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Evaluating the Effect of Organic Manure and Agricultural Lime on Chlorophyll and Oxidative Stress Enzymes of Dry Bean (*Phaseolus vulgaris*) and Moth Bean (*Vigna aconitifolia*)

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Background: Chlorophyll content in green plant is an important pigment that supports food manufacturing during the process of photosynthesis and enzymes are antioxidant system in plants that fight against oxidative stress generated by reactive oxygen species (ROS).
Aim: This study was designed to evaluate the effect of organic manure and agricultural lime on the chlorophyll and oxidative stress enzymes of two bean varieties.
Methodology: The three locations were: Akamkpa with pH 4.0, Calabar Municipality with pH 7.0 and Odukpani with pH of 9.0. The treatments were; control (0 g), OM_1 (100 g organic manure), OM_2 (200 g organic manure), AL_1 (100 g agricultural lime), AL_2 (200 g agricultural lime), $OM_1 + AL_1$ (50 g organic manure + 50 g agricultural lime) and $OM_2 + AL_2$ (100 g organic manure and 100 g agricultural lime.
Results: Chlorophyll a was highest in AL_2 (154.21 µg/gFW) of *P. vulgaris.* Chlorophyll b was highest in $OM_1 + AL_1$ (176.00 µg/gFW) grown in Akamkpa soil ($P<0.05$). In Akamkpa soil, $OM_1 + AL_1$ and OM_2 of *V. aconitifolia* had higher chl. b and total chl. (a + b) content than the control with mean

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values of 152.33 µg/gFW for OM_1 + AL_1 and 298.67 µg/gFW for OM_2. In Akamkpa soil, peroxidase activity was high in OM_1 (888.62 µmol/product/L/min) of *P. vulgaris* which was statistically higher than the control with mean value of (535.36 µmol/product/L/min). POD activity in *V. aconitifolia,* grown on Akamkpa soil treated with OM_1, OM_1 + AL_1 and OM_2 had significantly ($P<0.05$) higher mean values of (717.64, 708.39 and 694.27 when compared with the untreated soil (566.14^{d}±17.53 µmol/product/L/min). *P. vulgaris* grown on Odukpani treated with OM_1 + AL_1 and OM_2 + AL_2 had the highest PPO activity of 0.77 µmol/product/L/min. PPO activity of *V. aconitifolia* grown on Odukpani treated with of OM_1 + AL_1 revealed highest activity (0.78 µmol/product/L/min) compared to control (0.39 µmol/product/L/min).

Conclusion: From the findings of this study, organic manure and agricultural lime are two fertilizer sources recommended for incorporation into agronomic practices for enhanced plant performance.

Keywords: Organic manure; agricultural lime; chlorophyll; peroxidase and polyphenoloxidase.

1. INTRODUCTION

Phaseolus vulgaris (common bean) is an annual climber or sub erect pubescent with broad ovate leaflets, petiole 1.5-2.5 mm long, stipules 4 mm long. It grows well on soils of pH ranging from 4-9 [1]. It does better on well-drained, sandy loam, silt loam or clay loam which are rich in inorganic content. The leaf is occasionally used as a vegetable. It is a major food grain legume consumed worldwide for its edible grains and pod. It is a crop of commercial importance cultivated mainly for food and cash. Additionally, farmers grow the bean to be used as forage for livestock and mulching. Common bean is grown and consumed principally in developing countries with high prevalent poverty rate [2]. Consequently, efforts at improving its yield have increased over the years [3,4]. In some parts of Africa, it serves as the most important food grain legume, but is grown mostly by resource-poor farmers without fertilizer inputs [5] resulting in poor yield.

Similarly, *Vigna aconitifolia* commonly called mat bean, moth bean, matki, Turkish gram or dew bean is a herbaceous creeping annual crop that creates a low-lying soil cover when fully grown [6]. It is one of the most drought resistant pulses in Nigeria. Grown at altitudes up to 1300 m above sea level, it has a wide pH range (3.5-10) and can tolerate slight salinity. While dry sandy soil is most suitable for production, moth bean can tolerate a variety of soil types [6]. However, soil with balanced nutrient content will promote its performance resulting in high yield. Thus, for maximum performance of these two important bean varieties, it is sacrosanct that the best management practices are adopted by farmers.

