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Theobroma Cacao

Deploying Science for Sustainability of Global Cocoa Economy

Edited by Peter Osobase Aikpokpodion

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Edited by Peter Osobase Aikpokpodion

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Meet the editor

Dr. Peter Osobase Aikpokpodion is Professor of Plant Breeding, Molecular Genetics, and Genetic Resources Management at the Department of Genetics and Biotechnology. His research interest spans plant breeding and cultivar development, application of molecular techniques in genetic resources (diversity) management and utilization, marker-assisted selection, physiological genetics, host plant–pathogen interaction, breeding for resistance and end-use quality considerations, reproductive biology, plant adaptation studies, climate-smart agriculture, and participatory plant breeding. He obtained his BSc (Agric) Crop Science (1989), MSc Agronomy in Crop Science (1998), and PhD in Plant Breeding (2007) at the Department of Agronomy, University of Ibadan. He was Chief Research Officer (Plant Breeding) at the Cocoa Research Institute of Nigeria before joining the University of Calabar in 2010 and was a Norman Borlag LEAP Fellow at Pennsylvania State University, USA, in 2006. With a strong background in research, industry linkages, and knowledge-driven policy development, he continues to serve in many capacities in academia, government, and the global cocoa industry. Some of his research contributions include development of eight new cocoa hybrids officially registered and released in December 2010; assessment of genetic diversity in cacao (*Theobroma cacao* L.) germplasm in Nigeria and West Africa; and Nigerian field gene-bank and on-farm genetic diversity in cacao, *Theobroma cacao* L., for the management and utilization of the genetic resources available. A reputable cocoa breeder, Dr. Aikpokpodion's contributions to the cocoa industry include the development and official release of eight new cocoa hybrids (CRIN*Tc*1-8) now distributed to farmers in Nigeria and his unraveling of the genetic diversity of Nigeria's cocoa field gene-banks and farm plantations. Dr. Aikpokpodion has managed several research projects and is a recipient of scientific and industry awards, including the USAID-administered Norman Borluag LEAP Fellowship 2006 and the 2014 Nigeria's Cocoa Value Chain Team Player Award. Dr. Aikpokpodion also served as Technical Advisor and Team Leader, Cocoa Value Chain Development of Nigeria's Agricultural Transformation Agenda coordinated by the Federal Ministry of Agriculture and Rural Development (2011–2015).

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Preface

Cocoa is an important commodity in the global market for flavor. An industry worth more than $200 billion annually provides employment for more than 5 million people in producing countries of the world. Scientific contributions from many research scientists and institutions have been provided to support this industry, which continues to expand to meet the growing demand for cocoa-based products around the world. This book documents some of the research results that have framed the positive growth and development of the cocoa industry, especially in producing countries. Organized into five sections, the chapters present interesting research results across the value chain. It is my pleasure to present the interesting work that has been documented in this book. All the authors are hereby gratefully acknowledged for their time and effort in submitting these book chapters.

Sincerely,

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Section 1

Cocoa Agronomy and Agroforestry

Chapter 1

Cacao Growth and Development Under Different Nursery and Field Conditions

Idowu Babadele Famuwagun and Samuel Ohi Agele

Abstract

Experiments were conducted between 2004 and 2018 to examine cacao growth, development, establishment and yield under varying experimental conditions comprised of seed mucilage handling before sowing, sowing methods and its effects on seedling growth and development, timing of mycorrhizal inoculation on root and shoot growth and development and effects of shade and dry season drip irrigation on growth and yield of field-grown cacao. Results show that cleaning cacao seed mucilage before sowing enhanced sprouting rate and percent germination. The use of manure mixed with sawdust and loamy soil aided excellent seed germination, seedling vigor and root development. Inoculating cacao seeds with arbuscular mycorrhizal fungi (AMF) at point of sowing and early stages in the nursery aided root development and enhanced field establishment and survival during the dry season. Dense shade retarded cacao growth and development during the rainy season, while no shade enhances optimum growth and canopy development. The use of drip irrigation strategies in young cacao plantations increased seedling survival from less than 45% under no irrigation to above 95% at the end of the second dry season. This showed that irrigation during dry season can significantly enhance cacao establishment and survival.

Keywords: cacao, growth, irrigation, seedlings, shade

1. Introduction

Cacao (*Theobroma cacao* L.) is a tropical woody species which belongs to the family Malvaceae [1]. Under natural conditions the tree can attain a height of 20–25 m [2], whereas under cultivation, plant height varies from 3 to 5 m. The geographical origin of cacao is South America [3], where several wild populations can be found in the Amazon and Guyanian regions. It is considered one of the most important perennial crops with an estimated world output of 4.2 million tonnes in 2018 [4] while [5] reported an estimated annual yield of 3.2 million tonnes in 2009. Cacao is predominantly grown in the humid tropical areas of Central and South America, Asia and Africa [6]. Cocoa is propagated through the seed for plantation establishment, and the seedlings are raised in the nursery for about 3–4 months before transplanting on the field. Direct sowing or sowing at stake and vegetative propagation are also possible means of establishing the crop [7, 8].

In Nigeria, cocoa production is limited to the rainforest and savanna transition zones. At present, the level of cocoa production stands at 350,000 tonnes per annum [9], and Nigeria is endowed with vast land areas suitable for its cultivation. Adoption of good management practices can bring about increased bean production of up to 100–300% [10]. The country had its peak production of 304,000 metric tonnes of cocoa beans in 1970 [11]. However, by 1999 production had dropped to 225,000 metric tonnes. As a result of the drop in production, the federal government of Nigeria is making tremendous efforts to resuscitate this industry through programmes such as Cocoa Rebirth Program and Tree Crop Development Program in partnership with other international organizations like the World Cocoa Foundation and the International Institute of Tropical Agriculture (IITA) Sustainable Tree Crop Development in Ibadan. Their effort has led to an increase in production to 370,000 MT with average yield of 270 kg/ha [11]. The major reasons attributed to low productivity despite the huge effort of the government according to [8] were limited access to modern production technologies, limited access to input and credit facilities, low percentage of survival (less than 35%) of transplanted seedlings at the end of the second dry season due to soil moisture stress, poor field management and impact of changing climate. More so, cocoa farm sizes are relatively small ranging from 0.4 to 6.0 hectare per farmer with an estimated total cultivation area of about 2.25 million hectares. With the present ever-increasing rate of unemployment in the country, coupled with the dwindling growth in economy and increased rate of food insecurity and the attending impact of climate change on crop production, a more concerted effort is required to harness the full benefit accruable from growth (employment opportunities, climatic stability, etc.), consumption (as food and beverages locally) and exportation (for foreign exchange earnings).

Traditionally, plantains have been used by the farmers to complement the shade requirement of the freshly transplanted cacao seedlings across the cocoa-growing region of Nigeria [12]. The associated problems of this practice include lack of existing models to follow in terms of spatial arrangement of the shade plants/trees, densities and possible consequences on the plant growth and development as the relationship progresses. More so, despite the provision of shade by plantain for the young transplanted cacao seedlings, it was a known fact that the highest percentage of these seedlings died between the first and second dry seasons as a result of soil moisture deficit during the peak of dry seasons [13]. It is also established that the plantains that were planted to provide shade during this dry period do shed most of their leaves as a result of limited soil moisture in order to survive [13]. In solving the above-mentioned problems, more robust farm management strategies are therefore required.

An arbuscular mycorrhiza (AM fungi) is a type of mycorrhiza that penetrates the cortical cells of the roots of a vascular plant and aids root development and nutrient absorption. The involvement of biofertilizer in the experiment was to aid root development and water/nutrient absorption from the soil by the rapidly developing root. Also, it helps to build tolerance in the seedlings to moisture stress during the dry season as discussed by [8] that proper root development in cacao seedlings always aids nutrient and water absorption.

Nigeria agriculture like anywhere else in West Africa is completely rain-fed, and rainfall and humidity over the country as a whole have been in persistent decline since the mid-1970s [14], while temperature has also been on the increase. Given the increasing worldwide demand for cocoa and quest for obtaining sustainable production systems, it is imperative to understand the effect of some agronomic practices on the responses of cacao seedling to dry season environmental conditions especially the hydrothermal stresses [15]. Improved insights would be valuable towards the attainment of optimum seedling establishment and vigor on the field

and to compare attainable yield among agroecology conditions and growing season environmental condition.

The proposed agronomic practices may obliterate negative effects of environmental stresses especially in the dry season and increase cacao adaptation to stressful growing environments. Such practices may enhance the resilience of cacao in plantation to the impact of weather variability. In order to develop such systems, it is imperative to examine the value of agronomic practices in the amelioration of extreme growing environmental conditions especially the hydrothermal stresses of the dry season on seedling survival on the field. Improved insights are required in order to attain optimum seedling establishment on the field. Effective management of cacao seedlings on the field using agronomic practices like dry season irrigation and optimum shading regime to enhance root development will improve plantation establishment and cacao productivity.

This write-up comprises of major findings of four different experiments conducted over 10 years on sustainable cacao production:

Experiment 1: Effects of growth media and mucilage cleaning methods on sprouting rate, seedling growth and development of cacao in the nursery.

Experiment 2: Timing of arbuscular mycorrhiza fungi (AMF) application on growth and development of cacao seedling in the nursery.

Experiment 3: Cacao developmental pattern, soil temperature and moisture variation as affected by shade and dry season drip irrigation.

Experiment 4: Effects of shade regimes and varying seasons of irrigation on survival, developmental pattern and yield of field-grown cacao (*Theobroma cacao*).

2. Materials and methods

2.1 Experiment 1: effects of growth media and mucilage cleaning methods on sprouting rate, seedling growth and development of cacao in the nursery.

This was conducted twice in Akure, Nigeria, in 2009 and 2010 to investigate the effect of mucilage cleaning methods and growth media on sprouting rate and seedling development of cacao. The trial was a 3×3 factorial experiment laid out in three replicates. Factor 1 was growth media consisting of topsoil, mixture of topsoil and sawdust at ratio of 50:50 and mixture of sawdust and poultry manure at ratio of 50:50, while factor 2 was a mucilage cleaning method which consists of cleaning with water, cleaning with cloth and no cleaning. Nursery pots were filled in accordance with the treatments and seeds from freshly harvested cacao pods from Teaching and Research Farm, Federal University of Technology, Akure (FUTA), which were subjected to mucilage cleaning treatments and planted the same day. Watering continues on a 2-day interval throughout the period of the experiment. Data were taken on date of sprouting, number of leaves, plant height and leaf area which continued for 8 weeks. Root parameters which include taproot length, number of lateral roots and longest lateral root length were collected at the termination of the experiment. The measured data were subjected to analysis of variance, and the means separated using Tukey test.

2.2 Experiment 2: timing of arbuscular mycorrhiza fungi (AMF) application on growth and development of cacao seedling in the nursery.

This experiment evaluates timing of arbuscular mycorrhiza fungi (AMF) inoculation on growth and root development of cacao in the nursery and in the early stage

on the field. The experiment was conducted in Akure, Nigeria, between December 2009 and April 2010 and between December 2010 and April 2011 in a completely randomized design with three replicates. The treatments were applied at 4, 8, 12 and 16 weeks after sowing and the control treatment (with no AMF). Nursery sites were prepared close to a source of water on the field under a natural shade suitable for tree crop seedling production.

Polythene pots for cacao seedlings were filled using topsoil from a virgin forest. Cacao seeds were obtained from the Cocoa Research Institute of Nigeria in Owena substation. Fifty pots were used for each treatment, and the seeds were sown immediately after pot filling, and the first treatment was applied. Other treatments were also applied at 4, 8, 12 and 16 weeks after sowing of the seeds. Agronomic practices and watering were carried out for 5 months on the seedlings. Data collected include plant height, number of leaves and stem girth at 4-week interval. Leaf area, taproot length, number of lateral root, average length of lateral root and the distribution along the taproot were taken at the end of 5 months from five selected pots from each treatment. Spore count was carried out on the soil around the cacao roots at the end of each experiment to determine the population of AMF spores in the root zone of the plants. Data collected were subjected to statistical analysis of variance, and the means separated using Tukey Test.

2.3 Experiment 3: effects of plantain shade and irrigation on cacao seedling establishment, vigor of growth, root development, survival and soil temperature variation on the field

The field experiments were conducted in Akure between May 2010 and May 2012. The treatments are plantain shade alone, irrigation alone, irrigation + plantain shade and the control. The treatments were replicated three times in a completely randomized design. Cacao seedlings were raised between January and May 2010 in the nursery and were transplanted to a manually cleared forest land with 2-month-old established plantain suckers (for shade treatments) at a spacing of 3 x 3 metres on the two sites. Drip irrigation lines were laid out on the field at the base of the cacao seedlings (irrigated treatments) at the beginning of dry season (December 2010 and 2011) to enhance adequate moisture supply during the dry season. Water was applied once per week at 2 litres per plant for 4 months of dry season. The shade plant (plantain) was transplanted on the field at the ratio of one stand of plantain to one stand of cacao seedling. Growth and development of the seedlings, soil and air temperature of the experiment site and soil moisture variation both in the wet and dry seasons were monitored starting from the onset of the dry season for 5 consecutive months. The data collected include plant height, stem girth, number of leaves, number of branches, taproot length, number of lateral root, length of lateral root, percentage (%) of seedling survival and leaf area. The soil moisture was monitored using tensiometer while soil temperatures were measured using soil thermometer at the hour of 2.00-3.00 pm in the afternoon. The collected data were subjected to analysis of variance (ANOVA), and the means separated using Tukey test.

2.4 Experiment 4: effects of varying dry season irrigation and shade regimes on survival, development and pod yield of young cacao on the field

This trial was conducted in Akure in 2012–2013, 2013–2014 and 2014–2015 sowing seasons. The experiment design was a 3x4 factorial experiment involving four irrigation packages that were imposed on the young cacao over a 3-year period (1:1:1, 1:1:0, 1:0:0 and 1:0:1) with 1 indicating year of irrigation and 0 indicating year

of no irrigation. The shade treatments involved dense shade (one stand of cacao to one stand of plantain), moderate shade (two stands of cacao to one stand of plantain) and no shade (open sun).

The plants were monitored for 3 consecutive years after transplanting to the field, and irrigation treatments were imposed as appropriate during the dry season between December and March of each year of 2012–2013, 2013–2014 and 2014–2015. The plants were irrigated using water from a nearby stream dam that was piped to an overhead storage tank. Drip irrigation pipes were laid on the field having each cacao on drip point. The pipes were connected to the water source (overhead tank), and the field was irrigated for 2 hours on a weekly basis. The amount of water coming out of the emitter was measured to be 2 liters per hour per plant. The irrigation processes continued during the dry season for 3 years. Data were measured on root development, percentage survival/mortality and pod yield. Root count was taken by using pressurized water to wash off the soils around the selected plants in order to make the root available for counting before covering back with moist soil. The collected data were subjected to statistical analysis using GENSTAT, and the means separated using Tukey test.

3. Results

3.1 Experiment 1

Table 1 shows the effects of growing media on leaf area development (LAD); significant difference ($P \leq 0.05$) was obtained 3 and 6 weeks after planting. The topsoil growing media produced cocoa seedlings with the largest leaf area development during the weeks of the experiment, while the sawdust + poultry manure gave the least.

Table 2 shows the combined effects of cleaning methods and growth media on leaf number development. Significant differences ($p \leq 0.05$) were recorded at the second and sixth weeks after planting. Similar trend was also observed in **Table 3** where the combined effects of cleaning methods and growing media on plant height development of cacao were presented, although significant differences ($P \leq 0.05$) were only recorded at the second week after sowing while other weeks showed no significant differences ($P \leq 0.05$). The cacao seeds that were cleaned with water and planted in the SD + PM growing media gave the tallest cacao plant, while seeds that were not washed and planted in the topsoil growing media gave the shortest cacao seedlings.

Table 4 shows the combined effects of cleaning methods and growing media on leaf area development of cacao. Significant differences ($p \leq 0.05$) were obtained at 3 and 6 weeks after sowing; cacao seeds that were cleaned with cloth and water and were planted in the SD + PM growing media produced the largest leaf area, while

Growing media	WK2	WK3	WK4	WK5	WK6
Sawdust + topsoil	2.57a	44.10b	56.89a	62.22a	65.14b
Topsoil	2.19a	51.57ab	61.44a	63.72a	88.45a
Sawdust + poultry manure	2.59a	56.35a	60.26a	61.07a	64.21b

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.

Table 1.
Effects of growing media on leaf area development.

Cleaning methods	Growing media	WK2	WK3	WK4	WK5	WK6
	SD + TS	2.00b	4.00a	4.67a	5.67a	6.00b
Cleaning with cloth	Topsoil	1.33b	4.33a	5.00a	6.00a	6.67a
	SD+ PM	3.00a	4.67a	5.00a	6.00a	7.00a
	SD + TS	1.33b	4.00a	4.33a	5.33b	5.67b
Cleaning with water	Topsoil	1.96b	4.12a	4.51a	6.71a	7.03a
	SD + PM	3.33a	4.00a	4.66a	5.67a	6.33ab
	SD + TS	2.00b	4.33a	4.33a	5.33b	5.67b
No wash	Topsoil	3.00a	4.33a	4.67a	6.00a	6.33ab
	SD + PM	4.00a	4.33a	4.33a	6.33a	6.33ab

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.
Note: TS = topsoil; SD = sawdust; PM = poultry manure.

Table 2.
Combined effects of cleaning methods and growth media on leaf number development.

Cleaning methods	Growing media	WK2	WK3	WK4	WK5	WK6
	SD + TS	6.50b	15.80a	16.87a	17.20a	17.57a
Cleaning with cloth	TS	5.17b	15.13a	16.90a	17.27a	17.93a
	SD + PM	8.73a	16.87a	17.40a	17.93a	18.30a
	SD + TS	5.07b	14.40a	16.83a	17.63a	18.33a
Cleaning with water	TS	5.20b	13.68a	15.81b	16.37a	18.00a
	SD + PM	8.80a	16.53a	17.60a	18.40a	18.97a
	SD + TS	5.20b	15.43a	17.87a	18.07a	18.33a
No wash	TS	5.20b	14.10a	15.03b	15.93b	16.27a
	SD + PM	7.83a	16.37a	17.67a	18.00a	18.87a

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P= = 0.05.
Note: TS = topsoil; SD = sawdust; PM = poultry manure.

Table 3.
Combined effects of cleaning methods and growing media on plant height development of cacao.

Cleaning methods	Growing media	WK2	WK3	WK4	WK5	WK6
	SD + TS	3.06a	49.30b	53.79c	59.57b	60.98c
Cleaning with cloth	TS	2.50b	61.98a	67.29b	68.28b	99.46a
	SD + PM	1.78c	38.64c	41.18d	41.98c	44.45
Cleaning with water	SD + TS	3.79a	42.64b	63.34b	70.80a	73.48b
	TS	0.45c	31.94c	46.76	50.95c	88.80a
	SD + PM	3.07a	72.69a	76.13a	77.78a	78.37a
No wash	SD + TS	0.86c	40.37b	53.52c	56.30c	60.97c
	TS	3.66a	66.57a	71.02a	73.06a	76.85a
	SD + PM	2.92a	57.71bc	63.47b	63.47b	69.82bc

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.
Note: TS = topsoil; SD = sawdust; PM = poultry manure.

Table 4.
Combined effects of cleaning methods and growth media on leaf area development of cacao.

Growing media	Cleaning methods	Day 10	Day 14	Sprouting (%)
	No wash	2d	19d	63.3
Topsoil alone	C/cloth	5c	23c	76.7
	C/water	7c	28b	93.3
Topsoil + sawdust	No wash	2d	26b	86.7
	C/cloth	12b	28b	93.3
	C/water	15a	30a	100
Poultry manure + sawdust	No wash	7c	26b	86.7
	C/cloth	13b	30a	100
	C/water	14a	30a	100

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.

Table 5.
Cacao seed sprouting rate in day after sowing and in percentage.

cacao seeds that were cleaned with cloth and planted in the SD + PM growing media had cacao seedlings with the smallest leaf area at 6 weeks after sowing.

Table 5 represents cacao seed sprouting rate in numbers of day after sowing and in percentage. Seeds that had its mucilage cleaned with water and cloth had a highest and fastest sprouting rate of about 48–50% at day 10 after sowing in both soils mixed with sawdust + poultry manure and topsoil + sawdust, while those that had their mucilage intact had a delayed sprouting with less than 25% sprouting at 10 days. At 14 days after sowing, seeds that were cleaned with water and cloth sowed in combination of topsoil + sawdust and poultry manure + sawdust had above 98% germination, while those with mucilage sowed in topsoil had less than 65% germination under topsoil mix. There was a significant difference between germination rates among seeds washed with water and those cleaned with cloth over those with their mucilage intact. More so, the mixtures of topsoil + sawdust and poultry manure + sawdust supported early sprouting rate and higher germination percentage than topsoil substrate.

