% coconutwater (Mukhtar, 2008).

Vitamin C (ascorbic acid) has antioxidant properties in biological systems and restricts degenerative processes (Davey *et al*., 2000) and also helps the body absorb iron from plant foods (Vitamin, 2008).

The vitamin C levels in Capsicum fruits is affected by several factors: variety, production system, harvest ripening and storing conditions (Badia *et al*., 2017). In the present experiment, the effect of the treatments on vitamin C content of the pepper fruits did not significantly ($p \leq 0.05$) differ from that of the control. This is contrary to the reports of Thanaa *et al.* (2017) and Mukhtar (2008) that there was increase in the vitamin C content of Hollywood plum and redsorrel after a foliar spray of MLE and coconut water respectively. But it was also reported that CPPU treatment increased the flesh firmness of cucumber at harvest, but decreased phenolic acid and vitamin C content after storage (Qian *et al*.,2018).

Minerals are vital for the healthy growth of teeth and bones. They also help in such cellular activity as enzyme action, muscle contraction, nerve reaction, and blood clotting (Worthington-Roberts, 2008). In this study, the mineral elements content of the pepper fruits significantly increased in all the treatments when compared with the control, except phosphorus and iron which were found to be highest in the fruits of the control plants. While there was no significant difference in the copper content. This is in conformity with the work of Foidl *et al* .(2001) where he reported higher percentage of sugars and minerals in sugar cane and cantaloupe after treating the plants with *Moringa* leaf extract as a foliar spray. Additionally, the use of *Moringa* leaf extract in priming and foliar spray induced higher leaf total soluble protein and antioxidants in wheat (Azra *et al*.,2011). Mukhtar (2008) investigated the effects of growth regulator treatments on the growth, chlorophyll and biochemical contents of *Hibiscuss abdariffa*. Of all the treatments, 100ppm GA_3 and 15% coconut water treatments gave the greatest increase in height, chlorophyll, biochemical and some mineral elements. A spray made of a combination of CPPU, 5-aminolevulinic acid (5-ALA), sodium sulfate (Na_2SO_4) and GA_3 increased whole nitrogen and whole phosphorus contents in fruit flesh of Jun jujubes (Chen *et al*., 2015).

Conclusion

5% MLE resulted in greater vitamin A content. The calcium (Ca), sodium (Na) and manganese (Mn) levels were also higher in fruits of plants treated with 5% MLE. The levels of magnesium (Mg) and iron (Fe) were found to be higher in 10ppm CPPU whereas potassium (K) and zinc (Zn) levels were higher in 10% and 15% CW respectively. The study therefore suggests that single treatment of 5% MLE could increase the nutritive value of pepper. However, it is recommended that further study, especially field trial should be conducted in order to ascertain the findings of this study.

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