Increase in human population often drives up demand for food and new agricultural strategies are required to reduce the environmental costs of agricultural production. Acidic and alkaline soils stimulate abnormalities in metabolism of plants with photosynthesis, nitrogen and sulphur being the most affected. These effects reduce productivity and give rise to food scarcity, poverty and environmental degradation [7]. Many techniques have been developed to ameliorate the effects of soil acidity and alkalinity such as the use of inorganic fertilizer and urea, but this has recorded limited success coupled with further degradation of the environment and impacts on food chains and human health [7]. The use of organic manure has been recommended as ecologically friendly, enhances ecosystem nutrient cycles which are of great benefit to the environment. This in addition is also beneficial to agriculture as it facilitates nutrients availability and uptake by plants. Economically, the huge cost associated with inorganic fertilizer is drastically reduced through the use of organic manure and agricultural lime. This study, therefore seeks to investigate the potential of organic manure and agricultural lime in the amendment of acidic and alkaline soils for optimal production of *Phaseolus vulgaris* and *Vigna aconitifolia.*

2. MATERIALS AND METHODS

2.1 Study Location

The experimental site for this study was at the Greenhouse, Department of Plant and Ecological Studies, University of Calabar with an average temperature of 25±3ºC. Calabar is located between latitudes 4º78' and 5º09' N and longitudes 8º15' and 8º26' E and lies between the valleys of two rivers: The Great Qua River on the Eastern side and the Calabar River on the West. The total annual rainfall for the area is between 2109.5 mm and 4168.7 mm.

2.2 Seeds Collection and Planting Materials

Seeds of *P. vulgaris* and *V. aconitifolia* were obtained from Institute of Agricultural Research and Training (IAR and T) Moor Plantation in Ibadan, Nigeria. Polythene bags (planting bags) were obtained from Ministry of Agriculture, Calabar. Agricultural lime was obtained from Cross River Agricultural Development Project while organic manure (combination of poultry droppings, sawdust and cow dung) was obtained from the Department of Soil Science, Faculty of Agriculture, University of Calabar, Calabar.

2.3 Soil Sampling, Collection and Preparation

Soil samples (0-20 cm) depth were collected from three Local Government Areas of Southern Cross River State. Soils with pH 4.0 were collected from three villages in Akamkpa (Old Netim, Ayaebam and Awi), soils with pH 7.0 were collected from Calabar Municipality in designated locations (Forestry and Wild-Life Plantation, University of Calabar, Esuk Atu Community, Lemna dumpsite – Itung Effanga) and soils with pH 9.0 were collected from three villages in Odukpani Local Government Area (Akpan 18 Community, Akim-Akim and Okoyong-Usang Abasi) using an auger. Soils from the same Local Government area with same pH were properly mixed to give a composite soil sample. The map of Southern Cross River showing the geographical locations of the soil samples is shown in Fig. 1. Soil for germination and growth of plants were bulked air dried for three days, sieved through a 2 mm mesh to remove debris and were taken to Soil Science Laboratory, University of Calabar for the physico-chemical analysis.

2.4 Experimental Design and Layout

The experiment was conducted using a 2x3x7 factorial experimental layout in a Randomized Complete Block Design (RCBD) with 3 replicates. Factor one were the two plant varieties (*P. vulgaris* and *V. aconitifotia*), factor two were the three locations where soil samples were collected (Akamkpa-AK, Calabar Municipality-CM and Odukpani-OD) while factor three were the seven levels of treatment: control (0 g), OM_1 (100 g organic manure), OM_2 (200 g organic manure), AL_1 (100 g agricultural lime), AL_2 (200 g agricultural lime), OM1 + AL^1 (50 g organic manure + 50 g agricultural lime) and OM_2 +AL_2 (100 g organic manure and 100 g agricultural lime).

2.5 Planting Procedure and Treatment Application

One hundred and twenty-six experimental polybags (16 cm internal diameter) perforated at the bottom were filled with 5 kg of each soil sample. These were divided into three groups of 42 polybags based on the three soil samples. In each soil sample, there were 21 polybags each for the two plant varieties using randomized complete block design (RCBD) replicated three times. The soils were treated with agricultural lime (AL) and organic manure (OM) singly and in combinations. The treated soils were allowed to stay for two weeks before seed sowing. This time lapse before planting was to allow for microbial activities and interaction within treatment combinations. Each polybag was sown with three seeds each of *P. vulgaris* and *V. aconitifotia* at a depth of 2 cm. Following germination, seedlings stalk were watered and grown for 8 weeks.