3.2 Experiment 2

3.2.1 Results

Figure 1a and **b** represents the effects of timing of AMF inoculation on plant height from point of sowing (0 week after sowing) in nursery for 2009 and 2010. Inoculating cacao seeds at the point of sowing to 4 weeks after sowing showed significant effects on seedling height development over other periods of inoculation. No significant difference was observed among inoculating with AMF at the 12th week and 16th week after sowing and the control treatment. Similarly, **Figure 2a** and **b** represents the effects of varying time of AMF inoculation on stem girth development; the obtained results indicated that early inoculation (0–8 weeks) aided cacao stem girth growth and development significantly compared to late inoculation dates (10–20 weeks). More so, the numbers of leaves produced by the seedlings under early inoculation dates were significantly higher than those obtained with late inoculation dates (10–20 weeks after sowing) as shown in **Figure 3a** and **b**. The effects of timing of inoculation on root, shoot and leaf area development were shown in **Table 6a** and **b**; the results indicated that early inoculation at 0–4 weeks after sowing enhances leaf area development compared to other

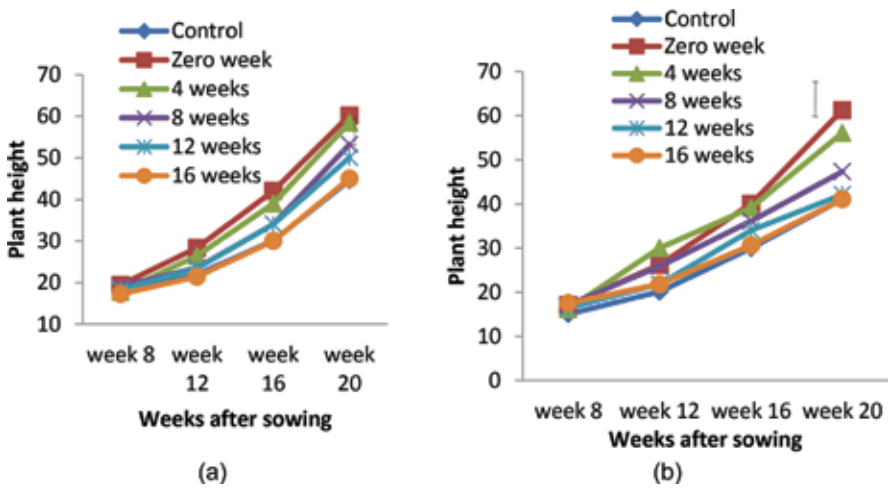

Figure 1.
(a and b) Effects of time of AMF inoculation on height development of cacao seedlings in the nursery (2009–2010, 2010–2011).

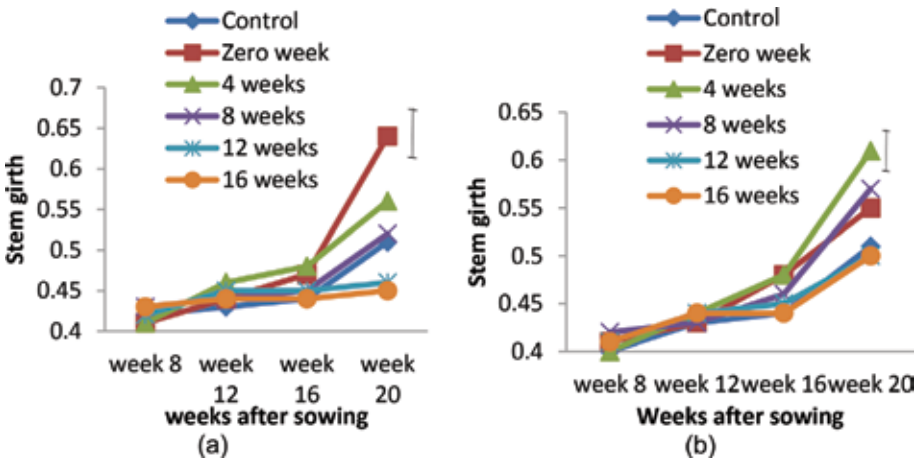

Figure 2.
(a and b). Effect of AMF inoculation on stem girth of cacao seedlings in the nursery (2009–2010, 2010–2011).

periods of inoculation. In addition, root development was higher significantly when cacao seedlings were inoculated at 0–8 weeks in terms of the number and length of lateral root and the taproot length compared with those inoculated later as shown in **Table 6a** and **b**.

3.3 Experiment 3

Effects of shade and AMF inoculation on vigor of growth and establishment of cacao seedlings on the field. Effects of combined use of plantain shade and AMF inoculation from nursery and at the point of transplanting were further studied to monitor growth, development, establishment and survival of field transplanted cacao seedlings in 2011–2012. The results obtained are as shown below.

Table 3 indicates the effects of treatments on the number of leaves produced by the cacao seedlings with treatments having AMF inoculation combined with plantain shade having the highest significant mean values over treatment at shade alone, AMF alone at transplant and the control. In addition, no significant difference was

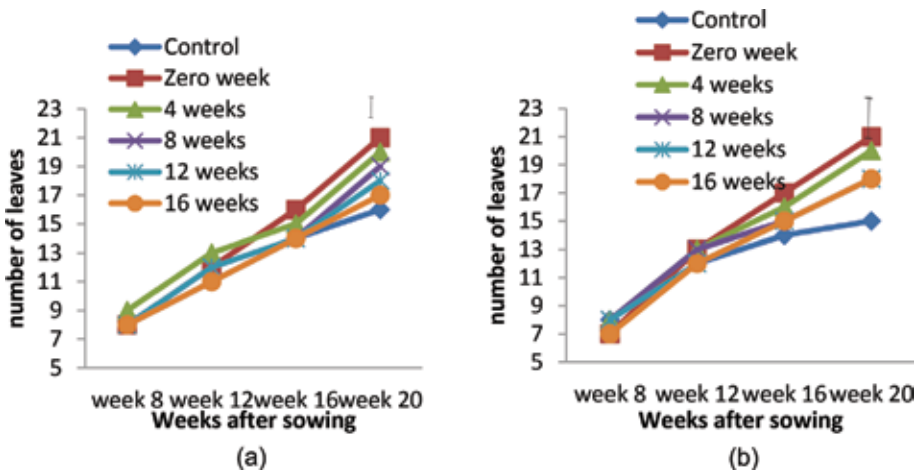

Figure 3.
(a and b). Effects of time of AMF inoculation on inoculation on leaf development in cacao seedlings in the nursery (2009–2010, 2010–2011).

Treatments	Leaf area (cm^2)	Number of lateral root (cm)	Lateral root length (cm)	Taproot length (cm)	
(a) First trial					
Control	105.03c	29c	7.1b	20.14b	
0 week	186.17a	83a	11.4a	26.45a	
4 weeks	182.11a	85a	11.6a	26.33a	
8 weeks	165.04b	80a	10.5ab	27.15a	
12 weeks	149.12b	71b	9.5ab	20.08b	
16 weeks	121.33c	65b	9.1b	21.12b	
Treatments	**Leaf area (cm^2)**	**Number of lateral root**	**Lateral root length (cm)**	**Taproot length (cm)**	**Number of AMF spores**
(b) Second trial					
Control	98.92c	22c	6.3b	18.4b	0c
0 week	176.5a	96a	11.0a	23.6a	117a
4 weeks	188.3a	104a	10.4a	26.4a	109a
8 weeks	162.5ab	88b	10.1a	22.4a	101b
12 weeks	143.5b	80b	8.3b	20.0b	91b
16 weeks	114.3c	69b	6.9b	20.2b	76b

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.

Table 6.
Effects of time of AMF inoculation on growth of cacao seedlings in the nursery (a, first trial and b, second trial).

observed between treatment of AMF alone from nursery and those with combined use of AMF and shade.

AMF inoculation from the nursery combined with plantain shade showed significantly higher mean values of stem girth development over other treatments. Other treatments except the control with a significantly lower mean value were not significantly different from each other in terms of plant height development as shown in **Table 7**.

Treatments	At transplant	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Shade + AMF at nursery	8a	10a	13a	16a	17a	18a	21a
Shade + AMF at transplant	8a	11a	13a	14a	16a	18a	20a
Shade alone	9a	10a	12a	13b	14b	15b	17b
AMF alone at nursery	8a	11a	13a	15a	15a	17a	20a
AMF alone at transplant	8a	11a	12a	14a	14b	15b	18b
Control	9a	12a	13a	14a	14b	15b	16c

Means in the same column followed by the same letter(s) are not significantly different at 5% probability.

Table 7.
Effects of treatments on the number of leaves produced during the first rainy season (0–7 months after transplanting) (2011 experiment).

Treatments	At transplant	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Shade + AMF at nursery	1.81a	1.88a	1.95a	2.06a	2.32a	2.35a	2.51a
Shade + AMF at transplant	1.78a	1.82a	1.93a	2.02a	2.22a	2.29a	2.33b
Shade alone	1.82a	1.83a	1.90b	1.99ab	2.08b	2.22b	2.24b
AMF alone at nursery	1.83a	1.84a	1.89b	1.94b	1.98b	2.07c	2.38b
AMF alone at transplant	1.81a	1.84a	1.87bc	1.96b	2.03b	2.20b	2.35b
Control	1.75a	1.83a	1.85c	1.91c	1.97c	2.05c	2.10c

Means in the same column followed by the same letter(s) are not significantly different at 5% probability.

Table 8.
Effects of treatments on stem girth development (cm) during the first rainy season (0–7 months after transplanting) (2011 experiment).

Effects of shade and AMF inoculation on plant height development. The results on **Table 8** show that treatments of AMF combined with shade and AMF alone from nursery were not significantly different from each other in terms of plant height development. It was also observed from the result that as the age of the inoculated cacao seedlings increases, the better the influence of AMF on them. This was evident in **Table 8** as the plant age increases the gap between treatments of AMF decreases.

Tables 9 and **10** show that the plant growth and developmental measurement at the beginning of the first dry season after transplanting with shade + AMF from the nursery and AMF alone at transplant have the highest significant mean values over others in terms of plant height development. No significant mean difference was recorded among the treatment combinations. Similar trend was followed in stem girth development with control treatment having the lowest mean values. The percentage survival at the onset of the first dry season was not significantly different among treatments.

Table 10 shows the results of the plant growth and developmental measurement at the end of the first dry season in April. The result indicated that inoculating with AMF from the nursery significantly increases the survival rate of the transplanted seedling with 64.7% survival compared with other treatments. Results also show

Treatments	Plant height (cm)	Number of leaves	Number of branches	Stem girth (cm)	Percentage (%) survival
Shade + AMF at nursery	67.5a	20.6a	1a	1.51a	100a
Shade + AMF at transplant	62.4b	18.3b	0a	1.33ab	95a
Shade alone	60.3b	22.5a	0a	1.24b	95a
AMF alone at nursery	64.3b	22.3a	2a	1.38ab	95a
AMF alone at transplant	66.3a	25.1a	0a	1.35ab	100a
Control	58.2b	17.3b	0a	1.10c	95a

Table 9.
Treatment effects on growth and survival of cacao at the onset of the first dry season 7 months after transplanting (December 2011).

Treatments	Plant height (cm)	Number of leaves	Number of branches	Stem girth (cm)	Percentage (%) survival
Shade + AMF at nursery	85.42a	60.6a	5.1a	2.2a	64.7a
Shade + AMF at transplant	83.18a	48.3b	4.6a	2.0a	50.3b
Shade alone	71.44b	32.5c	5.2a	1.4a	36.1c
AMF alone at nursery	69.20b	32.3c	5.1a	1.8a	29.4c
AMF alone at transplant	70.10b	45.1b	4.3a	1.7a	28.3c
Control	64.50c	9.3d	2.1b	1.1a	9.0d

Means in the same column followed by the same letter(s) are not significantly different at 5% probability.

Table 10.
Treatment effects on growth and survival at the end of the first dry season 7 months after transplanting (April 2011).

that shade alone is grossly inadequate to scale up the survival percentage of transplanted cacao seedling during the first dry season. Percentage survival of control treatment which is at 9% recorded the significantly lowest mean followed by AMF alone at transplant; AMF alone from the nursery and shade alone were not different significantly.

3.4 Experiment 4

Table 11 shows the impacts of varying shade regimes on cacao stand survival under the tested growing seasons of 2012–2013, 2013–2014 and 2014–2015. The results indicated that the sole use of shade does not guarantee optimum stand survival and establishment of cacao in the studied area as stand survival decreases with season having 99.5% decreased to 48.4% under dense shade, 99.9–40% under moderate shade and 100% to 27.1% under no shade. It was also recorded that the mortality rate after the end of the first dry season was least under no shade as plant stand survival and establishment are getting stabilized.

Shade	2012–2013		2013–2014		2014–2015	
	Onset of dry season	End of dry season	Onset of dry season	End of dry season	Onset of dry season	End of dry season
Dense	99.5a	68.2a	68.0a	52.1a	51.0a	48.4a
Moderate	99.9a	60.1a	58.9b	44.2b	44.2b	40.0b
No shade	100.0a	31.4b	31.4c	27.1c	27.1c	27.1c

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.

Table 11.
Effects of shade regimes on percentage (%) survival of cacao at the onset and end of dry seasons.

Irrigation treatment	2012–2013		2013–2014		2014–2015	
	Onset of dry season	End of dry season	Onset of dry season	End of dry season	Onset of dry season	End of dry season
Three season irrigation	99.8a	99.8a	98.5a	98.5a	98.5a	98.5a
Two season irrigation	99.8a	99.8a	99.8a	99.8a	99.8a	65.4b
One season irrigation	99.5a	99.5a	99.5a	75.2b	75.2b	55.5c

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.

Table 12.
Effects of varying seasons of irrigation on percentage (%) stand survival of cacao at onset/end of dry seasons.

Table 12 represents the effects of seasons of irrigation on survival of cacao at the onset and end of 2012–2013, 2013–2014 and 2014–2015 seasons. The results indicated that no significant difference was observed in the stand survival of the cacao during the 2012–2013 season. In 2013–2014, plots without the second dry season irrigation were significantly lower in percent stand survival under the three shade regimes. During 2014–2015 season, plots with three seasons of irrigation had the highest percentage survival above 97%, while those with two seasons of irrigation had a significantly lower surviving rate of about 63% which is significantly higher than those subjected to only one season irrigation. It was observed that percentage survival of cacao tends to improve under no shade after two seasons of dry season irrigation. It was also observed that moisture stress tolerance of cacao stands under no shade tends to increase after irrigation in the first two dry seasons.

Table 13 indicates the combined effects of shade regimes and varying dry season irrigation on pod yield of cacao during the main and midcrop harvest. Combination of no shade + two dry season irrigation and no shade + three dry season irrigation produced a significantly higher pod yield during the first main crop harvest (14th–18th month after transplant) over those combinations of moderate and dense shades. More so, between January and April, covering the 19th–22nd month after transplanting, combination of dense and moderate shade with two and three seasons of irrigation favored pod yield over those exposed to only one season irrigation. During the second main crop harvest, the 25th–29th month after transplant,

Shade treatment	Irrigation treatment	2012–2013		2013–2014		2014–2015	
		Onset of dry season	End of dry season	Onset of dry season	End of dry season	Onset of dry season	End of dry season
Dense shade	Three season irrigation	99.8a	99.8a	99.5a	99.5a	96.5a	98.5a
	Two season irrigation	99.8a	99.8a	99.5a	99.5a	99.5a	85.5b
	One season irrigation	99.5a	99.5	99.5a	73.5b	73.0b	54.7c
Moderate shade	Three season irrigation	99.8a	99.8a	97.6	97.5a	97.5a	97.5a
	Two season irrigation	99.7a	99.7a	99.5a	97.5a	97.5a	83.5b
	One season irrigation	100.0a	100.0	99.0a	73.5b	73.5b	52.5c
No shade	Three season irrigation	100.0a	100.0a	99.5a	99.5a	99.5a	99.5a
	Two season irrigation	100.0a	100.0a	98.0a	97.0a	97.5a	89.5b
	One season irrigation	100.0a	100.0a	99.0a	77.0b	57.0b	56.5c

Mean values in the same column followed by different letter(s) are significantly different by Tukey test at P = 0.05.

Table 13.
Effects of shade regimes and varying seasons of irrigation on percentage survival of cacao at the onset and end of dry seasons.

Figure 4.
Cacao seedlings facing moisture stress under no shade at the peak of the first dry season.

no-shade plots + two and three seasons of irrigation produced a significantly higher pod yield over those with dense and moderate shades.

Figures 4–6 present cacao response to varying environmental conditions of shade and no shade with or without AMF inoculation.

Figure 5.
Cacao seedling with AMF under no shade at the peak of the first and second dry season.

Figure 6.
Plantain shade + AMF at nursery at the onset of the first dry season.

4. Discussion

The results obtained indicated that plant height, number of leaf and leaf area of cacao plants were significantly influenced by both methods of mucilage removal and substrate mix used. In general, the best results, in terms of development of plant height, number of leaf, leaf area and percentage sprouting rate, were obtained from poultry manure mixed with sawdust and topsoil growing media whose cacao seeds were cleaned with water or cloth. This was a result of ease of sprouting in the absence of the mucilage coat on the seeds which was in conformity with the findings of [16].

The rapid growth and development that was recorded on the sawdust growing media at the early stage may be attributed to the high root aeration and porous soil media that allow easy root penetration and the presence of macrospores. This agree with [17] that there exist a higher numbers of macrospores (>100 mm) and high percentages of water retention (60%) in sawdust than composted media.

He further explained that the high amount of macrospores of sawdust specifies high air space (56.9%) and enhances root growth of seedlings.

From the results, the significant differences observed in AMF application at planting and at 4 weeks after sowing were a result of early colonization of the cacao roots and the formation of mycelium growing along depressions between epidermal cells of the roots. This was in line with the findings of [18] that inoculation of crops with mycorrhiza at early stages of growth aided quick colonization and subsequent nutrient absorption and development. More so, the significant mean values recorded under AMF inoculation at 0, 4 and 8 weeks after sowing were attributed to efficient nutrient absorption and partitioning of assimilates to the root and shoot region of the seedlings which was used for lateral root and shoot development. These findings were supported by [8] that cacao seedling growth, development and establishment are determined by the volume of the lateral roots/root hair of the seedlings.

The lower significant mean values recorded under inoculation at 12 and 16 weeks and the control in terms of number of leaves produced, stem girth development, lateral root length and number and taproot length were a result of late/no inoculation [19]. Early inoculation favored multiplication of the organism as supported by the result which also favors root and shoot development and subsequent field establishment.

The combined effects of moderate and dense plantain shade with continuous 3-year irrigation enhance field survival and establishment of cacao but with a significantly negative effects on some growth parameters like stem girth, branch number and canopy size compared to cacao with continuous 3-year dry season irrigation under no shade (open sun). This was in conformity with the findings of [20] that high-density shade impedes young cacao growth and developments as shade plant competes with both water and light, thereby leading to reduced photosynthetic rate and low assimilate production.

More so, [21] reported that fruit trees generally combined well with cacao though farmers said they provided fewer ecological services to cacao plants. Shade is not the most valuable feature according to farmers.

[22, 23] resolved that soil evaporation decreases proportionally over the growing seasons as the ground surface is increasingly shaded by crops and shade plant canopy. These facts validated the significant effects of shade treatments on increased percentage survival of cacao on the field after transplanting. Provision of water through dry season irrigation and unhindered access to sunlight positively enhanced early establishment, survival, development and speedy canopy development in the no-shade treatments which gave it a hedge over the shaded plots in shoot development and early production. This further confirms the early study of [13] that no-shade cacao under irrigation performed better than the shaded ones. Cacao requires shade during its early stages of growth. This may be provided by temporary plants or by mature trees. There is no absolute requirement for shade once the cacao tree is established, unless there is no irrigation, in which case shade trees preserve soil moisture. The significantly higher height of cacao plants under moderate shades came with a thinner stem girth than those under open sun (no shade) with a thicker girth, higher branch number, and better canopy sizes at first and second growing season was as a result of competition between the cacao and the shade plants. Meanwhile, that mortality were highest under plots of dense and moderate shades without irrigation in the second and third dry season (67%), followed by those without irrigation only in the second dry season (58%) and (52%) in those without irrigation in only the third dry season was as a result of diminishing soil moisture and shallow root development/penetration in the soil. This was in line with the findings of [24–26].

The reduction in stand mortality under moderate and dense shaded plots was traced to improved microclimate conditions occasioned by shade plants that aided reduced air and soil temperature, reduced moisture loss through evaporation and increased activities of microbial organism under shaded microclimate.

More so, the early canopy cover from individual cacao plant under no-shade plots may have contributed to reduced moisture loss to the atmosphere via evaporation which thereby helped in soil moisture conservation which thereby increase the amount of available moisture for growth and development. Irrigation may be implicated for the non-significant effects of shade on percent seedling survival at the end of the first dry season. Irrigation enhanced soil moisture availability during the dry season. These results were supported by [27, 28] that moisture is the principal requirement for crop survival during the dry season to supplement soil moisture loss due to transpiration, evaporation and diminishing soil water due to dry and hot air.

Soil evaporation decreases proportionally over the growing season as the ground surface is increasingly shaded by the crop canopy. The effect of both crop transpiration and soil evaporation is integrated into a single crop coefficient (Kc) incorporating crop characteristics and average effects of evaporation from the soil [22].

5. Conclusion and recommendation

Finally, results of this study have indicated that poultry manure mixed with sawdust proved to be a faster germination medium than topsoil mixed with sawdust and topsoil alone irrespective of the cleaning methods. In addition, the seeds cleaned with water sprouted faster than seeds cleaned with cloth and mucilage intact seeds irrespective of the media. Thus, adopting cleaning mucilage with water combined with using sawdust mixed with poultry manure will give a better sprouting rate and enhance seedling development.

It was concluded that cacao field establishment, growth and pod yield will improve significantly if dry season irrigation is provided for the first 3 years of establishment.

More so, stand mortality as a result of dry season soil moisture deficit in the first, second and third dry season can be avoided through dry season irrigation.

Shade can be considered to ameliorate the cocoa micro-environment.

It is therefore recommended that farmers who want to embark on cacao nursery production should adopt cleaning the mucilage of the seeds with water and using sawdust mixed with poultry manure, so as to reduce seed wastage.

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Chapter 2

Current and Potential Use of Timber and Non-timber Resources of the Cacao Agroforestry Systems

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Abstract

The cocoa agroforestry system (Cocoa-AFS) is a source of forest and forest non-timber resources. Forest timber resources (FTR) provide society with timber products. The most common uses for trees from the cocoa-AFS are shade for cocoa, firewood, medicinal, timber, fence posts, tool handles, ornamental, and supports and roofing for houses. Forest non-timber resources (FNTR) are those plant and animal products and services that can be obtained from the system. These resources include fruits, medicinal plants, ornamental plants, honey, and many others. Worldwide, FNTR may be the only source of personal income or food for the inhabitants of marginalized areas. Cocoa cultivation faces problems of low production and low prices. These problems induce growers to left-hand or to reduce their cocoa-AFS. Such reduction means the loss of FTR and FNTR that could complement grower incomes from the sale of cocoa. In this paper, we documented the forest tree species and determined the timber volume in cocoa-AFS in the municipality of Cardenas, Tabasco, Mexico. In addition, we determined and quantified the current use of FTR and FNTR. The emphasis of FNTR was on the associated flora and the stored carbon on aboveground biomass as environmental services by the shadow trees.

Keywords: cocoa agroecosystem, timber products, non-timber products, carbon sequestration

1. Introduction

Cocoa tree (*Theobroma cacao* L.) is cultivated in agroforestry systems (AFS) in Mexico. An AFS is a set of land management techniques that combines forest with crops, livestock, or both. It can be established simultaneously or stepwise over time and space [1]. In these systems, cocoa maintains close association with diverse tree species and other useful plants that potentially produce benefits for the families of cocoa growers [2]. In this way, cocoa-AFS possess a broad spectrum of plant associations and strong potential for production of timber, firewood, fruits, medicines, forages, oils, and ornamental plants [3]. Cocoa-AFS is possible since cocoa crop requires low radiation as a C3 plant [4]. Then, it can be established under

a tree canopy [5], although in Africa, Malaysia, Peru, Colombia, and Ecuador cocoa production systems under full sunlight have been developed [6]. Having a broad diversity of tree species, cocoa-AFS contain a high diversity of plants, microfauna, and macrofauna and have an important role in the protection and conservation of biodiversity and carbon storage [7, 8]. García [9] reported that the species *Erythrina americana* Mill*, Diphysa robinoides* Benth, *Gliricidia sepium* (Jacq.) Walp, *Samanea saman* (Jacq.) Merr. and *Colubrina arborescens* (Mill.) Sarg. are the most outstanding shade trees of the cocoa-AFS in Comalcalco, which is the first municipality cocoa producer in Tabasco, Mexico. Cocoa producers also introduce other species of their preference useful by their timber (*Cedrela odorata* L.) and fruits such as *Mangifera indica* L., *Citrus* spp. and *Pouteria sapota* (Jacq.) H. E. [9, 10].

Plant diversity in cocoa-AFS can be divided into forest timber resources (FTR) and forest non-timber resources (FNTR). Some of them are shown in **Figure 1**.

FTR provide the society with environmental services (conservation of water, soil and biodiversity, atmospheric carbon sequestration, mitigation of climate change, and global warming). These aspects have not been quantified in most of the cocoa-producing regions of the world [5, 8, 11]. Tangible contributions of FTR are timber products used in the production of lumber (boards, planks, beams, and packing material), paper, veneer and plywood, and for energy (firewood).

The most common uses for trees from the cocoa-AFS are medicinal, timber, pillars for constructing houses, fence posts, tool handles, fruit production, shade for cocoa, firewood, ornamental, and roofing for houses [12]. In the function of their diameter at breast height ($DBH_{1.3\,m}$), 34% of the trees in cocoa-AFS in Costa Rica and 15% in Bolivia are used for thick boards [13, 14].

Non-timber resources are those plant and animal products and services that can be obtained from the forest [15], that is, they are the set of biological resources that include fruit, medicinal plants, ornamental plants, honey, and many others [16]. In many parts of the world, these resources are indispensable for the inhabitants of marginalized areas, who are the main extractors of these products, which may be their only source of personal income [17, 18].

In Mexico, the largest cocoa-producing states are Chiapas and Tabasco occupying an area of 58,084.8 ha, on which 47,000 growers depend. In Tabasco, the area under cocoa is 40,848 ha, which produces 17,403.8 tons of dry cocoa [19, 20]. Of this area, 96% is in the Chontalpa region and 4% in the Sierra region [21].

Figure 1.
Cocoa agroforestry system and some of their timber and non-timber resources in Tabasco, México. Up, from left to right: Calathea lutea, Mangifera indica, Citrus sinensis, and Cedrela odorata logs. Down, from left to right: Persea Americana, Alpinia purpurata, Firewood, and Capsicum annuum.

Cárdenas with an area of 10,487 ha of cocoa-AFS is the second main cocoa-producing municipality of Tabasco [20].

Cocoa cultivation faces problems of low production and prices. These problems discourage growers who no longer maintain their plantations; thus, fewer and fewer number of farmers now cultivate cocoa. The reduction in area planted in cocoa means the loss of a production system that maintains tree cover and provides FTR and FNTR that could complement grower incomes from the sale of cocoa. In this paper we are reporting the forest tree species present in the AFS and determining the timber volume in cocoa-AFS in the municipality of Cárdenas, Tabasco. Also we are determining and quantifying the current use of FTR and FNTR in the cocoa-AFS.

2. Current use of forest timber resources and forest non-timber resources from cocoa-AFS

2.1 Materials and methods

2.1.1 Study area

The study was conducted in 20 plantations (cocoa-AFS) distributed in the populations C-20 (Miguel Hidalgo y Costilla) and C-28 (Gregorio Méndez Magaña) in the municipality of Cárdenas, Tabasco (**Figure 2**). This municipality is located between 17° 15′ and 17° 40′ N and 90° 59′ and 94° 06′ W, at an altitude of 2–17 m above sea level. Climate is hot-humid with mean annual precipitation of 2643 mm and a monthly mean of 355 mm; mean annual temperature is 26°C with a maximum of 45°C [22].

2.1.2 Quantification of the current use of FTR and FNTR from the cocoa-AFS

Twenty 50 × 100 m sampling sites were established in the same number of plantations (one site per plantation). In each site, age and area of the plantation and number of FTR and FNTR plant species were recorded. Common names of the species were recorded with the aid of people who depend on the cocoa-AFS. For scientific names of the species, the appropriate literature on the vegetation of Tabasco was consulted. A specific questionnaire was given to the owners of each plantation to elicit information on destination and use of FTR and FNTR, as well as on cocoa production. Only the principal use of each species was considered. The social part of the questionnaire included information on family makeup and land ownership. The economic section comprised questions on who works in the production activities, how much is invested in the plantation, and how much is the yield per hectare. The questions relative to the destination of the FNTR of the cocoa-AFS were what products are used in the plantation and how, how much is used of each, and what income is obtained. Average income from the FNTR in the cocoa-AFS was obtained by adding the income of each of the 20 plantations and dividing by the number of plantations. Income from sale of cocoa was obtained by multiplying yield (kg ha^{-1}) by the price per kilogram and subtracting production costs per hectare.

The data were analyzed with descriptive statistics in the Statistical Package for the Social Sciences (SPSS version 20).

2.1.3 Forest timber resources and estimation of C stored in aboveground biomass

In each sampling site, total height (Th) and diameter at breast height (DBH) of each tree were recorded. A Haga pistol was used to measure Th (m), the

Figure 2.
Location of the study area: C-20 town of Miguel Hidalgo y Costilla and C-28 town of Gregorio Méndez Magaña, Cardenas, Tabasco, Mexico.

DBH was measured with a diametric circumference tape, and the result was divided by 3.1415 (π). With the variables Ht and DBH, basal area (BA, m^2) and volume with bark (vwb, m^3) were calculated for each tree. The formulas used were BA = $(DBH/2)^2 \times \pi$ and vwb = BA × ff × Ht, where ff = form factor (0.70) [23, 24]. With vwb, the volume of bark per hectare was calculated (Vwb, $m^3 ha^{-1}$). Vwb was used to calculate the physical carbon inventory PCI, t ha^{-1}. The formula used was PCI = Vwb × FEB × FCC, where FEB is factor of expansion of biomass (1.6) and FCC is the factor of conversion of biomass to carbon (0.05).

2.2 Results and discussion

In the Cocoa-AFS sampled, a total of 3239 trees of 56 species and 27 families were recorded. The average tree density per hectare was 324, varying from 58 to 544. The families Fabaceae and Meliaceae predominate. **Table 1** shows the most frequent species. *Erythrina americana* Mill individuals (1678) account for 51.8% of the total.

FTR provide environmental services. Families obtain direct benefits, such as oxygen, lumber, fence posts, forked support posts, window and door frames, and firewood. Incomes per family reported by producers from sale of these items were US$ 155.4 a year. Of this amount, US$ 120.8 is from sale of timber for milling and US$ 34.6 from sale of firewood.

Common and scientific name		Num. trees	Percentage
Mote *Erythrina americana* Mill.	Fabaceae	1678	51.81
Cedro *Cedrela odorata* L.	Meliaceae	349	10.77
Tatuan *Colubrina arborescens (*Mill) Sarg.	Rhamnaceae	300	9.26
Chipilcohite *Diphysa robinioides* Benth	Fabaceae	188	5.80
Naranja *Citrus sinensis* (L.) Osb.	Rutaceae	188	5.80
Guácimo *Guazuma ulmifolia Lam.*	Malvaceae	87	2.69
Macuílis *Tabebuia rosea* (Bertol) *DC.*	Bignoniaceae	81	2.50
Cocoite *Gliricidia sepium (Jacq.) Kunth ex Walp.*	Fabaceae	68	2.10
Guarumo *Cecropia obtusifolia* Bertol.	Urticaceae	41	1.27
Cesniche *Lippia myriocephalus* Sch. y Cham.	Verbenaceae	36	1.11
46 other species		223	6.88
Total		3239	100

Table 1.
Most frequent forest species found in cocoa-AFS, according to the number of trees recorded.

2.2.1 Forest non-timber resources in cocoa-AFS

As FNTR, 6308 plants from 29 families and 53 species were recorded. The plants were grouped into six use categories (**Figure 3**), according to the families who depend on the cocoa-AFS in Cárdenas, Tabasco. The predominating categories were ornamental with 2719 plants, fruit tree with 1776, and vegetable with 1578 plants. Ornamental plants were more common because of their rapid growth and broad distribution. Moreover, since these types of plants do not require high solar radiation, the cocoa-AFS is an ideal habitat. The other highest use categories are those for home use: fruit, vegetable, and medicinal.

The 10 most frequent species are listed in **Table 2**. *Heliconia latispatha* Benth, considered an ornamental plant, accounted for 26.36% of total (1663 plants).

2.2.2 Use of forest and non-forest species

Of the people who depend on the cocoa-AFS, 90% have the same living conditions. They own their home, which has hard floors and concrete roofs. Three to six people live together and obtain income from sugarcane cultivation. However, 70% do not have any use for the products found in the cocoa-AFS, 20% gather them for home use, and 10% sell them. Cocoa production is the main reason for maintaining the system. What growers receive as income from the sale of cocoa is complemented with income from other crops, such as sugarcane (*Saccharum officinarum* L.), and from other activities. The 20% that use these products themselves use FTR for carpentry, supporting posts, fence posts, and roof beams, and

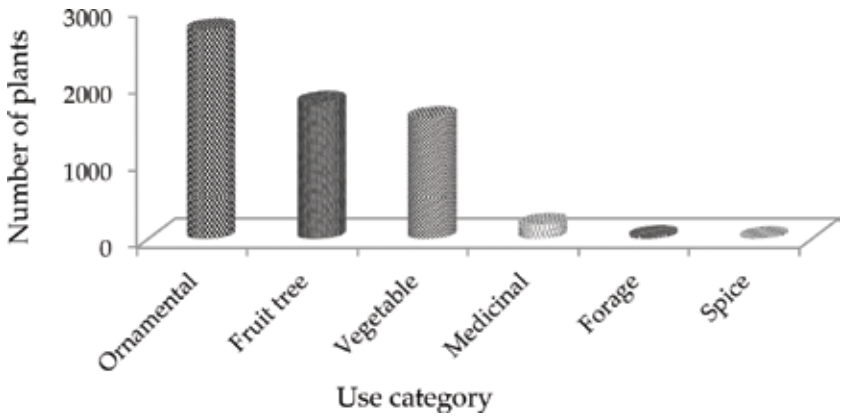

Figure 3.
Number of plants found in cocoa-AFS, in Cárdenas, Tabasco, Mexico, by use categories.

Common name	Scientific name	Num. plants	Percentage
Platanillo	*Heliconia latispatha* Benth	1663	26.36
Hoja de To	*Calathea lutea* G.F.W. Meyer	653	10.35
Platano Cuadrado	*Musa paradisiaca* L.	408	6.47
Platano Macho	*Musa balbisiana* L	386	6.12
Canna	*Canna indica* L.	351	5.56
Pitahaya	*Hylocereus undatus (*Haw.)	311	4.93
Macal	*Xanthosoma sagittifolium S.*	307	4.87
Heliconia Pie gallo	*Heliconia psittacorum* L. f	292	4.63
Hierba Mora	*Solanum tuberosum* L.	248	3.93
Papaya Silvestre	*Carica mexicana* (A.DC.)	237	3.76
43 remaining species		1452	23.02
Total		6308	100

Table 2.
Most frequent non-timber species found in cocoa-AFS.

the residues are used as fuel (firewood). It is estimated that these uses add up to a yearly average savings of US$ 197.50, which can be considered income since, if they were not obtained from the cocoa-AFS, the grower would have to pay out that amount. Some essential FNTR for home use were banana and banana leaves (*Musa* spp.), wild papaya (*Carica mexicana* A.DC.), "platanillo" (*Calathea lutea* Aubl Schult), purple maguey (*Tradescantia spathacea* Sw.), "amashito" chili (*Capsicum annuum* L.), yerba buena (*Mentha sativa* L.), arrowleaf elephant's ear (*Xanthosoma sagittifolium Schott*), Mexican cilantro (*Eryngium foetidum* L.), bitter watermelon (*Momordica charantia* L.), and achiote (*Bixa orellana* L.). Their fruits or leaves are used to prepare food. Moreover, the families save by not buying these products. Of the population that has cocoa plantations, 10% sell their FNTR at the local markets, generating incomes averaging US$ 41.9 per year. The producers that do not use products from the cocoa-AFS are interested only in the production of cocoa but not in the diversity of resources nor the uses they might have.

2.2.3 Economic value of cocoa and potential income from sale of carbon sequestration environmental services

Average cocoa yield reported by growers was 320.8 (±79.6 kg ha^{-1}) (**Table 3**). This yield provides an average net income from the sale of cocoa in pulp of US$ 456.3 ± US$ 140.1 ha^{-1}, which is not profitable since the estimated cost of production is US$ 345.8 (± US$ 135.9) ha^{-1}. The 70% of the growers reported yields above of the average.

Two growers have the highest yields and two the lowest. The former can be attributed to good management of their plantations, as the result of their participation in the ICCO-Nestle program. This program consists of training cocoa growers in good management of their cocoa plantations to ensure good yield, which is later marketed by the same program. The lowest yields can be attributed to two factors.

Plantation number	Production [a] ($kg.ha^{-1}$)	Net income cocoa ($US\$ ha^{-1}$)	AGB C [b] ($t\ ha^{-1}$)	Income [c] from C payments ($US\$ ha^{-1}$)
1	500	833	143.29	1074.67
2	333	417	87.92	659.41
3	233	417	86.57	649.30
4	333	667	122.07	915.53
5	267	500	111.90	839.27
6	200	333	148.98	1117.37
7	233	417	215.75	1618.13
8	333	417	165.79	1243.41
9	200	333	165.18	1238.83
10	333	417	122.45	918.36
11	500	667	117.46	880.98
12	333	417	99.85	748.87
13	333	417	115.29	864.70
14	333	500	160.98	1207.37
15	367	417	33.04	247.81
16	333	583	77.83	583.69
17	333	417	115.53	866.49
18	333	333	91.42	685.65
19	250	208	105.39	790.46
20	333	417	120.27	902.03
Media	320.8	456.3	120.38	902.6
D. E.	79.6	140.1	39.34	295.1
C. V	24.8	30.7	32.69	32.69

Source: Questionnaire given to cocoa growers 2014.
[a]*Dry cocoa.*
[b]*Carbon in aboveground biomass.*
[c]*Estimated price of a ton of C = US $ 7.5.*

Table 3.
Income (US $) from sale of cocoa in pulp and potential sale of carbon, from cocoa-AFS, in Cárdenas, Tabasco, Mexico.

First, these growers attach little importance to their plantations because they depend mainly on other crops such as sugarcane, while their cocoa-AFS is only an additional option. The second factor causing low yields is the moniliasis disease caused by the fungus *Moniliophthora roreri*, which attacks the cocoa pods directly and can cause 20–80% yield losses.

The average estimated production of carbon (C) in cocoa-AFS was 120.35 t ha^{-1}. At a price of US$ 7.50 per ton of C, average incomes were calculated at US$ 914.3 h^{-1}. Thus, sale of environmental services would provide 50% more income than cocoa production. Moreover, payment for C sequestration involves conserving trees, mainly young trees since protecting them increases the amount of C captured by the cocoa-ASF. However, because payment of environmental services is a slow process, an option for cocoa growers is to sell FTR and FNTR to obtain more income from their cocoa-AFS.

Cocoa-AFS have been caught up in a vicious cycle since 2005 when moniliasis came to Mexico depleting the cocoa production. When the grower considers only cocoa production and obtains low yields (and incomes), he stops investing in his plantation (time invested in its management decreases). With little or no care of the plantation (less pruning, little or no disease control, less or no fertilization), crop yield is reduced. The average yield in dry weight estimated in this study is below the national average (430 kg ha^{-1}) [20] and that of the Ivory Coast (550 kg^{-1}), the largest cocoa producer in the world [25].

3. Timber trees from the cocoa-AFS and their potential use

3.1 Materials and methods

3.1.1 Study area and sampling sites

The study was conducted in 20 cocoa-AFS in different localities of the municipality of Cárdenas, Tabasco. Experimental plots (50 × 100 m) were set up on Eutric Fluvisols (FLeu) and Eutri-gleyic Fluvisols (FLeugl). These soils are the typical soils of the study area and of the cocoa-AFS [26]. The cocoa-AFS for sampling were defined by interviews to local authorities in order to contact cooperating producers.

3.1.2 Tree sampling and taxonomic identification

For each one of the 20 cocoa-AFS, the age and surface were recorded before the tree sampling. Trees were numbered by painting on them; then they were identified taxonomically and located with a Global Positioner System (GPS, Garmin model GSmap60csx ®) [27]. Tree sampling consisted on record the variables: diameter at breast height ($DBH_{1.3\,m}$, cm) measured with a diametric tape, total height (Th, m), and commercial height (Ch, m) measured with a Haga® Pistol. Basal area (BA, m^2) was estimated with the equation $BA = (DBH/2)^2 \times \pi$. Total and commercial volume (TV, CV, m^3) were estimated with the equation $V = BA \times ff \times H$, where ff = form factor (0.70) and H = total or commercial height [23, 24].

3.1.3 Canopy classification and potential use of trees

Tree canopy was classified based on height [28]: <5 m high (very low stratum), ≥5–<15 m (low), ≥15–<25 m (medium), and ≥25 m high (high stratum). DBH data of trees was classified by categories (1–10 cm, 10–20, 20–30 cm, etc.) to calculate the frequency per class [14, 27]. The potential use per tree was defined in function

of DBH [29]: DBH < 5 cm (without use), ≥5–<10 cm (firewood), ≥10–<15 cm (posts), ≥15–<30 cm (narrow boards), and DBH ≥30 cm (thick boards).

3.2 Results and discussion

The total surface sampling was 10 out of 36.5 ha of cocoa-AFS visited. The mean surface per cocoa-AFS was 1.8 ha, varying from 0.5 to 5 ha, which indicate that cocoa-AFS belongs to smallholder producers. In Ref. [13], a mean surface of 1.3 ha per cocoa plantation, varying from 0.25 to 15 ha, is reported in Talamanca, Costa Rica.