2.6 Determination of Chlorophyll Content

The chlorophyll content of leaves of *P. vulgaris* and *V. aconitifolia* grown on acidic and alkaline soil treated with organic manure and agricultural lime was determined by the method of Strickland and Parsons, [8]. Chlorophyll content of leaves was extracted by homogenizing the 0.5 g of leaves sampled from the different experimental plots in 20 ml of 80% acetone using laboratory mortar and pestle. The homogenate was decanted into test tubes and centrifuged at 4000 rpm for 3 minutes. The supernatant was decanted after centrifugation and used for the determination of chlorophyll content of leaves using a cuvette. 2 ml of acetone was used to set the blank. This was then used to set the spectrophotometer to 1005 transmittance at 663 nm for chlorophyll “a” and 643 nm for chlorophyll “b”. The blank was eventually removed and to 3 ml of 80% acetone was added 0.1 ml of chlorophyll extract. The cuvette containing the chlorophyll extract was placed in the sample holder and absorbance readings taken. Chlorophyll content was estimated using the formula:

Chl a = (11.6 A663-1.3 A643) VX-1
Chl b = (19.6 A643-4.7 A663) VX-1
Chl a + b = (mg g-1 FW)

Where: A663 and A643 are absorbance at 663 and 643 nm.

A = absorbance,
V-1= volume of 80% acetone,
X-1 = sample fresh weight, mg = milligram and FW = fresh weight.

2.7 Assessment of the Impact of Two Oxidative Stress Enzymes

2.7.1 Enzyme extraction

Leaf tissues of 0.5 g fresh weight of *P. vulgaris* and *V. aconitifolia* grown on acid and alkaline soils treated with organic manure and agricultural lime were harvested randomly from experimental plots at 4 weeks after germination and homogenized using laboratory mortar and pestle in 5 ml of extraction buffer. The extraction buffer comprised of 100 mM mixed monobasic potassium phosphate salt (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4). To this was added 2 g of phenolic adsorbent polyvinyl polypyrrolidone (PVPP). The buffer was well stirred with the aid of a magnetic stirrer and adjusted to final pH of 7.0. The extraction was carried out at 4ºC. The homogenate filtered through cheese cloth and the filtrate centrifuged at 4000 rpm for 4 minutes. The supernatant was stored in boxes sustained in ice block and used as crude enzyme source in assaying for peroxidase (POD) and polyphenoloxidase (PPO) [9].

2.7.2 Purification of enzyme

To 0.5 ml of crude enzyme extract was added 2 ml of cold acetone. The mixture was allowed to stand for four minutes and then centrifuged at 4000 rpm for 4 minutes. The precipitate formed was re-suspended in 1 ml cold assay buffer (KH_2PO_4 and K_2HPO_4) as partially purified enzyme filtrate. Four milliliters of this partially purified enzyme filtrate was used to commence the purification process. To this 4 ml was added 0.8 g ammonium sulphate salt $(NH_4)_2SO_4$ and stirred with the aid of a magnetic stirrer for 10 minutes and the content poured into a test tube and centrifuged. After centrifugation the filtrate was decanted and the precipitate discarded. To the filtrate was added 2 g of ammonium sulphate $(NH_4)_2SO_4$ and stirred for 10 minutes. The