3.2.1 Tree flora composition

In the 10 ha of cocoa-AFS sampled, 2856 forest trees were found, belonging to 67 species, 58 genera, and 28 families. In another work [12], 6 ha in Tabasco Mexico were sampled and 38 species, 35 genera, and 24 families were found. Also in Tabasco, [9] recorded 40 species of 19 families in a survey of 72 producers, while [5] in the Soconusco region in Chiapas, Mexico, recorded 790 trees belonging to 23 families, 38 genera, and 47 species in 7.2 ha. In our study, we recorded more tree families and species than that reported in [9, 5]. In contrast, [30] in Brazil recorded 2514 trees belonging to 293 species and 52 families, i.e., less trees and more diversity than ours. It could be attributed to the cleared rainforests areas where cocoa-AFS were located or due to the larger sampling (15 ha). Besides, [31] in Nigeria reported 487 trees belonging to 45 species and 24 families in 1.3 ha sampled.

The mean number of species per hectare was 14, ranging from 6 to 35. The most common species were *E. americana* and *C. odorata*. For Bolivia, [14] reported the species Mahogany (*Swietenia macrophylla*), Brazilian firetree (*Schizolobium parahyba*), and Roble (*Amburana cearensis*) as the more frequent per hectare.

In our study, we found 286 trees ha^{-1}, ranging from 96 to 618 trees ha^{-1}, as mean density. Fabaceae and Meliaceae were the most frequent families. Somarriba et al. [32, 33] recorded 278 trees ha^{-1} in Panamá and Costa Rica; in Venezuela 300 trees ha^{-1} were reported [34], while in Brazil 47–355 trees ha^{-1} were reported [30]. Results obtained from our study are in agreement with four previous studies mentioned here concerning tree density per hectare, and that Fabaceae is the most commonly used tree family for shading cocoa.

3.2.2 Frequency of tree species by cocoa-AFS age

The age of cocoa-AFS sampled varied from 6 to 35 years old. In this entire range of ages, *Moté* (*E. americana*), Spanish cedar (*C. odorata*), and *Cocoite* (*G. sepium*) were outstanding (**Table 4**). These results agree with [10] who stated *E. americana* (25% of the shade trees) in Cárdenas, Tabasco, but differ with the same author for *G. sepium* (75% of the shade trees). It was cited for Brazil [30] that *Schefflera morototoni* (Aubl.) Maguire (8%) and *Artocarpus heterophyllus* Lam. (7%) are the most common shading species in cocoa. In Nigeria the tree species *Elaeis guineensis* Jacq., *Cola nítida* (Vent.) Schott et Endl., *C. sinensis, Mangifera indica, Anacardium occidentale* L., *Psidium guajava* L., *Persea americana* Mill., *Ricinodendron heudelotii* Muell. Arg., *Citrus reticulata* L., and *Cocos nucifera* L. summed 76% of the scored trees [31]. In Talamanca, Costa Rica, *C. alliodora, Citrus* spp., *C. nucifera*, *Inga* spp. and *C. odorata* were outstanding [13], but in Bolivia, the outstanding species were *S. macrophylla, S. parahyba*, *A. cearensis*, *Centrolobium ochroxylum*, and *C. odorata* [14]. Comparing our results with those of such studies, we found that

Common and scientific name of the species	Number of trees	Frequency (%)
Moté, *Erythrina americana* Mill	812	28.4
Spanish cedar, *Cedrela odorata* L.	573	20.1
Cocoite, *Gliricidia sepium* (Jacq.) Walp	247	8.7
Tatúan, *Colubrina arborescens* (Mill.) Sarg.	246	8.6
Chipilcohite, *Diphysa robinioides* Benth	188	6.6
62 other species	790	27.6
Total	2856	100.0

Table 4.
Amount and frequency of the most common trees in cocoa-AFS from 6 to 35 years old in Cardenas, Tabasco, Mexico.

species are similar, but tree density is different. It is because the shading species in the cocoa-AFS vary among countries and among regions into the same country.

In 6, 20, and 25-year-old cocoa-AFS, *C. odorata* was the most frequent shading tree species at 78.0, 41.9, and 15.1% frequencies, respectively. In 15- and 20-year-old cocoa-AFS, the most common species was *G. sepium* (35.1 and 27.1%). At 18-, 25-, and 30-year-old cocoa-AFS, the most frequent species was *Erythrina americana* (46, 45.4, 56.6%, respectively). In 18- and 35-year-old cocoa-AFS, *Tabebuia rosea* was the most frequent species (15.2 and 15.8%). *Diphysa robinioides* with 47.8 and 38.5% frequencies was the most common species in 27- and 33-year-old cocoa-AFS, whereas *C. arborescens* with 17.5, 12.8 and 16.9% frequencies was the most common shading tree species in 30-, 33-, and 35-year-old cocoa-AFS (data not tabulated).

3.2.3 Species with greater basal area by age of cocoa-AFS

The main tree species by the largest BA in cocoa-AFS from 6 to 35 years old were *E. americana* with 6 m^2 ha^{-1}, *E. poeppigiana* 3.8 m^2 ha^{-1}, and *C. odorata* with 1.6 m^2 ha^{-1}. Each one of the other 64 species had ≤1 m^2 ha^{-1} of BA. By age, the smallest BA (12.2 m^2 ha^{-1}) and the largest BA (22.7 m^2 ha^{-1}) were recorded on the 20- and 25-year-old cocoa-AFS, respectively (**Figure 4**).

In 6- and 20-year-old cocoa-AFS with 15.7 and 2.2 m^2 ha^{-1}, respectively, a main species by BA was *Cedrela odorata*. In 6- and 18-year-old cocoa-AFS with 0.6 and 1.9 m^2 ha^{-1}, respectively, the main species was *Guazuma ulmifolia*. In 15-, 25-, and 30-year-old cocoa-AFS, with 7.8, 9.1, and 1.9 m^2 ha^{-1}, respectively, a main species was *E. poeppigiana*. In 15-, 20-, and 35-year-old cocoa-AFS with 4.3, 2.7, and 3.5 m^2 ha^{-1}, respectively, the main species was *Gliricidia sepium*. In 15-, 18-, 25-, and 30-year-old cocoa-AFS with 3.3, 8.3, 8.2, and 14 m^2 ha^{-1}, respectively, the main species was *E. Americana*. *Mangifera indica* was the main species in 18-, 30-, and 33-year-old cocoa-AFS with 1.4, 0.9, and 2.6 m^2 ha^{-1} BA. *Samanea saman* in 25- and 33-year-old cocoa-AFS with 1.7 and 7 m^2 ha^{-1} BA was the main species. *Diphysa robinioides* with 3.5, 2.5, and 1 m^2 ha^{-1} BA was the main species in 27-, 33-, and 35-year-old cocoa-ASF (**Figure 4**).

The mean basal area (BA) of all the scored trees was 18.5 m^2 ha^{-1} and a range of 8.3–34.6 m^2 ha^{-1}. In Cárdenas, Tabasco [12] stated 48.2 m^2 ha^{-1} as average BA and *S. saman* with 12 m^2 ha^{-1}, *D. robinoides* 7.8 m^2 ha^{-1} and *G. ulmifolia* with 5.6 m^2 ha^{-1} as the main species. Such BA values are greater than ours because their reported species have higher frequency and bigger diameter, e.g., *S. saman*.

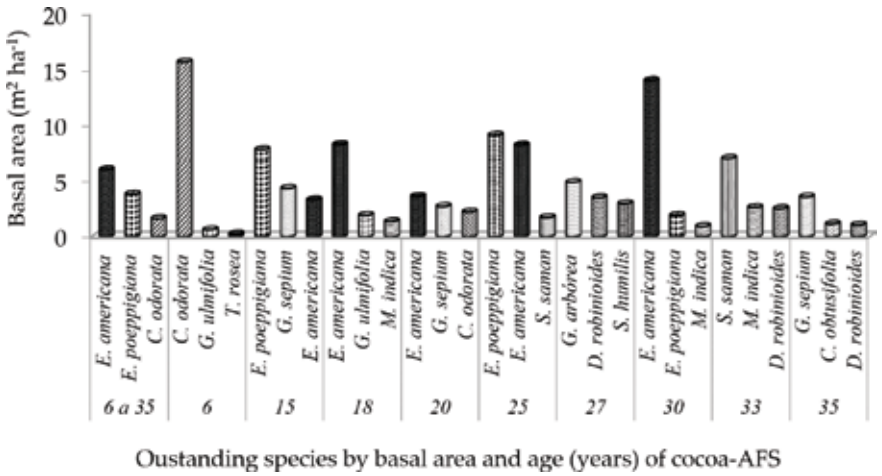

Figure 4.
Tree species with the largest basal areas (m^2 ha^{-1}) in cocoa-AFS of different ages in Cardenas, Tabasco, Mexico.

In Panamá, a mean of 11 m^2 ha^{-1} was reported; the species *C. alliodora* (12 m^2 ha^{-1}), *T. ivorensis* (11 m^2 ha^{-1}), and *T. rosea* (10 m^2 ha^{-1}) had the largest BA [32]. In Lima, Peru, a mean BA of 5.71 m^2 ha^{-1} was reported; the species with the largest BA were *Inga* sp., *Citrus nobilis*, and *Piptadenia favia* [35]. In Costa Rica, Somarriba and Domínguez [36] reported 4.1 m^2 ha^{-1} of mean BA; *T. ivorensis* with 52 m^2 ha^{-1}, *T. rosea* 4.5 m^2 ha^{-1}, and *C. alliodora* with 2.8 m^2 ha^{-1} were the main species. By the way, [37] stated 4.8 m^2 ha^{-1} of mean BA in Honduras. Our recorded BA values are higher than those of the four previous authors cited maybe because their sampled trees were younger and smaller on diameter.

3.2.4 Total timber volume and commercial volumes (TV, CV)

There is a large quantity of timber in cocoa-AFS that can and should be used in a sustainable way. A TV of 1923.8 m^3 in logs was found in the 20 sampled sites. The average TV was 192.4 m^3 ha^{-1}. It ranged from 70.4 to 619.86 m^3 ha^{-1}. Ten species accounted for 87.4% of the TV; the outstanding species were *E. poeppigiana* 33.5% (64.4 m^3 ha^{-1}), *E. americana* 20.9% (40.3 m^3 ha^{-1}), and *C. odorata* 8.1% (15.5 m^3 ha^{-1}) (**Figure 5**). In Panama, the species *C. alliodora* (90 m^3 ha^{-1}), *T. ivorensis* (81 m^3 ha^{-1}), and *T. rosea* (46 m^3 ha^{-1}) were reported as having larger volumes than those of our study [32], as they were established preferentially for shade. The values were similar to those reported in Honduras for the species *Cordia megalantha* 118 m^3 ha^{-1}, *Tabebuia donnell-smithii* 33.9 m^3 ha^{-1}, *Cojoba arborea* 33.5 m^3 ha^{-1}, and *Vitex gaumeri* 31.6 m^3 ha^{-1} [37]. In Costa Rica, *Terminalia ivorensis* with 35 m^3 ha^{-1}, *Cordia alliodora* with 21 m^3 ha^{-1}, and *Tabebuia rosea* with 19 m^3 ha^{-1} TV were reported [36].

In the 20 cocoa-AFS sampled, we recorded 526.29 m^3 log of CV; the mean was 52.6 m^3 ha^{-1} varying from 21.9 to 146.7 m^3 ha^{-1}. The ten main species summed 82.9% of CV; among such species, *E. poeppigiana*, *E. americana*, and *C. odorata* with 27.4% (14.4 m^3 ha^{-1}), 18.7% (9.9 m^3 ha^{-1}), and 11.9% (6.1 m^3 ha^{-1}), respectively, were the most outstanding species (**Figure 6**). For cocoa-AFS in Costa Rica, the species *C. alliodora* was stated with the greatest CV (31 m^3 ha^{-1}), which is due to its preference for shading tree; in the same study, the species with smaller CV was *Cedrela odorata* [38], which is in agreement with our results.

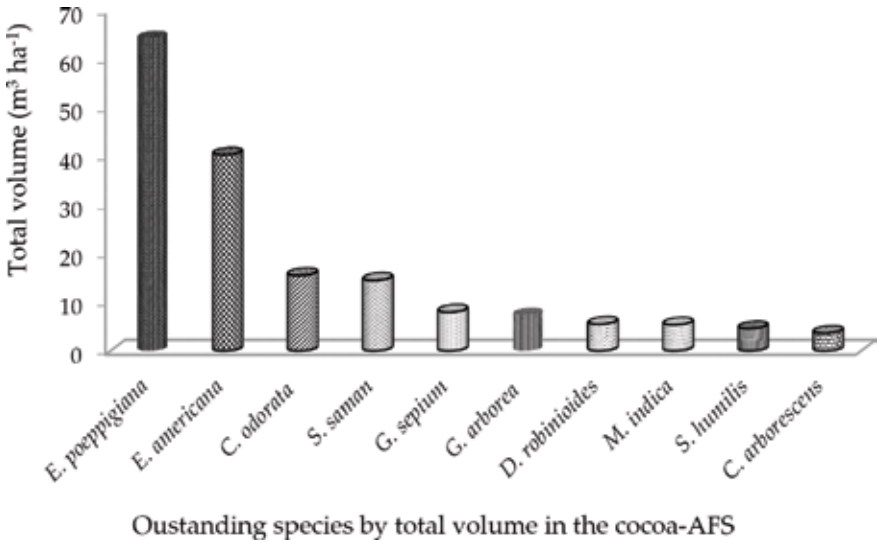

Figure 5.
Tree species with the highest timber volumes (m^3 ha^{-1}) in cocoa-AFS in Cardenas, Tabasco, Mexico.

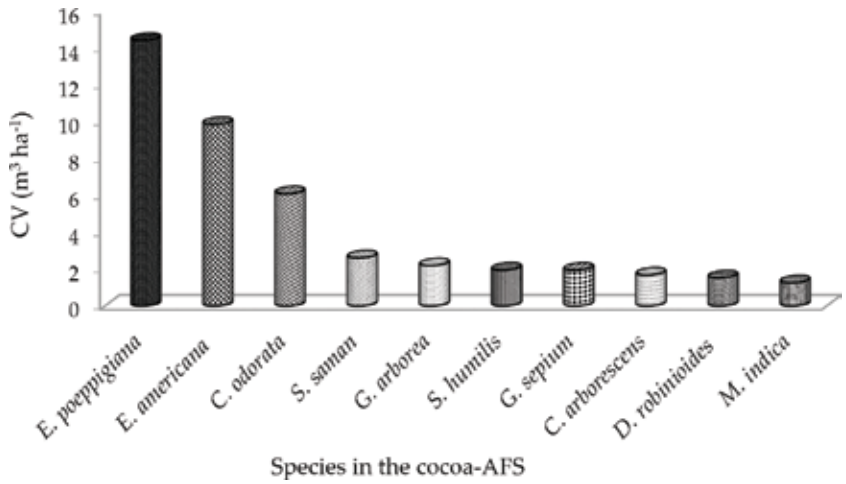

Figure 6.
Main tree species by commercial volume (CV, m^3 ha^{-1}) in the cocoa-AFS, in Cardenas, Tabasco, Mexico.

3.2.5 Classification of tree canopy by height

A mean total height of 10.1 m varying from 2 to 35.5 m was registered for trees in the cocoa-AFS sampled. In the very low stratum, the 5.8% of trees were classified. These trees were *C. odorata*, *T. rosea*, and *C. arborescens* young plants. The low canopy stratum included 84.2% of trees, whereas the high canopy included 1% of trees (**Figure 7**). This 1% grouped species such as *E. poeppigiana* (Erythrina), *S. saman* (Samán), and *A. altilis* (Chestnut). In Cárdenas, Tabasco, some trees of 36 m, mainly of the species *S. saman, G. ulmifolia*, and *C. arborescens,* were reported [12], evidencing that cocoa-AFS contains tree species of similar height to those found in the tropical rainforests. In Talamanca, Costa Rica, up to 30 m for the upper canopy of cocoa-AFS is reported [13]. In Panama and Honduras, average heights of 17 and 13 m have been reported [32, 37]; such heights are higher than those recorded in our study.

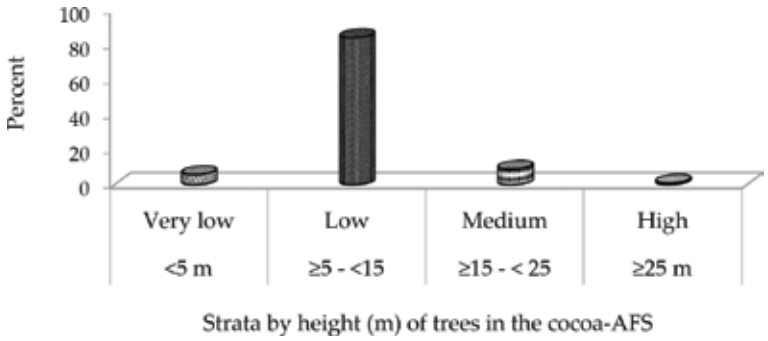

Figure 7.
Trees distribution by height (m) in cocoa-AFS in Cardenas, Tabasco, Mexico.

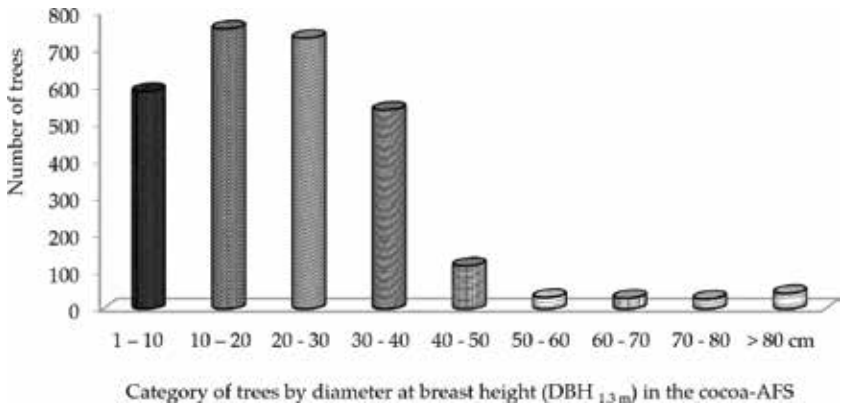

Figure 8.
Categories of trees by diameter at breast height ($DBH_{1.3m}$) of the cocoa-AFS in Cardenas, Tabasco, Mexico.

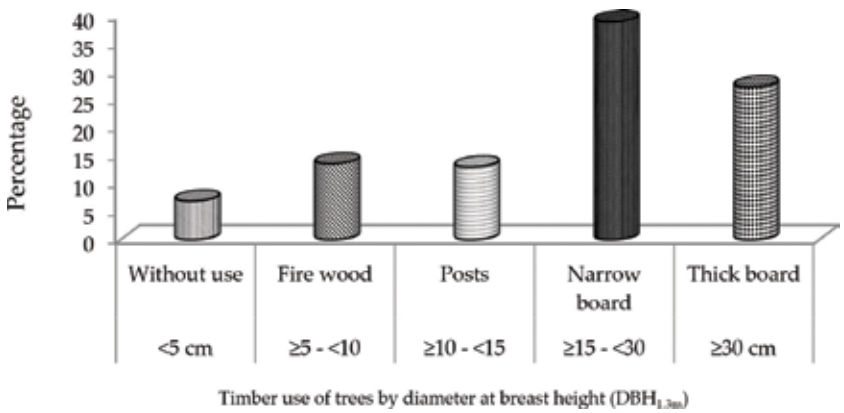

Figure 9.
Potential timber use by diameter at breast height ($DBH_{1.3m}$) of trees from the cocoa-AFS in Cardenas, Tabasco, Mexico.

3.2.6 Tree classification by diameter at breast height and potential use

The mean diameter at breast height ($DBH_{1.3\ m}$) was 23 cm varying from 1 to 146 cm. The 91% of the 2856 scored trees had a DBH of between 1 and 40 cm. Among these trees, 53% had 10–30 cm DBH (**Figure 8**). In Bolivia, 45% of the

trees in cocoa-AFS had DBH of between 10 and 20 cm [14], while a maximum DBH of 137 cm for some species was reported in Cárdenas, Tabasco [12]. In Panamá [32] and Honduras [37], average DBH of 25 and 28 cm were reported, which are larger than those found in our study, probably because the authors averaged only three timber species, while we averaged all the timber species found in the cocoa-AFS.

According to the DBH, the main timber uses of the registered trees were narrow and thick boards, 39 and 27.4%, respectively, and 6.9% of the trees were recorded without any use (**Figure 9**) because they were reforestation species established in areas without shade. Use varies with species, age, diversity, and culture, among other factors. In Talamanca, Costa Rica, 34% of the trees with use for thick boards were registered [13], and in Bolivia 15% with this use were reported.

4. General conclusions

Cocoa agroforestry systems are made up of a large number of timber and non-timber resources. From cocoa-AFS in Cárdenas, Tabasco, Mexico, information on current use was obtained, and species were quantified. The most common species used as shade trees are *E. americana*, *C. odorata*, *G. sepium*, *C. arborescens*, and *D. robinioides*. The species with the largest timber volumes are *E. poeppigiana*, *E. americana, C. odorata, S. saman*, and *G. sepium.* This timber resource can be used sustainably in different ways. The principal uses of these timber resources were in making narrow boards and thick boards, a function of their diameter at breast height ($DBH_{1.3\,m}$).