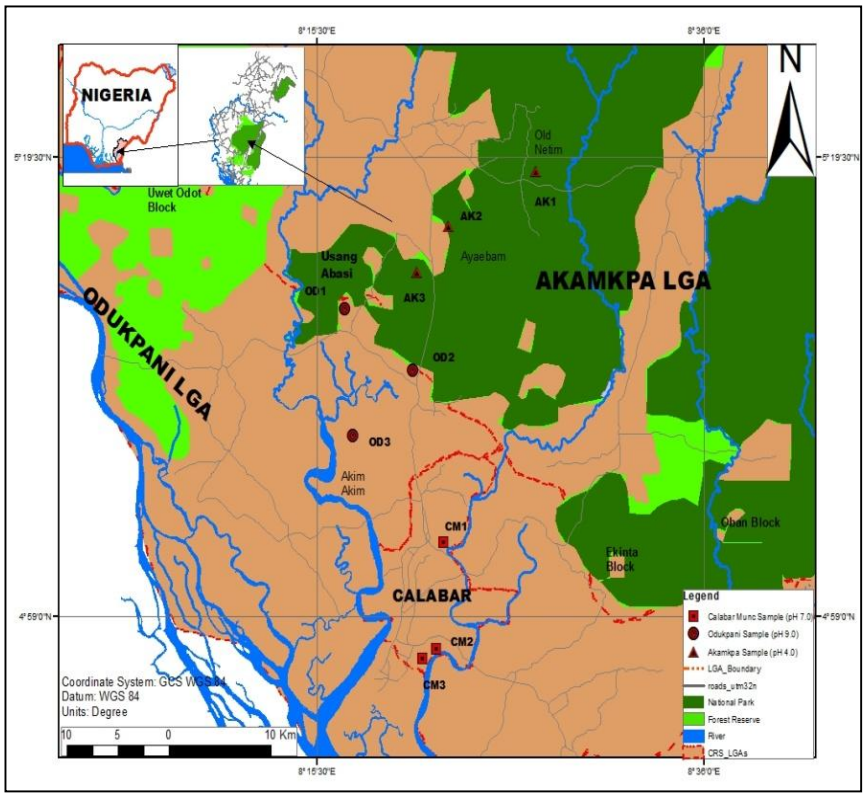

Fig. 1. Map of Southern cross river showing geographical locations of soil samples

content was again poured into the test tube and centrifuged at 4000 rpm for another 4 minutes. The filtrate was decanted and the precipitate used for purification. The assay buffer 0.5 ml was then added and used in assaying POD and PPO [9].

2.7.3 Peroxidase (POD) assay

The assay buffer used to measure peroxidase activity consisted of 100 mM of mixed phosphate buffer containing 0.7 g of KH_2PO_4, 0.9 g of K_2HPO_4, pH 7.2. The assay mixture had in a final volume of 2.8 ml, made up of 2 ml of assay buffer, 0.1 ml of 10 ml guaiacol and 0.6 ml enzyme preparation. The reaction was initiated with the addition of 0.1 ml of 10 ml H_2O_2. Absorbance readings were taken after 1 minute at 436 nm spectrophotometrically with the assay buffer as blank. The POD activity expressed was calculated using an extinction coefficient of 6.39 $mol^{-1}cm^{-1}$ for guaiacol dehydrogenation product [10].

2.7.4 Polyphenoloxidase (PPO) assay

To 1.6 ml of assay buffer (100 mM mixed phosphate salts, pH 7.0) in a cuvette was added 0.6 ml of 10 mM dihydroxyphenylalanine (DOPA) and 0.1 ml enzyme preparation. The reaction was started with the addition of 0.1 ml of 10 mM H_2O_2 in a final volume of 2.8 ml. The absorbance of the mixture was measured after 30 minutes at a wavelength of 470 nm [11]. The activity of PPO was calculated using an extinction coefficient of 2.9968 $nMcm^{-1}$ for the quinone product [12].

2.8 Statistical Analysis

Data obtained on the chlorophyll and oxidative stress enzymes of the two bean varieties were taken as the mean measurements of three replicates. Statistical analysis was performed using the statistical package for social sciences (SPSS) version 20.0 Significant means were separated using the least significant test at $p<0.05$.