Non-timber resources included 6308 plants belonging to 53 species grouped in 29 families. The 53 species were classified by use into five groups: ornamental, fruit, vegetable, medicinal, and forage. Seventy percent of the people do not use the cocoa-AFS resources; 20% use them in their homes, and only 10% sell them. The products of the cocoa-AFS that are used or sold are square banana and plantain, arrowleaf elephant's ear, banana leaves, papaya, platanillo, Moses in the cradle, amashito chili, yerba buena, parsley, bitter melon, achiote, and firewood. Cocoa production is complemented with income obtained from the sale of forest timber and non-timber resources. Income from the sale of environmental services could be up to 50% higher than income from cocoa production.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Section 2

Cocoa Pests and Diseases Management

Chapter 3

Diversity of Cacao Pathogens and Impact on Yield and Global Production

Dele Adeniyi

Abstract

Cacao, *Theobroma cacao* L., an important cash crop in foreign exchange earnings and also a major income source for many smallholder farmers in growing ecologies of West Africa. Global cocoa production has been rising fairly steadily over the years by increasing production in growing countries with most of the production taking place in areas of high pathogen biodiversity. Thus, the sustainability of the cocoa economy is under threat as diseases of various statuses now constitute the most serious constraint to production. Most important among these is the black pod disease caused by *Phytophthora* genus with annual losses of 30–90% of the crop. This economically important pathogen is very diverse in nature and varied across growing countries including species such as *palmivora*, *megakarya*, *capsici* and *citrophthora* distinguished based on chromosome number, sporangial characteristics and pedicel length. World losses of 20–25% in cacao production are due to black pod disease, an estimate of 700,000 metric tons on global scale reducing global cocoa production. High cacao loss to diseases is a prime factor limiting production; consequently, significant effort is required to deal with problems associated with disease control to ensure a sustainable cacao. The effective and sustainable management of black pod disease requires integrated approach encompassing different control measures.

Keywords: *Phytophthora*, pathogen, diversity, yield, production, management

1. Introduction

Cacao, *Theobroma cacao*, is a major cash crop in the tropics and the source of chocolate, one of the world's most popular foods. In addition, cacao-based agroforestry systems provide a promising means to address the challenges of deforestation and create a habitat for biodiversity while simultaneously providing a profitable crop for agricultural communities [1]. Cocoa is mainly grown by smallholder farmers in West Africa and around the world where favourable tropical environments occur. The farmers plant their cocoa traditionally at random under thinned forest and/or plantain as shade crop. Moreover, when grown in traditional form under thinned, forest shade, cacao affords sustainable benefits not only to the farmer but also to the environment [2]. This low-input cultivation system uses the forest soil fertility and the existing shade.

2. Cocoa production status

The West Africa region has some 6 million hectares of cocoa and provides around 70% of the total world production. In recent time, Côte d'Ivoire, Ghana, Nigeria and Cameroon have been rated as top cocoa-producing countries and the production from around 2,000,000 metric tons to around 3,000,000 metric tons in 10 years plus [3]. The world cocoa production is around 4.3 million tons, and almost 71% of it produced in a relatively small region of West Africa which comprised of Cote d'Ivoire, Ghana, Nigeria and Cameroon with 56, 29, 8 and 7% productions, respectively [3].

However, the average yields remain low, and this could be attributed to many factors ranging from pests and diseases to old and moribund farms and extensive cultivation methods, among others. Steady growths in cocoa production have been reported in Nigeria; production increase from 165,000 metric tons in 1999–2000 to 250,000 metric tons in 2013–2014 has been linked to high grower prices and government support to a limited extent through the 2011 Cocoa Transformation Action Plan [4]. The total harvested area in Nigeria was reported as 640,000 hectares with the average yield of about 400 kg per hectare.

The Cocoa Transformation Action Plan of the Federal Government of Nigeria envisaged improving cocoa situation and rising production to 500,000 metric tons by 2015; however, the yield improvement constrains were required to be better managed. The crop is seriously affected by the impact of diseases and the low-yielding potential of most plantations due to genetic and management reasons [5]. The sustainability in cocoa is currently under an increasing threat as both coevolved and new-encounter diseases of various statuses now constitute the most serious constraint to cacao production [6, 7] (**Figure 1**).

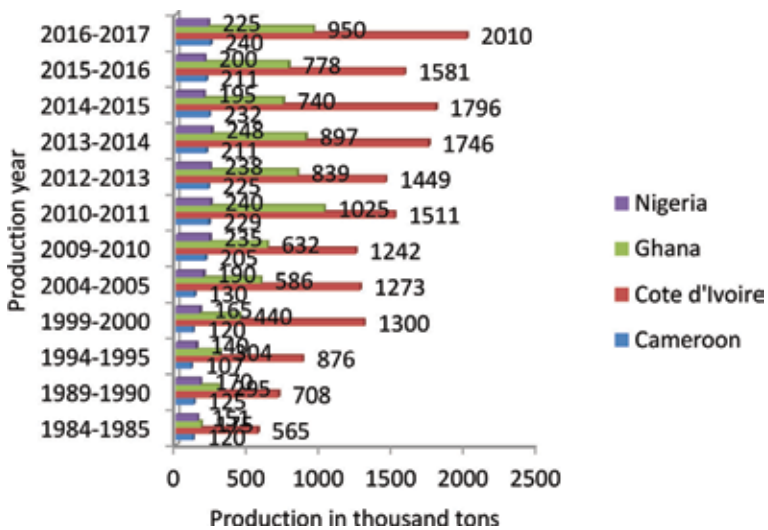

Figure 1.
Shows the status of cocoa production in growing countries of West Africa. (Sources: [8–10]).

3. Pathogen and disease distribution

The cacao trees in the absence of disease infestation and good farm management provide improved productivity to the maximum of the potential of the crop, under ideal field condition (**Figure 2**).

Figure 2.
Healthy cocoa trees at varied stages (A, B and C) of maturity.

However, many factors including poor farm management can introduce diseases or reawaken the inoculum from their resting stage for infection. *Phytophthora* pod rot, commonly called "black pod", is the most economically important disease of cocoa. Four species of *Phytophthora* are mainly responsible for this disease, *P. palmivora*, *P. megakarya*, *P. capsici* and P. *citrophthora*, while four additional species of *Phytophthora* have also been isolated from cacao, *P. katsurae*, *P. arecae*, *P. nicotianae* and *P. megasperma* [11], but no economic impact of these has been reported. *Phytophthora palmivora* is the most common, being present in most of the cacao-growing countries around the world, causing yield losses of 20–30% and tree deaths of 10% annually. *Phytophthora megakarya* occurs only in West and Central Africa countries but considered to be the most virulent among other species on cacao. *Phytophthora capsici* is widespread in Central and South America and prevalent in Brazil.

The estimate of genetic diversity and structure of *Phytophthora* isolates from Ghana, Togo, Nigeria, Cameroon, Gabon and Sao Tome using the isozyme and RAPD markers [12] separated the isolates into two different genetic groups, with one located in Central Africa and the other in West Africa. The two centres of major diversity are located in Cameroon and on the Cameroon/Nigeria border region. This distribution however coincides with two major biogeographical domains reflecting an ancient evolution of *P. megakarya.* A lower genotypic diversity was also found in isolates from Ghana, Togo and Nigeria when compared with isolates form Gabon and Sao Tome. Again, four intermediate marker patterns were observed which correspond to isolates near the border between Nigeria and Cameroon and assumed it is a genetic exchange between the Central and West African groups. Black pod disease incidence in the field is influenced by environmental conditions. Numerous studies have established the role of climatic factors on incidence of black pod disease, caused by *Phytophthora* spp. [13, 14]. Rainfall, high relative humidity and low temperature are known to create favourable humid conditions for the development of the disease. [13] showed that in Ghana, black pod disease developed when the relative humidity, particularly within the day, remained above 80% under the cocoa canopy and that the rate of disease development was influenced by the frequency and amount of rainfall. [14] also reported a significant positive correlation between rainfall when assessed after 1-week lag, and *P. megakarya* pod rot incidence in Cameroon, and emphasised the role of rainfall in the disease epidemics [14]. Further, the time and/or period for black pod peak infection in Ghana varied annually and also with location depending on the rainfall [15].

In Ghana, it is known that primary infections usually occur around June, but the peak of *P. megakarya* black pod disease generally occurs between August and October [16, 17]. Such information on periods for attaining disease infection peaks is useful in forecasting the pattern of disease development, and it is an important tool for disease management since conditions immediately preceding the infection peaks must be favourable for disease development. The black pod disease situation in Nigeria is similar to that of Ghana and depends on growing ecologies, pattern of rainfall, high humidity and farmers' management practices. The disease inoculum can remain in the soil for a long time, the spores are brought back to viability at onset of rain and other conditions are suitable. Thus, rain splashes, infected tools and equipment and poor farm management, among others, contribute to the spread of the pathogen in the field.

3.1 Expression of black pod and *Phytophthora* on cacao

Phytophthora pathogens are ubiquitous and so cause economic loss to a greater or lesser extent in all cocoa-producing countries but most especially in those with prolonged periods of high relative humidity at, or near to, saturation levels. It infects every part of cacao plants at different developmental stages [18] and under wet and humid atmospheric conditions. *Phytophthora palmivora* is present in most of the cacao-growing countries around the globe and has a broad host range [19]. *Phytophthora megakarya* occurs only in the countries of West and Central Africa and is considered a significant pathogen only on cacao. Black pod was originally thought to be caused by a single species, *P. palmivora*, but increased knowledge have shown otherwise over the past few decades and that each continent has a complex of species of *Phytophthora* which can induce black pod symptoms in cacao. Thus, the main pathogen especially in Nigeria is *P. megakarya*, which was thought to be a variant form of *P. palmivora* but was first identified taxonomically as a species [20].

Under nursery operation, seedling infection leads to blight and root rot, while infections of stem, chupons and branches cause cankers [21, 22]. Infection of the pod leads to black pod which can occur at any stages of pod development, and all parts of the pod are also susceptible to infection [22]. However, immature pods of 10 and 20 weeks have the highest disease incidence when the dynamics of pod production and black pod disease were evaluated in relation to environmental factor impact, chemical fungicide and biological control [14]. Infected immature pods are rendered useless, while infection of ripe pods reduces the bean quality [4].

The black pod caused by all *Phytophthora* species is developed by an initial symptom showing appearance of a small translucent spot on cocoa pods [22], appearing around 2–3 days after infection, then turns brown, eventually darkens and the spot covers the entire pod between 7 and 14 days under humid conditions. Whitish spores are produced 3–5 days after the appearance of the first symptom. These species attack pods of all sizes and are harboured in the roots of cocoa during the dry season making it very hard to control [23].

Black pod symptoms due to *P. megakarya* are, however, characterised by multiple lesions which spread fast and coalesce (**Figure 3**) showing abundant bloom of white zoosporangia on the lesion except for about a centimetre from the advancing margin of the lesions (arrowed). Pods at every stage of development may be infected, and infection may start from the proximal, distal or lateral (**Figure 4A–C**) portion of the pod, and extreme cases of black pod could also affect pods at different stages of development.

Cacao fruits can become infected at all stages from setting to maturity. Observations in Nigeria suggest that the relative frequencies of different infection sites may be affected by fruit length. It was found that the mean length of distally

Figure 3.
Coalescing lesions.

Figure 4.
Sites of pod infection in black pod disease. A, proximal; B, distal; and C, lateral.

infected fruits tended to be less than the mean length of either laterally or proximally infected fruits. These observations can be interpreted as indicating that distal infections tend to occur on relatively shorter and younger fruits, as compared with laterally and proximally infected fruits [24]. It was suggested that proximal infection might be favoured through moisture being retained in the annular depression where the stalk is inserted and at the distal ends of young fruits [25]. However, in Nigeria, the annular depression at the base may not necessarily be favourable for infection as compared with the distal end of the pod.

Figure 5.
Densely sporulating cacao pods indicating the presence of Phytophthora megakarya.

The predominance of *P. megakarya* on cacao in Nigeria started in the 1980s, alongside Cameroon, Equatorial Guinea, Gabon and Togo [22]. Recent studies showed that *P. palmivora* is no longer routinely isolated from cacao in Nigeria and Cameroon [12, 26, 27]; however, the displacement of *P. palmivora* by *P. megakarya* from cacao in these countries remains unclear [28]. However *P. megakarya* continues to be the major actual and potential threat to cacao in West Africa [16]. The much denser sporulation exhibited by *P. megakarya* on the surface of cacao pod (**Figure 5A–C**) indicates greater virulence of this species than *P. palmivora* and such significantly increases inoculum loads of *P. megakarya* [7, 29].

4. Characteristic and genomic diversity of *Phytophthora* species

Five major diseases of cacao (*Theobroma cacao* L.), *Phytophthora* pod rot (black pod), witches broom, swollen shoot virus, vascular streak dieback and monilia pod rot, account for over 40% annual loss of cocoa [30]. Correct identification of plant pathogens is critical and fundamental to population genetics, epidemiological studies and the development of disease control strategies. Due to the similarity in growth patterns of *Oomycetes* including *Phytophthora* species and fungi, *Oomycetes* were previously considered as a class within the fungi. Fundamental differences between *Oomycetes* and fungi have been established [31–33], and the two are now known to be taxonomically distinct in spite of their common infection strategy [34]. As a result of the initial consideration of *Oomycetes* as a class within the fungi, [35] reported that researchers have for several decades pursued a wrong track in addressing the menace caused by *Phytophthora infestans.* For example, chitin was earlier reported as a minor component of oomycete cell walls and, therefore, insensitive to chitin synthase inhibitors, but it is now known to be an important component of hyphal tips in *Oomycetes* [36]. Isolations of *P. palmivora* from diseased cacao pods in Nigeria have been found to be "typical" in culture [37] with respect to

general characters including early production of sporangia. *Phytophthora palmivora* tends to have a more rapid growth rate than *P. megakarya* in culture, possibly contributing to its ability to cause accelerated necrosis in mechanically wounded cacao tissues compared to *P. megakarya* [38]. There have been no indications of important local variations with respect to the characters of this pathogen nor as regards the nature of the black pod rot infection. Variations in dimensions of sporangia were in respect to age and nature of substratum.

Phytophthora palmivora was first considered the only causal agent of black pod disease. However, a reclassification of some of the isolates previously described as *P. palmivora* into distinct species was suggested [39, 40]. Classification of species within the genus *Phytophthora* has progressed through the use of several criteria, including morphological dataset of colony, sporangium and oogonium characteristics; the presence or absence of chlamydospores and hyphal swellings, physiology [20, 41], isozyme patterns [42]; and lately the combined use of molecular markers and morphological characteristics [43]. Consequently, based on the size and number of chromosomes, they introduced the S- and L-type designations, which represented isolates having comparatively smaller chromosomes with n = 9–12 and isolates having large chromosomes with n = 5, respectively. However, the earlier work of [20] and recently [28] has established the variation in genetic characteristics of *Phytophthora* species commonly associated with cacao in Nigeria, and *P. megakarya* was found as the most virulent of the species.

Consequently, the species were reclassified into three types: chromosome number, sporangial characteristics and pedicel length [20]. The S-type was regarded as *P. palmivora* sensu Butler (MF1) with 9–12 small chromosomes, papillate sporangia varying from near spherical to ovate-elongate shape and a short pedicel (2–5 μm) and being worldwide in distribution. The L-type was reclassified as *P. megakarya* (MF3), with five to six large chromosomes, papillate near spherical sporangia shape and pedicel of medium length (10–30 μm) and found only in West and Central Africa. Thus, the name "megakarya" is derived from the relatively large (mega) chromosomes. The third group was classified as *P. capsici* (MF4), with characteristics similar to *P. capsici* from black pepper [44, 45], and had longer pedicel (20–150 μm). The MF2, however, remains a variant of *P. palmivora.* The occurrence of hybridization is an important phenomenon in *Phytophthora*, given that hybridization may result in genetic variation that will adapt to new hosts or environments. Further confusion in the *P. palmivora* complex can occur due to heterothallic mating behaviour of the species. Sexual reproduction in *P. megakarya* and *P. palmivora* results in the production of oospores, and this requires the two opposite mating types, A1 and A2. Mating types in *P. megakarya* and *P. palmivora* show a curious imbalance, with A1 predominating in *P. megakarya* and A2 in *P. palmivora* [20]. This imbalance in mating types might favour hybridization between species, but not sexual reproduction within species. In spite of the two species coexisting on cocoa fields, no hybrids have been observed. The differences in chromosome numbers between *P. megakarya* and *P. palmivora* may also hinder hybridization and, hence, the rare occurrence of oospores in nature. *Phytophthora megakarya* was first described as a new *Phytophthora* species on *cacao* in West Africa based on chromosome number, sporangial characteristics and pedicel length. *Phytophthora megakarya* is indigenous to West and Central Africa, and it has become the main yield-limiting factor for cocoa production in affected areas [17] and rapidly surpassing *P. palmivora*.

In a susceptible cacao genotype, mechanical wounding may not be required for infection establishment in *P. megakarya* [38]. The genome size of *Phytophthora*

megakarya and *P. palmivora* was estimated at 126.88 and 151.23 Mb, respectively and number of genes 42,036 and 44,327, respectively [46]. *Phytophthora palmivora* appeared to have gone through whole genome duplication and subsequent gene diversification which expanded its genetic capacity for nutrient acquisition and breakdown of complex structures like the cell walls. This capacity may have influenced *P. palmivora* vigorous growth and broad host range, even without extended co-evolution with cacao. *Phytophthora megakarya* on the other hand has undergone amplification of specific gene families, some of which are clearly virulence-related like RxLRs, CRNs, elicitins and NPPs [46]. During *Phytophthora* infection, appressoria release effectors even before penetrations that enter host cells in an attempt to suppress pathogen-associated molecular pattern (PAMP)-triggered immunity [47]. Under the conditions of high and frequent rainfall in Cameroon, *P. megakarya* can cause yield losses of up to 100% when no control measures are taken [48]. In Ghana, losses ranging between 60 and 100% have been reported [15].

Some other species of *Phytophthora* have been reported on *cacao* and such include *P. botryosa*, causing cacao pod rot in Malaysia, and *P. citrophthora* in Bahia, Brazil [49, 50]. *P. capsici*, *P. citrophthora* and *P. heveae* were reported in Mexico [51], *P. katsurae* in Côte d'Ivoire [52] and *P. megasperma* in Venezuela [45]. Apart from the cosmopolitan *P. palmivora*, the other species have only been recorded in certain countries or geographical location/regions. However, factors responsible for the geographical separation of the *Phytophthora* species are yet to be elucidated, but it is possible that lack of intensive surveys, coupled with isolation of isolates from the same location, from a few plant species and on a narrow range of media could be responsible for this observation. Another possibility is that these species occur rarely on cacao; nonetheless, more investigations are required to ascertain these claims.

5. Impact of *Phytophthora* on cacao yield and bean quality

Major economic losses in cocoa production are caused by pests and diseases, particularly in the many small and isolated farms that lack adequate control measure across West Africa region. These species cause mean annual pod losses of about 40% and even higher in parts of Ghana and Côte d'Ivoire [17, 53]. The highest incidence of black pod disease is found in the shaded cocoa in Cameroon. World losses in cacao production due to black pod disease caused by various species of *Phytophthora* have been estimated to cause about 450,000 metric tons [7]. This disease probably accounts for 20–25% of the expected crop and making it the biggest constraint to production. **Figure 6** shows the usual harvesting exercise/activities in cocoa farms where both mature healthy cocoa pods and the disease black pods (arrowed) are usually lumped together on farmer's field and processed.

Losses due to black pod can be especially severe in West and Central Africa, an area that contributes 60–70% to the worldwide cacao production [7]. In Africa, it can cause 30–90% annual crop loss for farmers, and, thus, it poses a severe threat to the cacao industry and to producers. Part of the contributory factors which is major in limiting productivity in cacao is related to the practices of farmers. Apart from the practice indicated in **Figure 6**, the observation of piles of cocoa pod husks on different locations on farmers' fields serves as the sources of inoculum of the pathogen of black pod disease. The spores of *Phytophthora* species on infected cocoa pods are usually left on the field after extraction of the beans (**Figure 7**) and are reactivated under suitable conditions to infect fresh cocoa pods.

Figure 6.
Harvested cocoa pods on farmer's field with black pod (arrowed).

Figure 7.
Cocoa pod husk pile on farmer's field.

In the year 2012, Ghana lost more than 200,000 tonnes of cacao beans (25% of the annual output) due to black pod, costing the nation $230 million (Ghana Cocoa Board report). Until the mid-1980s, only *P. palmivora* was present in all the cacao-growing regions of Ghana, causing limited crop losses of 4.9–19% [54]. After 1985, black pod became a major disease in Ghana and was attributed to the emergence of *P. megakarya* as a pathogen of cacao [55]; various reports from Ghana indicated a rapid spread of *P. megakarya* to various cacao districts by the late 1990s causing 60–100% crop losses [17].