3. RESULTS

3.1 Effect of Agricultural Lime and Organic Manure on Chlorophyll Content

Table 1 shows the results obtained on the chlorophyll contents of *P. vulgaris* and *V. aconitifolia* grown on soils from three locations. In Akamkpa soil, there were significant differences ($P<0.05$) in the amount of chlorophyll a and b contents in both *P. vulgaris* and *V. aconitifolia.* Chlorophyll a was highest in AL_2 (154.21 µg/gFW) of *P. vulgaris.* Chlorophyll b was highest in $OM_1 + AL_1$ (176.00 µg/gFW) grown in Akamkpa soil ($P<0.05$) compared to the control values of (141.76 µg/gFW and 73.67 µg/gFW), respectively. The highest total chlorophyll content (a + b) was obtained from *P. vulgaris* ($P<0.05$) treated with $OM_1 + AL_1$ (320.33 µg/gFW) compared with the untreated soil with mean value of (2.15.67 µg/gFW) of *P. vulgaris*. While in Calabar Municipality, chlorophyll a, b and a + b of *P. vulgaris* grown on soil treated with AL_1 and AL_2 had lower chlorophyll contents than the untreated soils. OM_l, $OM_1 + AL_1$, OM_2, $OM_2 + AL_2$ had significantly ($P<0.05$) higher chlorophyll contents in *P. vulgaris* grown on treated soils. Chlorophyll a, b and total chlorophyll (a + b) contents of *P. vulgaris* grown on untreated alkaline soil from Odukpani were significantly ($P<0.05$) lower than chlorophyll contents of *P. vulgaris* grown on all treated soils. Chlorophyll a, b and a + b for treated soils OM_l had the lowest mean values of 94.73, 26.33 and 121.00±1.73 µg/gFW compared to untreated soils values of 104.46, 109.67 and 214.33 µg/gFW, respectively. Chlorophyll b and total chlorophyll a + b content of *V. aconitifolia* grown on Akamkpa soil treated with AL_1, AL_2, OM_1, and $OM_2 + AL_2$ were significantly lower than the untreated control while $OM_1 + AL_1$ and OM_2 had higher chl. b and total chl. (a + b) content than the control with mean values of 152.33 µg/gFW for $OM_1 + AL_1$ and 298.67 µg/gFW for OM_2.

3.2 Effect of Agricultural Lime and Organic Manure on Peroxidase Activity

Table 2 shows the result of the effect of agricultural lime and organic manure on peroxidase activity of *P. vulgaris* and *V. aconitifolia* grown on soil from three locations. In Akamkpa soil, peroxidase activity was high in OM_1 (888.62 µmol/product/L/min) of *P. vulgaris* which was statistically higher than the control with mean value of 535.36 µmol/product/L/min. POD activity in *V. aconitifolia,* grown on Akamkpa soil treated with OM_1, $OM_1 + AL_1$ and OM_2 had significantly ($P<0.05$) higher mean values of 717.64, 708.39 and 694.27 when compared with the untreated soil (566.14^{d}±17.53 µmol/product/L/min). Also, in Calabar Municipality and Odukpani soils, there was significant different ($P<0.05$) in POD activity of

Table 1. Effect of agricultural lime and organic manure on the chlorophyll content (µg/gFW) of *Phaseolus vulgaris* and *Vigna aconitifolia*