5.1 Impact of *Phytophthora* on cocoa beans

The *Phytophthora* species affects different parts of the cacao, but infection of the pod is the major economic loss as pods or cherelles may be infected at any parts on the surface. Observation of the disease indicates a firm, spreading, chocolate-brown lesion which eventually covers the whole pod. The beans inside the pod may remain undamaged for several days after initial infection of the husk, but in advanced infections, *Phytophthora* invades the internal pod tissues and causes discoloration and shrivelling of the cocoa beans (**Figure 8A–C**), thus tampering with the mucilage colouration (**Figure 9**) and affecting quality of the cocoa bean.

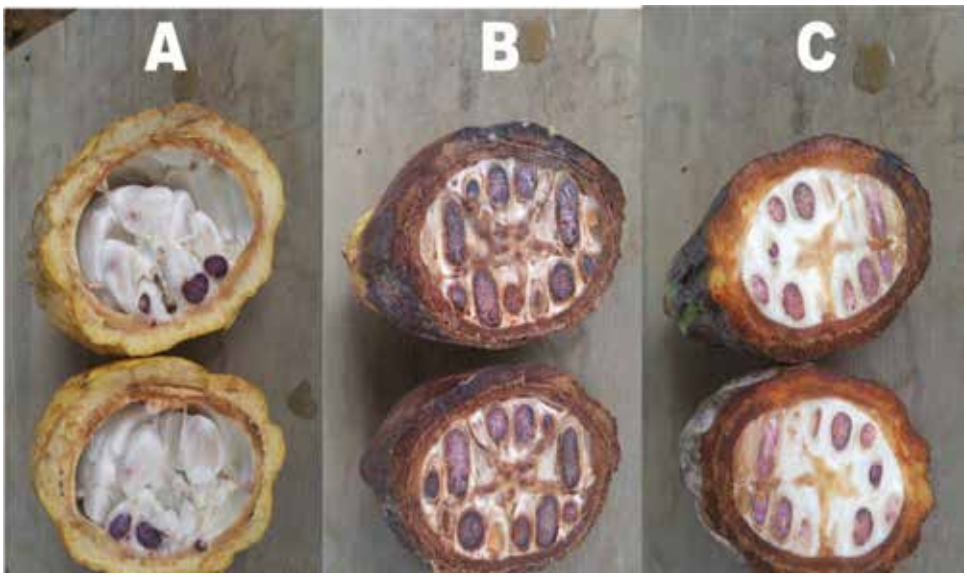

Figure 8.
Status of black pod disease on cocoa bean. A: Ripe and healthy cocoa pod with quality mucilage and beans. B: Ripe but diseased cocoa pod with infected beans. C: Unripe but diseased cocoa pod with infected beans.

Figure 9.
Effect of black pod disease on cocoa mucilage and beans in ripe cocoa pod.

6. Management strategies for *Phytophthora* species

Phytophthora can persist in soil and debris for several years making the control of black pod difficult [56]. Also, since susceptible pods may be present on the trees most of the year, the pathogen may always be present in the canopy, ready to cause major epidemics when environmental conditions become favourable for sporulation and dispersal [29]. Although much research has been published over the past few decades on black pod disease, sustainable management strategies that are applicable to smallholder farms are still lacking in most producing countries. Crop losses and cost of controlling *Phytophthora* (black pod) diseases constitute a significant financial burden on agricultural enterprises and have serious socioeconomic and environmental consequences wherever these pathogens are found. Neglect of cocoa

farms infected with *P. megakarya*, cultivation of crops other than *T. cacao* in infected areas [16] and establishment of *T. cacao* in *P. megakarya*-free forest areas have significant impacts on the economies of the cocoa-producing countries in West Africa. It also has effects on biodiversity and functioning of the natural ecosystems.

Phytophthora megakarya has spread within the West and Central African subregions, and it is still in its invasive phase. The spread of this pathogen from one location to another in Ghana has been linked with the movement of planting materials [16, 57, 58]. The faster communication, travel and trade links and the relatively free movement of people and commodities all over the world pose a serious risk of introducing *P. megakarya* to other cacao-growing regions. On the other hand, there is a risk of introduction of other major cocoa diseases from other cocoa-producing areas into West and Central Africa [59]. This will have negative impact on world cocoa production. A devastating impact on the world's cocoa supply is eminent and extremely serious in social, economic and environmental problems. Such pathogen introduction can also be experienced within a growing country from the region of high incidence to low one. To minimise such risks, preventive measures and effective testing procedures and exchange of materials through intermediate quarantine facilities must be enforced. Consequently, there is an urgent need for effective and sustainable control of *P. megakarya*/black pod disease. The effective and sustainable management of this disease requires integrated approach of several methods, including quarantine, cultural, chemical and biological control and use of resistant cocoa varieties.

6.1 Cultural practices to combat black pod disease

Activities to combat the menace of yield losses and decline in cocoa production resulting from black pod disease incidence in cocoa-growing communities are enormous. Cultural control practices that promote crop growth, inhibit and obstruct pathogen establishment, growth and development is one of the first approaches in plant disease control. Cultural practices are not only essential for increasing yield but also for providing the right environment for efficient performance of fungicides [60]. For the small holdings, low-input and low-income cocoa farmer use of cultural practices remains the least expensive disease control option for managing black pod disease. However, frequent harvesting saves partly infected mature pods, removal of infected pods, and reduces sources of sporangial inoculum. The regular and timely removal of infected pods and reduction in the shade to increase the humidity which in turn reduce pod losses however, additional chemical control by regular spraying of fungicides is required.

In Nigeria, frequent removal of diseased pods complemented sprayed programmes in controlling *P. megakarya*, but, often, excessive tree heights hampered the effectiveness of the technique [61]. Similarly, in Togo, *P. megakarya* diseased pod removal was recommended as part of a package to reduce disease incidence [62]. In Cameroon, inoculum levels were successfully reduced by the pruning and weekly removal of pods but only in concert with spraying [63]. Another cultural method occasionally recommended is the removal or spraying of pod husk piles where they occur on farms (**Figure 7**). It is known that these pod husk piles serve as disease foci on *P. megakarya* farms. In Nigeria and Sao Tome, burying of husks was recommended, but its limited effectiveness and expense caused this option to be dropped [64]. However, in Ghana the husks are burnt into potash and used in the production of soap. Cultural practices on cacao farms are labour intensive and inadequate when applied alone for *P. megakarya* control. They need to be supplemented with other control methods, such as spraying of fungicides to reduce losses on farms [58, 65–67]. Most farmers, however, are unable to adopt this technology because of

the high costs of the fungicides and application problems. In practice little fungicide is used [17, 68]. However, removal of black pods from the soil surface would be a simple strategy to reduce inoculum spread by ants, as well as by flying vectors [69]. Reduction in canopy humidity and consequent sporulation can be achieved by pruning and appropriate tree spacing to increase aeration. Maintenance of leaf litter or mulches to prevent soil inoculum of *P. megakarya* reaching pods has been suggested [70], while leaf litter was found to reduce pod infection from soil inoculum [71]. The spread of the disease from infected pods can be reduced by frequent harvesting to lessen the danger of spread of disease from infected pods. Black pod disease is also managed by regular pruning to remove infected chupons and increase air circulation. Other measures, such as the removal of infected pods and husk piles, may have some effect on inoculum levels.

6.2 Chemical strategies to combat black pod disease

Fungicides have been used to control *Phytophthora* pod rot of cocoa for over a century, and several experiments on different chemical control measures have been conducted in all cocoa-growing countries. The history of the development of fungicides on cocoa has been extensively reviewed [72–74]. Recommendations adopted in the different countries are based on local factors, such as species of pathogen, climatic conditions, cocoa variety, planting density and social and economic considerations [64]. Traditionally, expensive copper-based fungicides (or systemic) have been applied frequently (sometimes every 10 days) in areas of high infection. Without prophylaxis, the losses could reach 100% in areas of continuous high humidity and high disease incidence, although the disease has a normal range of 5–90%. In order to limit yield loss to black pod disease in Ghana, three preventive rounds of copper fungicide were applied during the rainy season under a national spray programme in *P. megakarya* prevalent areas. This however puts immense pressure on resource-poor farmers in the form of reduced farm-gate prices, leading to ecological, socioeconomic and possibly political instability. In Nigeria, many fungicides of varied active ingredients are used by farmers across growing ecologies. The Cocoa Research Institute of Nigeria has the national mandate to screen *in vitro* and *in vivo* such fungicides among other pesticides prior to being used on cacao in Nigeria. Many of the active ingredients (product of different agrochemical companies) that have undergone a 3-consecutive-year field trials include but not limited to copper hydroxide, cuprous oxide + metalaxyl-M, cuprous oxide and metalaxyl-M + copper-1-oxide and recently are copper-1-oxide + metalaxyl, mandipropamid + mefenoxam, initium + dimethomorph and pyraclostrobin + dimethomorph + ametoctradin. The relative effectiveness of certain treatments and inconsistencies in results between countries and locations depends on the different combinations of these factors. For example, while fungicides are applied at two weekly intervals in Cameroon to control black pod disease, due to the relatively high and frequent rainfall, fungicides are applied at three to four weekly intervals in Ghana [16], and spray interval of 3 weeks is also advised in Nigeria. The reason for the difference among countries has to do with the amount and frequency of rainfall.

In West Africa, protectant fungicides that are mainly "fixed" copper compounds, e.g., copper hydroxides and copper oxides, or systemic fungicides containing copper and metalaxyl as mixtures are routinely sprayed onto pods with lever-operated knapsack sprayers for *Phytophthora* pod rot control. These fixed copper compounds are finely divided molecules that are readily mixed and easy to apply at low volumes. This is in contrast to earlier products such as the Bordeaux mixture, which had to be applied in relatively large volumes. These copper fungicides form a

chemical barrier on the surface of the pod and guard against infection [58, 75]. The spraying of copper and metalaxyl mixtures is to take advantage of multisite action of the different active ingredients and to reduce the possible build-up of metalaxyl resistance in *Phytophthora* species on cocoa. Furthermore, it must be emphasised that correct dosage of fungicides, timing of initial application in relation to the epidemic, frequency and target of application are all critical factors to ensure successful and economic chemical control.

6.3 Alternative practices to combat black pod disease

Many natural substances, including plant extracts and bioactive compounds produced by microorganisms, have been tried to control *Phytophthora* on cacao [76, 77]. Rosemary (*Rosmarinus officinalis*) and lavender (*Lavandula officinalis*) leaf extracts when supplemented to agar plates at different dilutions were found to inhibit germination of *P. capsici*, *P. megakarya* and *P. palmivora* zoospores. Rosemary extracts, containing caffeic acid, rosmarinic acid or derivatives, thereof, reduced necrosis of cacao leaf discs caused by *P. megakarya* zoospores [77]. Another promising class of natural microbial compounds with activity against *Phytophthora* species is the cyclic lipopeptides (CLPs) [78–81]. Studies showed that massetolide A (massA) produced by *P. fluorescens* strain SS101 caused zoospore lysis through induction of pores, reduced sporangium formation and increased branching and swelling of hyphae of *P. infestans* [78, 82]. MassA also induced systemic resistance in tomato plants and reduced the number and expansion of late blight lesions on tomato caused by *P. infestans* [83, 84]. Given that hyphae, sporangia and zoospores are important sources of inoculum and play major role in cacao black pod epidemic, there is the need to investigate if CLPs or CLP-producing microorganisms can be exploited for the management of black pod disease caused by *P. megakarya*.

7. Conclusion

Phytophthora megakarya infestation of cacao is a threat to the economies of growing countries in West Africa. This diverse pathogen is spreading fast in the subregion, displacing the original populations of the less severe *P. palmivora*. The mechanisms for this shift in population composition of the black pod disease complex remain unknown, although the possibility of further spread to other cacao-producing regions is a great concern. The available and fast-emerging genomic and genetic information on oomycete pathogens and their hosts, including *Theobroma cacao*, should be utilised and explored for the development of new sustainable management practices for *Phytophthora* species. Current methods of control through routine spraying of inorganic fungicides are expensive, hazardous and environmentally unfriendly. Programmes of integrated pest management with precise fungicide application which give less residue in the cocoa beans, combined with field sanitation and proper farm management, should be encouraged in all areas where *Phytophthora* species cause significant losses on cocoa.

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Section 3

Cocoa Value Added and By-Products for Consumption

Chapter 4

Unsweetened Natural Cocoa Powder: A Potent Nutraceutical in Perspective

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Abstract

Unsweetened natural cocoa powder is a pulverized high-grade powder of compressed solid blocks which remains after extraction and removal of the cocoa butter. The authors determined the elementary composition of UNCP, investigated its effect on nitric oxide levels, toxicity, and its protective effect on the heart, kidney, and liver during simultaneous administration with high dose (HD) artemether/lumefantrine (A/L). Macro- and microelements in UNCP were analyzed with energy dispersive x-ray fluorescence spectroscopy (EDXRF). Adult male guinea pigs were administered various doses of UNCP alone and also simultaneously with A/L. Phytochemical analysis of UNCP showed the presence of saponins, flavonoids, tannins, cardiac glycosides, and 38 macro- and microelements. Histopathological analysis showed no toxic effect on the heart, liver, kidney, lungs, testis, and spleen. Administration of various doses of UNCP increased white blood cell counts and lymphocyte count ($p > 0.05$) compared with the controls. Additionally, UNCP and A/L combination caused an increase in nitric oxide levels when compared with the control group and restores some hematological disorders induced by the 3-day HD A/L administration. Even though UNCP appears to be relatively safe, care should be taken due to the high content of copper element to avoid the possibility of intestinal lining erosion.

Keywords: unsweetened cocoa powder, artemether-lumefantrine, malaria, *Theobroma cacao*, nutraceutical, dark chocolate

1. Introduction

Nutraceuticals, a word coined by DeFelice [1], can be described as any nontoxic food extract scientifically proven to have health benefits for the treatment and prevention of certain diseases.

Natural products have been a good source of drug leads from time immemorial for as long as history has been recorded. The most common among these natural products are plants, which have been exploited for their medicinal purposes [2–4]. Interestingly, many consider plant products to be devoid of adverse effects; thus, the demand for herbal remedies has been on the increase in industrialized

countries as it has been with developing countries [5]. Currently, extensive research is being carried out on natural products including plants from the rain forests, among other places for their potential medicinal value as well as potential toxic effect [6].

One such plant that has generated a lot of interest is *Theobroma cacao,* belonging to the class *Equisetopsida* and family *Malvaceae*. For some decades now, Ghana and Cote D'voire, two neighboring countries in West Africa, have been the world's leading producers of cocoa. Seeds from the fruit of *Theobroma cacao* are used to make cocoa powder and chocolate. Cocoa seeds (or the powder) are major nutraceuticals in Ghana and most parts of Africa. As food, it is consumed by most indigenous people in the raw state (the bean or the pod), as dark chocolate, and as a beverage prepared with powder obtained from cocoa beans. The cocoa powder is prepared by the removal of cocoa butter from the beans by fermentation, which is then followed by the following processes: drying and bagging, winnowing, roasting, grinding, and pressing. After the final process, solid blocks of compressed cocoa are obtained (press cake), which are pulverized into a powder to produce a high-grade cocoa powder. Most of the marketed natural cocoa powder in Ghana is sweetened, even though it has been proven that regular intake of unsweetened natural cocoa powder as a beverage has immense health benefits. Pharmacologically, cocoa is known to exert antioxidant [7], anti-inflammatory [8], antimalarial [2, 3], and anti-asthmatic properties [8, 9].

1.1 Composition of unsweetened cocoa powder

The chemical composition of cocoa powder is well documented. Cocoa contains water-soluble polyphenols (also called flavanols), which include catechins, epicatechin, procyanidins, anthocyanins, and leukoanthocyanins [10]. The antioxidant properties of cocoa are partly ascribed to its structural characteristics, and this is the basis of its role as a free-radical scavenger [10]. Quantitative analysis of unsweetened natural cocoa powder (UNCP) has shown that flavanol contents do not change during different manufacturing processes [11, 12]. Several polyphenols such as 14 N-phenylpropenoyl-L-amino acids, N-[4′-hydroxy-(E)-cinnamoyl]-L-tryptophan, and N-[4′-hydroxy-3′-methoxy- (E)-cinnamoyl]-L-tyrosine have also been found to be present in cocoa powder [13–15].

1.1.1 Phytochemical analysis

A study conducted to ascertain the phytochemical components of unsweetened cocoa powder in Ghana used various pharmacopoeias tests to confirm the presence of the various components. To test for saponins, about 0.5 g of the UNCP was added to water in a test tube and shaken to observe foam formation. To test for the presence of tannins, about 0.5 g of UNCP was dissolved in 80% of aqueous methanol (10 mL). Freshly prepared iron III chloride solution was added and observed for a color change. The presence of alkaloids was confirmed by performing the Dragendorff's, Mayer's, and Wagner's reagent tests as previously described [16].

To identify flavonoids in the UNCP, about 0.1 g of the sample was added to 80% ethanol and filtered. Subsequently, magnesium turnings were added to the filtrate, followed by concentrated HCl. A color change was observed within 10 min. A test for cardiac glycosides was conducted by dissolving about 0.5 g of UNCP in chloroform (2 mL) in a test tube, after which concentrated sulfuric acid was carefully added down the side of the test tube to form a lower layer. This result obtained

confirmed work by Oracz et al. [11], Andres-Lacueva et al. [12], and Stark et al. [17] that the flavanol contents of cocoa does not change during different manufacturing processes.

1.1.2 Micro- and macro-elemental composition

Besides the phytochemical components, micro- and macro elements have been shown to be present in most plant products. The most toxic of these are the heavy metals. These have the potential to interfere with the availability of secondary metabolites [18]. For the safety of consumers, the World Health Organization states maximum permissible levels of heavy metals in raw plant materials for only cadmium (0.3 mg kg^{-1}), arsenic (1 mg kg^{-1}), and lead (10 mg kg^{-1}) [19].

To establish the elemental components of marketed UNCP, energy dispersive X-ray fluorescence method was used. Briefly, samples of the powder were well dried and pelleted using Licowax C micropowder PM-Hoechstwax as binder. Simultaneous measurements of the elemental components were measured with a SPECTRO X-Lab 2000 spectrometer (Geological Survey Department, Accra, Ghana) as previously described [20]. The results revealed a total of 38 elements, of which 12 were macro, and are listed in **Table 1**.

1.2 Toxicity of unsweetened cocoa powder

Rarely are toxicological studies conducted on nutraceuticals. This could be because such products are generally termed "natural" and perceived to be devoid of any adverse effects. Interestingly, cardiotoxic and teratogenic potentials have been reported for cocoa [21, 22]. Against this background, a study was conducted to elucidate the safety of cocoa powder in Sprague Dawley (SD) rats. The experimental procedure for this study was approved by the departmental ethical and protocol review committee and the Noguchi Memorial Institute for Medical Research, University of Ghana. Institutional Animal Care and Use Committee also approved the protocol for the study. The study was conducted in accordance with international ethical guidelines.

The design of the experiment was such that the rats were randomly assigned to either the experimental group or the control group. There were 20 rats in all, 10 in each group. All rats had access to water and food except for a 12-hour fasting period before the administration of the unsweetened natural cocoa powder. The experimental group of rats received UNCP at a dose of 2000 mg/kg, while the control group received an equal volume of distilled water only. After the study period, effects of UNCP on selected organs, as well as on biochemical indices of blood, are described below.

1.2.1 The effect of UNCP on the body and relative weight of organs

The effect of UNCP on the body and relative weight of organs was determined as described previously [20]. Selected organs, the lungs, heart, liver, spleen, kidney, intestines, and testis, were excised. The organs were then placed in ice-cold saline to wash off blood. They were trimmed of fat, connective tissues, blot dried, and then weighed on a balance. The organ-to-body weight index, OBI, was determined as the ratio of organ weight and the body weight of the animal before sacrifice × 100. Body weight of rats was also taken dosing, a week after dosing on day 7 and before sacrificing them on day 14 (**Tables 2** and **3**).

	Element	Mean/SD mg/4000 mg
Macroelements	Na	2.4666 ± 0.00
	Mg	33.0133 ± 0.02
	Al	14.0093 ± 0.01
	Si	15.3880 ± 0.02
	P	64.3866 ± 0.00
	S	30.9120 ± 0.00
	Cl	2.3616 ± 0.00
	K	149.0667 ± 0.03
	Ca	11.0146 ± 0.00
	Ti	0.0232 ± 0.00
	Mn	0.4093 ± 0.00
	Fe	1.0309 ± 0.00
Microelements	V	0.2320 ± 1.73
	Cr	0.4200 ± 17.44
	Co	0.0108 ± 0.10
	Ni	0.0638 ± 1.16
	Cu	0.2984 ± 1.71
	Zn	0.4086 ± 0.74
	Ga	0.0024 ± 0.00
	As	0.0020 ± 0.00
	Rb	0.1698 ± 0.49
	Sr	0.1064 ± 0.20
	Y	0.0016 ± 0.00
	Zr	0.0125 ± 0.42
	Nb	0.0070 ± 0.29
	Mo	0.0044 ± 0.00
	Sb	0.0043 ± 0.06
	I	0.0133 ± 0.15
	Cs	0.0232 ± 0.10
	Ba	0.0620 ± 5.81
	La	0.0480 ± 0.00
	Ce	0.0849 ± 4.97
	Hf	0.0148 ± 0.17
	Ta	0.0213 ± 0.06
	Pb	0.0036 ± 0.00
	Bi	0.0024 ± 0.00
	Th	0.0020 ± 0.00
	U	0.0112 ± 0.10

Table 1.
Mean and standard deviation (SD) of measured elements (mg/4000 mg).