		Locations								
		AK			CM			OD		
Plant species	**Treatment**	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**
P. vulgaris	Control	141.76^{b}±0.71	73.67^{h}±0.58	215.43^{e}±0.38	125.34^{b}±0.38	72.00^{g}±0.00^{g}	197.00^{e}±0.00	104.46^{c}±0.34	109.67^{d}±1.53	214.33^{ab}±0.52
	AL_1	146.38^{b}±0.10	105.00^{h}±0.00	251.33^{e}±0.28	103.00^{c}±0.00	41.00^{i}±0.00	144.10^{g}±0.00	143.90^{a}±0.42	158.00^{b}±0.00	301.67^{a}±0.58
	AL_2	154.21^{a}±14.38	139.67^{c}±4.93	294.00^{b}±9.53	105.00^{c}±0.00	41.67^{i}±0.58	146.33^{g}±0.58	143.77^{a}±0.35	95.67^{f}±0.57	239.33^{a}±0.58
	OM_1	145.10^{b}±0.33	112.33^{f}±0.58	257.00^{d}±1.00	129.67^{a}±0.58	97.33^{d}±0.00	227.67^{c}±0.58	94.73^{c}±0.15	26.33^{h}±1.33	121.00^{d}±1.73
	OM_1+ AL_1	144.53^{b}±0.41	176.00^{a}±0.00	320.33^{a}±0.58	129.67^{a}±0.58	140.67^{ab}±0.58	270.67^{a}±0.58	117.67^{b}±0.58	45.00^{g}±0.00	162.67^{c}±0.58
	OM_2	144.64^{b}±1.06	84.67^{g}±0.58	229.33^{g}± 0.58	130.00^{a}±0.80	90.00^{de}±0.00	220.00^{cd}±0.00	125.56^{b}±0.63	48.00^{g}±1.00	173.67^{c}±1.53
	OM_2 + AL_2	146.78^{b}±0.17	148.67^{b}±0.58	295.67^{b}±0.58	130.67^{a}±0.58	144.00^{a}±0.00	244.33^{b}±0.58	141.00^{a}±0.00	79.33^{c}±0.58	220.00^{b}±0.00
V. aconitifolia	Control	146.33^{b}±0.58	129.00^{d}±1.73	275.33^{c}±2.08	131.00^{a}±0.00	136.00^{b}±0.00	257.00^{b}±0.00	114.33^{c}±0.58	119.33^{b}±1.53	234.00^{ab}±1.00
	AL_1	106.67^{c}±0.58	34.67^{j}±4.61	141.33^{i}±4.04	117.67^{b}±0.58	54.00^{h}±1.00	171.00^{f}±1.00	146.00^{a}±1.00	104.00^{e}±0.00	250.00^{g}±1.00
	AL_2	146.00^{b}±1.00	115.33^{e}±0.58	261.33^{d}±1.15	105.00^{c}±0.00	42.00^{i}±0.00	146.33^{g}±0.58	143.67^{a}±0.58	174.00^{a}±1.00	317.67^{a}±0.58
	OM_1	145.33^{b}±0.58	89.00^{g}±0.00	234.33^{g}±0.58	129.67^{a}±0.58	84.00^{ef}±0.00	213.67^{d}±0.00	146.33^{a}±0.58	164.00^{b}±1.00	310.67^{a}±0.58
	OM_1+ AL_1	146.33^{b}±0.58	152.33^{b}±0.58	298.67^{b}±1.53	128.67^{a}±0.58	86.33^{f}±0.58	215.00^{d}±0.00	143.67^{a}±0.58	104.00^{e}±0.00	247.00^{a}±0.00
	OM_2	147.33^{b}±0.58	148.67^{b}±0.58	295.67^{b}±0.58	130.00^{a}±0.00	90.00^{de}±0.00	220.00^{cd}±0.00	145.67^{a}±0.58	141.67^{b}±0.58	287.00^{a}±0.00
	OM_2 + AL_2	146.00^{b}±0.00	92.67^{g}±0.58	238.67^{f}±0.58	130.67^{a}±0.58	114.00^{c}±0.00	244.33^{b}±0.58	143.67^{a}±0.58	114.33^{d}±9.24	258.00^{a}±8.67

Mean values with different superscripts along the same vertical axis are significantly different from each other (p<0.05). WAP – Weeks after planting, Control - 0 g, AL_1 -Agricultural lime , OM_1 -Organic manure, AL_2 -Agricultural lime, OM_1 - Organic manure, OM_1 + AL_1 – 50% organic manure + 50% Agricultural Lime, OM_2 - Organic manure, OM_2 + AL_2 – 100% organic manure + 100% Agricultural Lime. AK: Akamkpa, CM: Calabar Municipality, OD: Odukpani

Table 2. Effect of agricultural lime and organic manure on peroxidase activity (µmol/product/L/min) of *Phaseolus vulgaris* and *Vigna aconitifolia*

Plant species	Treatment	Locations		
		AK	CM	OD
Phaseolus vulgaris	Control	535.36^{d}±9.58	545.39^{f}±5.53	648.51^{d}±12.48
	AL_1	552.11^{d}±8.89	567.68^{f}±3.04	541.39^{f}±2.23
	AL_2	616.86^{c}±3.35	629.52^{e}±5.26	702.55^{c}±2.92
	OM_1	888.62^{a}±5.06	692.81^{c}±3.68	539.45^{f}±3.67
	OM_1+ AL_1	447.92^{e}±8.04	785.80^{a}±4.38	598.36^{f}±6.59
	OM_2	630.98^{c}±5.26	720.08^{bc}±1.46	899.23^{a}±3.04
	OM_2 + AL_2	403.61^{f}±3.04	629.03^{e}±3.67	517.57^{f}±4.74
Vigna aconitifolia	Control	566.14^{d}±17.53	581.32^{f}±1.46	687.95^{c}±73.93
	AL_1	521.43^{d}±9.58	669.44^{de}±5.13	644.61^{d}±6.59
	AL_2	539.45^{d}±5.53	629.53^{e}±5.26	746.89^{b}±115.51
	OM_1	717.64^{b}±4.70	655.32^{e}±4.46	737.60^{b}±12.98
	OM_1+ AL_1	708.39^{b}±9.12	702.07^{c}±6.59	759.03^{b}±10.36
	OM_2	694.27^{b}±10.57	807.71^{a}±2.92	649.97^{d}±2.92
	OM_2 + AL_2	660.19^{c}±1.46	629.03^{e}±3.67	864.68^{a}±3.87

Mean values with different superscripts along the same vertical axis are significantly different from each other (p<0.05).Control - 0g, AL1 -Agricultural lime , OM1 -Organic manure, AL2 -Agricultural lime, OM1 - Organic manure, OM1 + AL1 – 50 g organic manure + 50 g Agricultural Lime, OM2 - Organic manure, OM2 + AL2 – 100 g organic manure + 100 g Agricultural Lime. AK: Akamkpa, CM: Calabar Municipality, OD: Odukpani

Table 3. Effect of agricultural lime and organic manure on Polyphenoloxidase (PPO) activity (µmol/product/L/min) of *Phaseolus vulgaris* and *Vigna aconitifolia*

Plant species	Treatment	Locations		
		AK	CM	OD
Phaseolus vulgaris	Control	0.54^{d}±0.006	0.64^{c}±0.006	0.61^{d}±0.000
	AL_1	0.67^{c}±0.000	0.64^{c}±0.001	0.62^{d}±0.001
	AL_2	0.71^{b}±0.006	0.76^{a}±0.000	0.73^{b}±0.001
	OM_1	0.70^{b}±0.006	0.73^{a}±0.003	0.71^{b}±0.000
	OM_1+ AL_1	0.74^{a}±0.000	0.70^{b}±0.002	0.77^{a}±0.000
	OM_2	0.43^{f}±0.000	0.66^{c}±0.002	0.69^{bc}±0.001
	OM_2 + AL_2	0.49^{e}±0.006	0.71^{b}±0.003	0.77^{a}±0.000
Vigna aconitifolia	Control	0.47^{e}±0.000	0.70^{b}±0.001	0.39^{f}±0.001
	AL_1	0.72^{a}±0.000	0.74^{a}± 0.001	0.70^{b}±0.000
	AL_2	0.46^{f}±0.000	0.76^{a}± 0.000	0.52^{e}±0.001
	OM_1	0.67^{c}±0.000	0.74^{a}± 0.001	0.73^{b}±0.001
	OM_1+ AL_1	0.35^{g}±0.001	0.65^{c}±0.002	0.79^{a}±0.000
	OM_2	0.73^{a}±0.006	0.71^{b}±0.001	0.68^{c}±0.000
	OM_2 + AL_2	0.67^{c}±0.000	0.71^{b}±0.003	0.78^{a}±0.006

Mean values with different superscripts along the same vertical axis are significantly different from each other (p<0.05).Control - 0g, AL1 -Agricultural lime , OM1 -Organic manure, AL2 -Agricultural lime, OM1 - Organic manure, OM1 + AL1 – 50 g organic manure + 50 g Agricultural Lime, OM2 - Organic manure, OM2 + AL2 – 100 g organic manure + 100 g Agricultural Lime. AK: Akamkpa, CM: Calabar Municipality, OD: Odukpani

P. vulgaris treated with OM1 + AL1 (785.80 and 899.23 µmol/product/L/min) compared to control (545.39^{f}±5.53 and 648.51 µmol/product/ L/min).