1.2.2 Histopathology examination

The histopathology examination was performed as previously reported by Asiedu-Gyekye et al. [20] The lungs, heart, liver, spleen, small intestinal organs, and testis were placed in a 10% buffered formaldehyde solution for 24 h. Tissue samples from the organs were paraffin embedded and sectioned at 5 μm thickness. The sectioned tissues were stained with hematoxylin and eosin (H&E) and evaluated under a light microscope (Olympus BX 51TF) for histological changes.

1.2.3 The effect of UNCP on hematological parameters

The effect of UNCP on hematological parameters was studied as described previously by Asiedu-Gyekye et al. [20]. Blood (2 mL) from euthanized SD rats was drawn out by cardiac puncture. This was then transferred into ethylene diamine tetra acetic acid (EDTA) test tubes. An automated hematology analyzer was used for the evaluation. Peripheral blood smear was used to examine the nature of blood cells.

DAY	CTRL	UNCP	*p* value
Day 1	112.5 ± 12.50	142 ± 8.000	0.0526
Day 7	115.0 ± 10.00	142.0 ± 7.176	0.3618
Day 14	112.5 ± 7.500	135.0 ± 7.000	0.0522

Table 2.
Changes in body weight of SD rats after administration of unsweetened natural cocoa powder solution.

ORGANS	CTRL	UNCP	*p* value
Liver	8.605 ± 4.225	7.454 ± 1.263	0.3260
Kidney	0.6000 ± 0.05000	0.5500 ± 0.02739	0.3618
Heart	8.605 ± 4.225	7.454 ± 1.263	0.3534
Lungs	0.8500 ± 0.05000	0.7600 ± 0.02915	0.3618
Spleen		7.454 ± 1.263	0.0829
Testis		1.224 ± 0.02502	0.3618

Table 3.
Changes in organ weight of SD rats after administration of unsweetened natural cocoa powder solution.

1.2.4 The effect of UNCP on serum biochemistry

The effect of UNCP on serum biochemistry was studied as described previously by Asiedu-Gyekye et al. [20]. Blood (1 mL) of sacrificed rats was collected via cardiac puncture. The blood sample was allowed to stand for a while, this was then centrifuged at 4000 rpm for 15 minutes using a Wiperfuge centrifuge. The serum was then collected for the measurement of the biochemical parameters (**Table 4**).

Herbal medicines are thought to have no side effects or the potential to cause harm due to their natural origins and are often considered as healthy food supplements and not drugs. Additionally, most herbs used for medicinal purposes lack specific instructions concerning dose, frequency, and route of administration. Evaluating UNCP as nutraceutical, the assumption was that the average African weighs 60.70 kg.

Lead and arsenic limits permissible by the WHO are 0.00016 and 0.0010 mg/kg, respectively [23]. Heavy metals determined in UNCP were Pb—0.0036 mg and As—0.002 mg corresponding to 0.0002 and 0.0001 mg/kg, respectively, which are far below WHO guidelines. There was a high content of copper (0.2984 mg per 4 g UNCP) observed which should be of concern especially when high doses of UNCP are consumed. This is due to the fact that copper has been shown to play a role in the pathogenesis of Wilson's syndrome and liver damage [16, 24], while the high content of chromium could have beneficial effect in the management of diabetes mellitus and cardiovascular disorders [18, 25, 26]. Thus, the relationship between these elements, nutrition, and medicine observed is indicative of the fact that micro- and macro-elements of herbal products does influence certain body processes and hence should not be envisaged always as contaminants.

Body weight changes between the control group and the experimental group were observed on day 1, day 7, and day 14. However, with respect to dosing, they were found not to be statistically significant ($p > 0.05$). Body weight of the SD rats decreased by 8.3 and 22.2% ($p < 0.05$) in the 2nd week after dosing for the control and test groups, respectively. Although the decrease in body weight with the control animals and the test animals is consistent with the corresponding decrease in their food and water intake, that for the test animals might partly be due to the ability of UNCP to react with nutrients in the body including stored fat, carbohydrate, and protein.

Parameter	UNITS	CTRL	UNCP	*p* value
Creatinine	μmol/L	43.25 ± 2.925	40.61 ± 1.158	0.3618
Urea UV	mmol/L	8.605 ± 4.225	8.854 ± 1.263	0.2880
Bilirubin total	μmol/L	0.795 ± 0.0144	0.625 ± 0.165	0.3960
ALT	U/L	125 ± 0.722	120 ± 5.01	0.1999
Albumin	g/L	40.5 ± 0.442	39.0 ± 0.692	0.2046
AST	U/L	2.49 ± 0.358	2.52 ± 0.541	0.3263
Total protein	g/L	73.1 ± 1.02	70.6 ± 3.09	0.6061
Triglycerides	mmol/L	1.28 ± 0.160	0.943 ± 0.116	0.1185
Bilirubin direct	μmol/L	0.555 ± 0.0240	1.38 ± 0.489	0.1791
ALP	U/L	707 ± 7.86	535 ± 40.7	0.0103
GGT	U/L	1.20 ± 0.200	2.40 ± 1.44	0.4316
HDL cholesterol	mmol/L	0.560 ± 0.0372	0.755 ± 0.0349	0.0060
Cholesterol	mmol/L	2.08 ± 0.0854	2.15 ± 0.129	0.6616
LDL cholesterol	mmol/L	1.27 ± 0.0740	0.934 ± 0.124	0.0810
Na^+	mmol/L	137 ± 0.479	133 ± 0.477	0.0002
K^+	mmol/L	5.75 ± 0.132	6.44 ± 0.293	0.1098
Ca^{2+}	mmol/L	0.845 ± 0.0132	0.858 ± 0.0180	0.5200

Table 4.
Changes in serum biochemistry of SD rats after administration of unsweetened natural cocoa powder solution.

A reduction of 10.90% ($p > 0.05$) in the organ weight was observed in the test group as compared to the control group. These variations in the relative organ weight of the control and experimental groups of SD rats were not significantly different.

High-density lipoproteins (HLD) increased slightly with a p value <0.05, a decrease in the level of triglycerides and low density lipoprotein (LDL) cholesterol of the UNCP group ($p > 0.05$) was observed. In comparison with that of the control, the cholesterol levels remained relatively unchanged. This is consistent with the speculation that UNCP possess lipid lowering abilities.

Hematological results (**Table 5**) revealed a decrease (28.44%, $p > 0.05$) in the level of platelet in the UNCP group in comparison with the controls. Polyphenols in cocoa have been found to reduce platelet count. Neutrophil and lymphocyte polymorph of white blood cells showed a slight decrease of 8.02 and 18.73%, respectively ($p > 0.05$).

Histopathology evaluation of the organs studied; liver, kidney, heart, lungs, spleen, and the testis of the animals that received UNCP showed no toxic effect as compared to that of the control group. UNCP solution therefore is not likely to have toxic effects on the kidney when administered in a single oral high dose of 2000 mg/kg. Notable changes were observed on the small intestines in the form of erosions of the mucosal lining of the villi (**Figure 1**). These effects were, however,

Parameter	Ctrl	UNCP	*p* value
WBC	8.605 ± 4.225	7.454 ± 1.263	0.3618
Neut. number	2.020 ± 0.4400	1.858 ± 0.3836	0.4109
Lymph number	5.985 ± 3.435	4.864 ± 1.111	0.3422
Mono. number	0.3700 ± 0.220	0.3740 ± 0.1225	0.4936
Eosin. number	0.2250 ± 0.125	0.3520 ± 0.07439	0.2045
Baso. number	0.0050 ± 0.005	0.0060 ± 0.002449	0.4228
Neut.%	27.65 ± 8.450	25.38 ± 4.450	0.4021
Lymph.%	65.80 ± 7.600	63.90 ± 6.119	0.4348
Mono.%	4.000 ± 0.6000	5.700 ± 1.642	0.2828
Eosin.%	2.500 ± 0.2000	4.940 ± 0.9553	0.0941
RBC	7.360 ± 1.060	7.156 ± 0.5533	0.4290
HGB	12.20 ± 1.500	12.38 ± 1.048	0.4646
HCT	36.05 ± 4.050	37.82 ± 3.269	0.3877
MCV	49.20 ± 1.600	52.76 ± 0.9405	0.0515
MCH	16.65 ± 0.3500	17.28 ± 0.2354	0.1037
MCHC	33.80 ± 0.4000	32.76 ± 0.3696	0.0862
RDW-CV	17.05 ± 3.150	15.74 ± 1.105	0.3106
RDW-SD	27.00 ± 2.000	27.70 ± 1.064	0.3746
PLT	696.5 ± 324.5	498.4 ± 166.3	0.2855

Table 5.
Changes in hematological indices in rats after receiving 2000 mg/kg bwt of UNCP.

not observed in the control animals those that received equivalent volumes of the vehicle (distilled water). This could be due to the high concentration of proanthocyanidins contained in the 2000 mg/kg dose of UNCP (approximately 2.5 g in man). Proanthocyanidins have been found to instigate the destruction of the mucosal lining of the gastrointestinal tract.

1.2.5 Conclusion

In conclusion, the aqueous solution of unsweetened natural cocoa powder administered at the single oral high dose of 2000 mg/kg appears to be relatively safe in male SD rats. Caution should however be taken when using UNCP especially in high quantities or amounts since it is capable of causing considerable damage to the mucosal lining of the small intestines.

1.2.6 Reproductive toxicity

Since UNCP is widely consumed by individuals in their reproductive period of life, it is worth investigating the generative toxicity, genotoxic, and aspects of the reproductive toxicity potential of UNCP in both male and female white wistar rats. As a preliminary study, the genotoxic potential of UNCP was assessed using the DNA comet method. DNA breaks in the rectal epithelium, liver, bone marrow, and kidney of the mice were quantified and compared with the negative control (2% starch). Pre-implantation loss, viable fetuses, corpora lutea and post-implantation

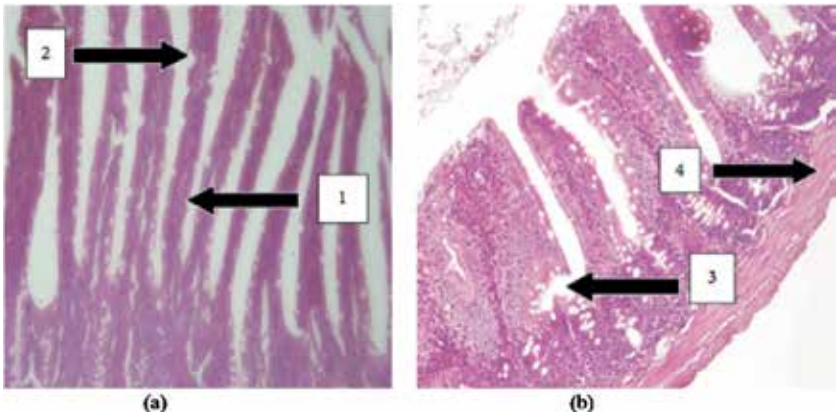

Figure 1.
H&E stained sections of the small intestine of 20× magnification. (a) Small intestine sections of control male SD rates showing villi (1) goblet cells (2) basement membrane with no proliferation (4). (b) Small intestine sections of the group treated with UNCP showing moderate changes and erosion of the mucosal lining of the villi (3).

loss, and implantation sites/number of implants were assessed. Results show that UNCP does not exhibit any reproductive toxicity potential.

1.3 Organoprotective effect of unsweetened cocoa powder against high-dose artemether-lumefantrin-induced effects

Artemisinin and its derivatives, derived from *Artemisia annua* (**sweet** wormwood), have impressive parasiticidal properties *in vivo* and *in vitro* [27, 28]. Despite the efficacy of artemisinin in reducing the malaria parasite load in the body, monotherapy was discouraged in an attempt to delay or prevent the development of drug resistance. Artemisinin-based combination therapy (ACT) was recommended by the WHO for use in the treatment of malaria after resistance was developed to the quinine derivatives used at the time [29, 30]. Increased therapeutic efficacy was reported to be associated with the use of the combination. Treatment failure to these therapies is suspected, and in recent years, some countries have considered increasing the dose of the A/L in treatment in order to arrest the issue of resistance [31], but increase in dose implies that there will be increased side effects, adverse reactions, and hepatotoxic effects [32]. In fact, there are already concerns about frequent usage of A/L on some organ systems [28]. Considering the fact that, so far, A/L is one of the most effective combination therapies, the issue of drug-induced hepatotoxicity needs to be addressed. Another effect of A/L is its effect on nitric oxide levels, where it has been found to reduce nitric oxide levels. However, other studies show that A/L increase nitric oxide levels as a compensatory mechanism in cases of reduced nitric oxide levels [28]. Additionally, some studies of artemether in rats have shown changes in the hematological profile of the rats [33]. It is therefore suspected that artemether may aggravate anemia in malaria patients. In another study, A/L was found to reduce red blood cell count (RBC), HGB, and packed cell volume (PCV) in patients taking treatment [34].

Ghana is the second producer of cocoa in the world. It is therefore not surprising that many consume cocoa beverage at one time or the other during the day. It is also one of the endemic regions for malaria. Thus, a high possibility of consuming a cocoa product while being treated for malaria using any of the ACTs is

recommended by WHO. Artemether/lumefantrine (A/L) is one of the combination therapies used more frequently for treating malaria in Ghana.

It is interesting to note that anecdotal reports of the ability of cocoa to prevent malaria exist. Following these reports, regular intake of cocoa powder as a beverage has been shown to be associated with reduction in the incidence of episodic malaria and its use as diet-mediated malaria prophylaxis [3]. The antiplasmodial activity of different fractions especially the nonpolar solvent fractions have also been confirmed. Thus, simultaneous consumption of cocoa beverage during antimalarial treatment with A/L is expected to have dual benefits such as rapid clearance of the malaria parasites.

Regular intake of unsweetened natural cocoa powder as a beverage has immense health benefits including both cardiovascular and neurodegenerative disorders, reduces platelet aggregation, and improves lipid profile [25, 35].

Determination of the major elemental composition and toxicity of unsweetened cocoa powder was conducted. Another set of projects were aimed at assessing whether UNCP will worsen or is able to prevent some common cardiovascular and renal side effects associated with the use of A/L, its effect on nitric oxide levels and to assess its hepatoprotective potential against A/L-induced liver toxicity during their simultaneous ingestion in male guinea pigs, and finally to investigate the effect of UNCP on the hematological parameters and NO levels during high-dose (HD) A/L administration.

The methods used in the assessment of the parameters listed above are as described previously by Asiedu-Gyekye et al. [26] as follows: 30 adult male guinea pigs weighing between 300 and 350 g were purchased from the Animal Experimentation Department of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. The guinea pigs were acclimatized to laboratory environment (20–23°C) with a 12 h light-darkness cycle for 7 days prior to experimentation. The guinea pigs had access to standard laboratory diet and water *ad libitum*. The adult male guinea pigs were randomly assigned to five groups with each group containing six guinea pigs, 500 mg/kg per body weight, respectively, for 14 days, with two other groups serving as controls. One control group (negative control group [NCG]) received 75 mg/kg body weight A/L, and the other group was given distilled water (vehicle control group [VCG]).

The dosage regimen was as follows:

Group 1: Control (distilled water only)/vehicle control (CTRL).
Group 2: 75 mg/kg A/L(last 3 days)/negative control(COARTEM).
Group 3: Cocoa 300 mg/kg (14 days) + 75 mg/kg A/L (last 3 days) (300).
Group 4: Cocoa 900 mg/kg (14 days) + 75 mg/kg A/L (last 3 days) (900).
Group 5: Cocoa 1500 mg/kg (14 days) + 75 mg/kg A/L (last 3 days) (1500).
The guinea pigs were, thus, observed daily for a total period of 14 days.

Conversion of animal doses to HED was based on basal surface area. HEDs were calculated according to a study by Reagan-Shaw et al. [24] and Asiedu-Gyekye et al. [26] using the formula:

Human equivalent dose (HED) (mg/kg) = Animal dose (mg/kg) × (Human km/Human km).

The value of km factors (i.e., body weight, kg/surface area, m^2) for adult and guinea pigs to be 37 and 8, respectively, and an average weight of Ghanaian to be 70.0 kg.

After day 14, the guinea pigs were euthanized with 50 mg/kg chloroform by exsanguination, and 2 ml of blood was sampled by cardiac puncture and transferred into EDTA-2 k test tubes for immediate analysis.

1.3.1 Potential of unsweetened natural cocoa powder to attenuate high-dose artemether-lumefantrine-induced hepatotoxicity in nonmalarious guinea pigs

1.3.1.1 Biochemical assays

Blood samples were collected from the descending aorta and aliquoted into EDTA-2K tubes and plain tubes, respectively, at the end of the dosing period. This was done after euthanization of the animals under ether anesthesia. The EDTA blood was immediately analyzed for hematological parameters using the SYSMEX Hematology Autoanalyzer (Kobe, Japan), while sera prepared from blood in plain tubes were used for biochemical examinations including clinical chemistry measurements such as alkaline phosphatase (ALP), alanine aminotransferase (ALT) or glutamic pyruvic transaminase (SGPT) levels, serum glutamic oxaloacetic transaminase (SGOT) or aspartate transaminase, and gamma glutamyl transpeptidase (GGT). These were measured as liver function tests (LFT), which gives an indication of the state of the liver.

Nitric oxide levels were also measured using the Griess Reagent System. The total nitric oxide kit by R&D Systems was used in this study, and the assay reported previously by Bryan and Grisham [36] was employed for this purpose. In this assay, nitrate is converted to nitrite by nitrate reductase after which colorimetric detection of nitrite as an azo dye is carried out. The Griess reaction involves a two-step diazotization reaction, acidified NO_2^- to produce a nitrosating agent, which then reacts with sulfanilic acid to produce the diazonium ion. The diazonium then couples to *N*-(1-naphthyl) ethylenediamine to form azo-derivatives, which are chromophoric. These azo-derivatives are absorbed within the range of 540–570 nm.

Elevation of serum and plasma enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate transferase (AST) has been shown to be reliable markers of acute hepatocellular damage. This occurs due to hepatocyte membrane distortion leading to membrane leakage of the hepatocyte cytosolic contents. AST is abundant in the cardiac muscles, skeletal muscles, kidneys than in the liver; thus, ALT is the most reliable marker.

This study revealed that A/L increased ALT, AST, GGT, and bilirubin levels but witnessed a reduction in albumin and total protein levels indicating the presence of hepatotoxicity (**Figures 2**–**4**). The increases in AST and ALT were found to be dose dependent.

Usually in patients with hepatotoxicity, ALT levels increase by more than three times the upper limit of normal, ALP levels also increase by more than twice the upper limit or total bilirubin more than twice when associated with increased ALP or ALT. It is important to mention that liver damage could manifest with either predominately initial alanine transferase elevation (hepatocellular) or initial alkaline phosphatase rise (cholestatic). The two are, however, not mutually exclusive.

The synthetic function of the liver can be assessed by the levels of total protein and albumins. Administration of A/L leads to a decrease in total protein and albumin levels supporting the hepatotoxic effects of A/L (**Figures 5**–**7**).

UNCP significantly increased the levels of total proteins, which further supports its hepatoprotective effect. Elevation of albumin levels after administration of UNCP was significant. For the first time, this study reports the hepatoprotective effect of UNCP against A/L-induced hepatic damage in guinea pigs.

1.3.1.2 Histopathological studies

Guinea pigs were euthanized, and their livers were swiftly excised and washed with 0.9% saline. The livers were stored in 10% neutral buffered formaldehyde.