3.3 Effect of Agricultural Lime and Organic Manure on Polyphenol-oxidase (PPO) Activity

Results of polyphenoloxidase activity of *P. vulgaris* and *V. aconitifolia* grown on acid, alkaline and neutral soils treated with organic manure and agricultural lime are presented in Table 3. Results revealed a trend of significantly (P<0.05) higher PPO activity in plants grown on treated soils from the three locations than plants grown on the untreated control soils. Results showed that PPO activity of *P. vulgaris* grown on Akamkpa soil treated with OM_1+AL_1, Calabar Municipality soil treated with AL_1, Odukpani treated with OM_1+AL_1 and OM_2+AL_2 had the highest PPO activity of 0.74, 0.76 and 0.77 µmol/product/L/min, respectively compared to control. PPO activity of *V. aconitifolia* grown on Akampka soil treated with OM_2, Calabar Municipality treated with AL_1 and Odukpani treated with OM_1+AL_1 revealed highest activity with mean values of 0.73, 0.76 and 0.78

µmol/product/L/min, respectively compared to control (0.47, 0.70 and 0.39 µmol/product/L/min).

4. DISCUSSION

Chlorophyll content in green plant is an important pigment that supports food manufacturing during the process of photosynthesis. Plants grown on soil from Akamkpa showed more chlorophyll "a" with 200 g agricultural lime (AL_2) while chlorophyll "b" was higher with the combination of both treatments (OM_1 + AL_1). The highest net chlorophyll yield was observed in *P. vulgaris* grown on Akamkpa soil treated with organic and agricultural lime (OM_1 + AL_1). It has been reported that increase soil acidity hampers the chlorophyll content in plants [13,14,15]. This was evidence in the reduced amount of chlorophyll measured from plant in the control acidic soil from Akamkpa. However, treatment with agricultural lime and organic manure ameliorated this condition and enhanced the chlorophyll content. Viscas, et al. [14] Eguagie [15] Cavalcante, et al. [16] have reported an increase in chlorophyll content of plants grown on acidic soil following administration of organic manure. It was also observed that, when the soil from Odukpani (alkaline) was amended, the net chlorophyll contents across the treatments were generally higher compared to soil from Akamkpa (acidic) suggesting a better performance of *P. vulgaris* and *V. aconitifolia* on alkaline soil.

Alteration in chlorophyll content of plant will have a conspicuous effect on photosynthetic activities [14]. Apparently, increased chlorophyll content of plant will have a positive effect on yield and yield components. Following the application of organic manure and agricultural lime to the soils, plants grown on them depicted significant increase in chlorophyll contents than the control. This is in agreement with previous report by Ekpo, et al. [17] that reduction in chlorophyll content was due to increase transpiration caused by acidic soil. The pH of the soil causes stress on the plants that grow on such soils.

Peroxidase and Polyphenoloxidase are two important enzymes in the plants antioxidant system. In the present study, peroxidase activities were lower in the control in both plants grown on soil from Akamkpa (acidic). However, the application of organic manure at 100 g increased peroxidase activity of *P. vulgaris* significantly. In a similar way, OM_1, OM_2 and OM_1+AL_1 also increased peroxidase activity in *V. aconitifolia*. Increase in the activities of these enzymes in plants grown on acidic and alkaline soils may be attributed to tolerance enhancement of these plants due to application of soil amendments. Similar results were obtained in plants grown on soils from Calabar Municipality and Odukpan (Alkaline). Peroxidase activity of plants grown on Odukpani soil (alkaline) were comparatively higher than plants grown on Akamkpa (acidic) soil which further support the view that alkaline soil may support cowpea performance than acidic soil. Polyphenoloxidase activity was also higher in the treated plants than in the control soils from Akamkpa and Odukpani, especially when treatments were combined (OM_1 +AL_1 and OM_2 + AL_2) implying that treatments contributed to enhancing the antioxidants of the plants to enable them withstand oxidative stress. With the critical role of polyphenoloxidase in fighting oxidative stress, these treatments may contribute immensely in crop improvement and enhancement of food security if properly harnessed into agricultural scheme.

The plant cells have complex antioxidant system. The primary role of such antioxidant system is to fight against oxidative stress generated by reactive oxygen species (ROS). The amount of these enzymes could be an indication of oxidative stress in plant tissues [18,19]. Generally, reduction in the amount of these oxidative stressed enzymes indicate a reduced antioxidants available to fight against the deteriorating reactive oxygen species and in such cases the affected plants are said to be experiencing oxidative stress. At such state, plant productivity is drastically reduced.

5. CONCLUSION

From the results put together, it was clear that organic manure and agricultural lime were more effective in enhancing the chlorophyll content and oxidative stress enzymes of *P. vulgaris* and *V. aconitifolia* when combined together.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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