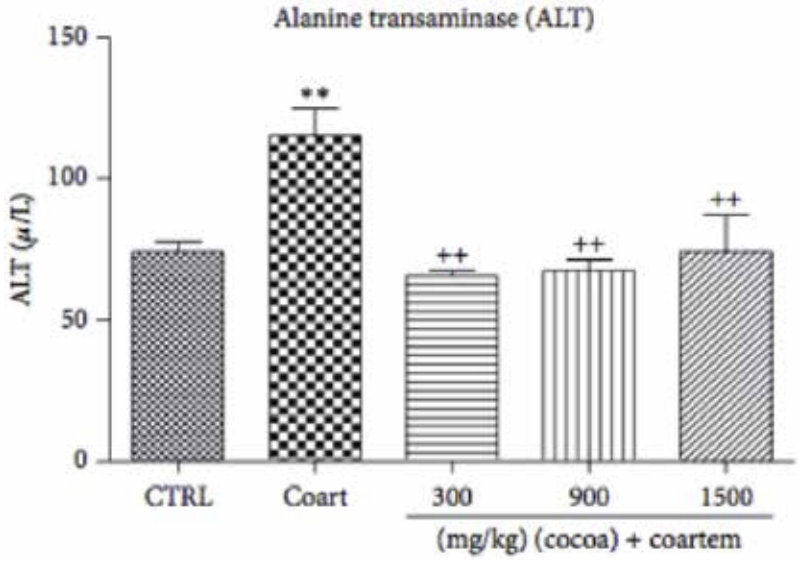

Figure 2.
*Changes in ALT levels of guinea pigs during a 14-day administration of UNCP followed by a 3-day A/L administration. The differences among the means were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc analysis, where ** means $p < 0.001$ when compared with the control (distilled water) and $^{++}p < 0.001$ when compared with the A/L group.*

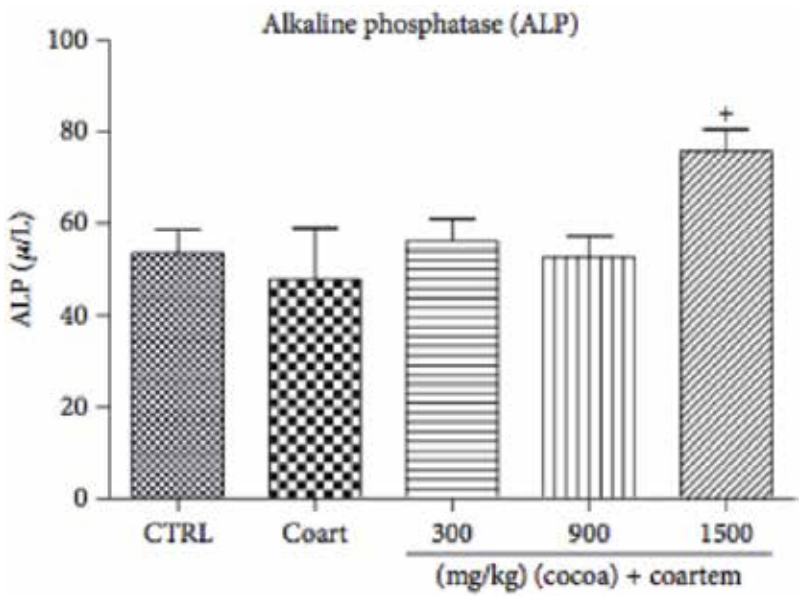

Figure 3.
Changes in ALP levels of guinea pigs during a 14-day administration of UNCP followed by a 3-day A/L administration of UNCP followed by a 3-day A/L administration. The differences among the means were analyzed using one-way ANOVA followed by Newman-Keuls post hoc analysis, where $^{+}$ means $p < 0.05$ when compared with the A/L group.

The liver tissues were then cut and sectioned using a microtone into 2 μm thick liver slices and stained with hematoxylin-eosin for examination. The stained tissues were observed with an Olympus microscope (BX-51) and photographed by INFINITY 4 USB Scientific Camera (Lumenera Corporation, Otawa, Canada).

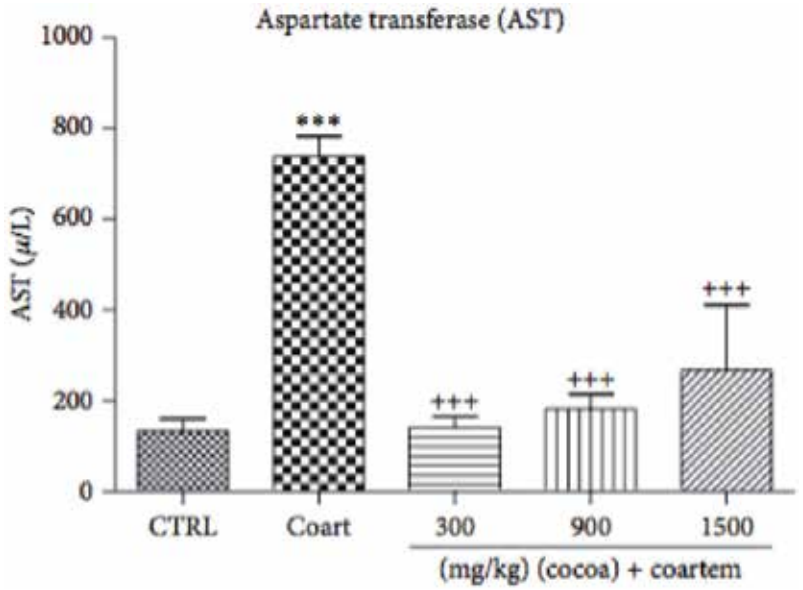

Figure 4.
*Changes in AST levels of guinea pigs during a 14-day administration of UNCP followed by a 3-day A/L administration. The differences among the means were analyzed using one-way ANOVA followed by Newman-Keuls post hoc analysis, where *means $p < 0.05$, ** means $p < 0.001$ when compared with the control (distilled water) and ++ $p < 0.001$ when compared with the A/L group.*

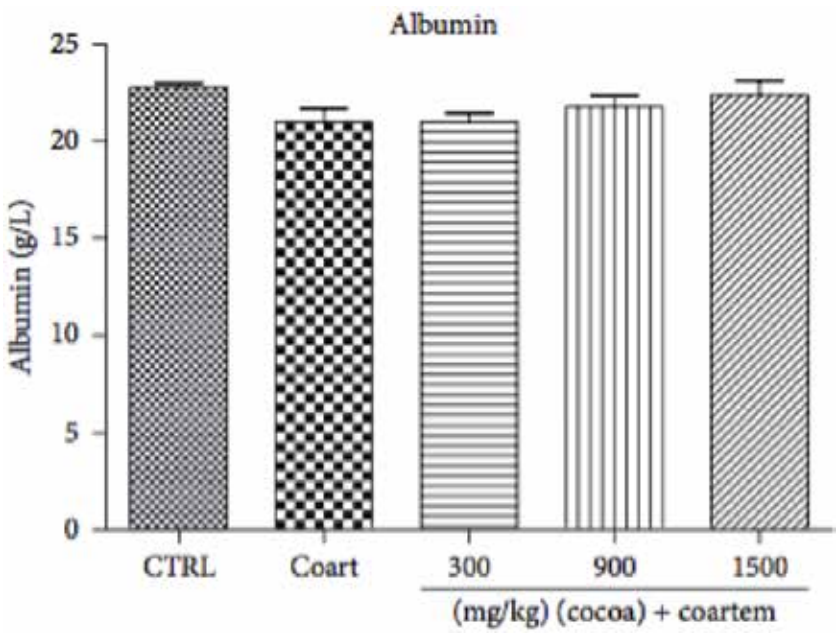

Figure 5.
Changes in albumin levels of guinea pigs during a 14-day administration of UNCP followed by a 3-day A/L administration. The differences among the means were analyzed using one-way ANOVA followed by Newman-Keuls post hoc analysis.

The hepatoprotective effect of UNCP was further confirmed in the histological data presented above, where very insignificant abnormalities were observed in the group that received UNCP before A/L. Damaged liver tissues in animals that received A/L alone was observed, which is evidenced by disturbed (necrotic)

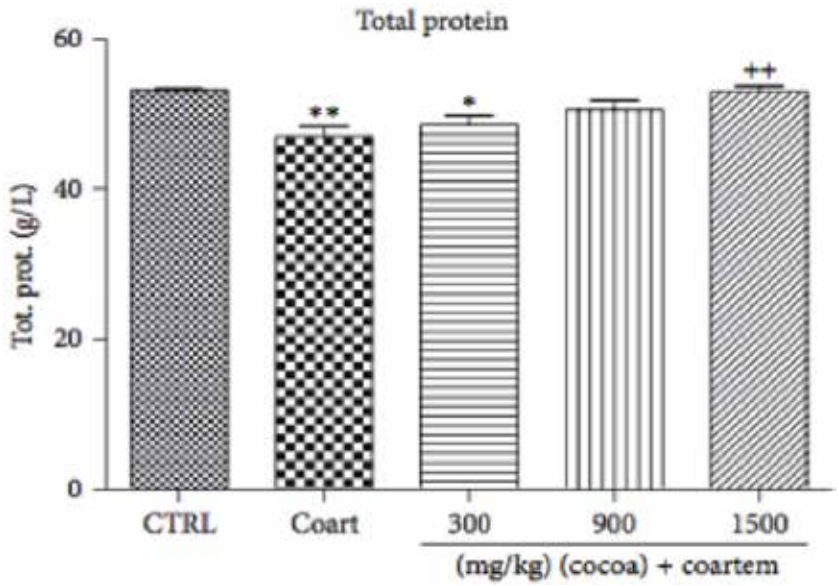

Figure 6.
*Changes in total protein levels of guinea pigs during a 14-day administration of UNCP followed by a 3-day A/L administration. The difference among the means were analyzed using one-way ANOVA followed by Newman-Keuls post hoc anlaysis, where * means $p < 0.05$, ** means $p < 0.001$ when compared with the control (distilled water) and ++$p < 0.001$ when compared with A/L group.*

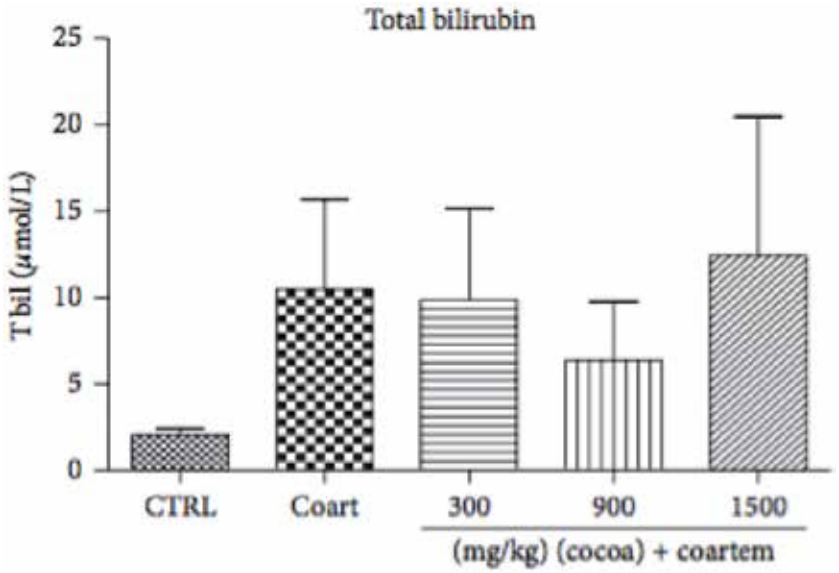

Figure 7.
Changes in total bilirubin levels of guinea pigs during a 14-day administration of UNCP followed by a 3-day A/L administration. The differences among the means were analyzed using one-way ANOVA followed by Newman-Keuls post hoc analysis.

liver parenchyma (NeLP), a highly congested and dilated central vein (CCV), and lymphocytic infiltration (LYM) in all animals (**Figure 8(b)**). Administration of UNCP before A/L reduced the extent of liver damage evidenced by the undisturbed liver parenchyma with an uncongested but dilated central vein (mild liver damage).

1.3.1.3 Conclusion

Unsweetened natural cocoa powder has hepatoprotective potential during high-dose A/L administration. The simultaneous consumption of UNCP and A/L is not likely to result in liver injury or dysfunction. However, care must however be taken during high daily consumption due to the high copper content.

1.3.2 Cardio and renal toxicity

Artemether-lumefantrine is one of the fixed-dose combination therapies recommended by the WHO for the treatment of malaria falciparum in Africa. Administration of this medication, however, generates free radicals that have the potential of causing cellular damage and other organ toxicity, characteristic being cardio-hepato- and renal toxicity.

It is speculated that simultaneous consumption of natural cocoa during antimalarial treatment with A/L is expected to rapidly clear the malaria parasites as well as ameliorate the A/L-induced toxic injury to heart and kidneys. Thus, the consumption of natural antioxidants such as found in cocoa could be beneficial in rectifying such damage in humans. The study also aimed at assessing whether UNCP will worsen or is able to prevent some common cardiovascular and renal side effects associated with the use of A/L. To establish the protective effect on the heart and

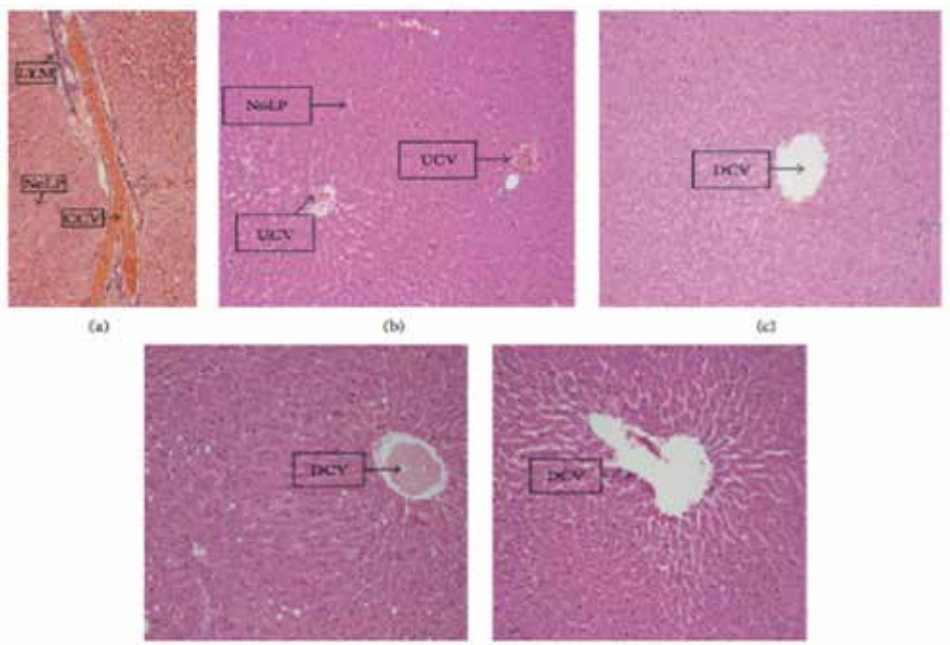

Figure 8.
Microtome sections of liver from guinea pigs that received (a) only a 3-day HD A/L administration (75 mg/kg/bwt) showing disturbed (necrotic) liver parenchyma (NeLP), a highly congested and dilated central vein (CCV), and lymphocytic infilteration (LYM). (b) Distilled water, control showing undisturbed liver parenchyma (NoLP) with uncongested central veins (UCV). Note the regularity of liver cell plates, microcirculatory zones, and sinusoids. (c) A 14-day UNCP administration (300 mg/kg/bwt) followed by a 3-day A/L administration (75 mg/kg/bwt) showing undisturbed liver parenchyma with uncongested but dilated central vein (DCV). (d) A 14-day UNCP administration (900 mg/kg/bwt) followed by a 3-day A/L administration (75 mg/kg/bwt) showing undisturbed liver parenchyma with uncongested but dilated central vein (DCV). (e) A 14-day UNCP administration (1500 mg/kg/bwt) followed by a 3-day A/L administration (75 mg/kg/bwt) showing undisturbed liver parenchyma with uncongested but dilated central vein (DCV) (H&E stain ×40).

kidney against (A/L) administration, various disease markers were assayed after concomitant administration of cocoa and artemether/lumefantrine (A/L).

The biochemical assays were performed as reported previously by Asiedu-Gyekye [16]. Blood samples collected in plain gel tubes were allowed to clot. It was then centrifuged at 3000 rpm for 15 min, sera removed and stored at −20°C. The sera were analyzed for biochemical parameters such as the total cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins, very low density lipoproteins (VLDL), creatinine, urea, blood electrolytes, creatine kinase (CK), and aspartate transferase [35, 37]. Measurements were carried out on the Selectra Junior Autoanalyzer (Vital Scientific BV, Version 04, Netherlands).

Euthanized guinea pigs were dissected, their hearts and kidneys were removed. The tissues were kept in 10% buffered formalin. The tissues were embedded in paraffin wax and sectioned at 4 μm thickness, stained with hematoxylin-eosin. The light microscope was employed for the histological studies of the study animals as well as two control groups. A total of 30 photomicrographs at a magnification of ×40 were used for each group.

1.3.2.1 Biochemical assays

In the medium and high UNCP dose groups, there was a decrease in the mean serum levels of low density lipoprotein by 11.6 and 10.6% ($p < 0.05$), respectively, in comparison with the negative control group (NCG) (0.662 ± 0.269 mmol/L) [16].

A dose-dependent increment was observed for both VLDL and triglycerides upon administration of UNCP [16]. The triglyceride changes were as follows: controls (1.075 ± 0.360 mmol/L), A/L-administered group (0.966 ± 0.619 mmol/L), 300 mg/kg UNCP (0.980 ± 0.391 mmol/L), 900 mg/kg UNCP (1.208 ± 0.317 mmol/L), 1500 mg/ kg UNCP (1.478 ± 0.487 mmol/L) ($p < 0.05$) [16].

Groups, which received 900 mg/kg UNCP, showed 12.9% ($p < 0.05$) increase in the mean serum levels of high density lipoprotein (HDL) in comparison with the NCG 0.148 ± 0.046, ($p < 0.05$) [16].

The levels of coronary risk was high in animals that received 1500 mg/kg UNCP exhibited high levels of coronary risk (11.778 ± 1.167), but those on 900 mg/kg UNCP showed low levels (8.470 ± 2.624) in comparison with the NCG (9.08 ± 2.894, $p < 0.05$) [16].

Significant changes in serum levels of total cholesterol, low density lipoproteins cholesterol, and triglycerides were not observed after administration of both A/L and UNCP. It must however be noted that lipid profile including cholesterol in general takes considerable time to show significant changes even with cholesterol lowering agents. The authors conclude that the 14-day administration of cocoa may not have been long enough to produce significant changes expected.

1.3.2.2 Creatine kinase (CK)

Asiedu-Gyekye's group observed a significant difference in the mean levels of CK in VCG (598.0 ± 382.425 μmol/L) and NCG (1039.0 ± 749.494 μmol/L) [16]. The groups that received 300 mg/kg UNCP, 900 mg/kg UNCP, and 1500 mg/kg UNCP had their CK as follows: 552.2 ± 399.968 μmol/L, 318.5 ± 122.516 μmol/L, and 366.8 ± 174.921 μmol/L, respectively (**Figure 8**). The LD, MD, and HD cocoa groups hence reduced the creatine levels by 46.9, 69.3, and 64.7%, respectively ($p < 0.05$).

Creatine kinase (CK) or creatine phosphokinase is a marker of damaged tissue. Myocardial injury is often associated with an increase in CK levels. In this study, it was observed that animals that received 900 mg/kg bwt + A/L had 69.3% reduction

in serum CK showing the greatest mitigating activity against coartem toxicity. CK plays a crucial role in the conversion of creatine to phosphocreatine and adenosine diphosphate; thus, it might also protect or enhance myocardial bioenergetics.

1.3.2.3 Renal function test

Asiedu-Gyekye's group reported a reduction in urea by 53% in 1500 mg/kg when compared with the VCG ($p < 0.05$) [16]. Those of groups 3 and 4 were reduced by 14 and 10.64% in comparison with the VCG.

For the creatinine levels, significantly increments was observed; a 24.08% increase in NCG compared with VCG and decrease of 21.27, 17.54, and 11.05% in groups 3, 4, and 5, respectively, when compared with group 1 ($p < 0.05$) [16] .

The sodium, potassium, and chloride levels remained relatively unchanged in all study groups when compared with the control that received distilled water only [16].

The 1500 mg/kg group showed a significant reduction in urea levels as compared to the Coartem® only group. Creatinine levels decreased in all the groups compared with the control group. The authors attribute the observed effects to the antioxidant and nephroprotective effects of cocoa. Guinea pigs that received only the 75 mg/kg Coartem® group showed high levels of renal damage.

1.3.2.4 Histopathological examination

Aspartate transferase, one of the enzymes mentioned earlier in this chapter, is distributed mostly in the heart followed by the liver and skeletal muscles. Elevated serum aspartate transferase values are hence indicative of cellular injury and may present in myocardial disease, shock, hypoxia, among others. The group administered with of distilled water +A/L showed a significantly increased serum levels of aspartate transferase. The reverse (significant reduction) was observed in all animals administered unsweetened natural cocoa powder extract.

These observations are corroborated by histopathological examination of the myocardial tissues of the guinea pigs (figure not shown), where tissue sections from animals that received only A/L 75 mg/kg showed evidence of inflammation and degeneration of the myocardial tissue. Normal cardiac tissue structures of myocardial tissue were observed in animals administered UNCP extract except those of animals that received 900 mg/kg UNCP where there was a single case observed with suspected ongoing tissue necrosis at the initial stages. Similar observations of the cardioprotective effect have also been made by other researchers.

Photomicrographs of myocardial tissues of animals from the different experimental groups revealed patchy areas of congestion, edema, extensive nuclear, and tissue degeneration leading to loss of microstructure of myocardial tissues for animals receiving 75 mg/kg A/L [16]. Animals that received 300 mg/kg UNCP and the control group retained the normal branching of myocardial cells characteristics [16].

Coronary risk ratio is an important indicator of cardiovascular health. Assessment of it showed that groups on 1500 mg/kg cocoa +A/L were at a higher risk compared with the control groups [16]. This observation corroborates the findings that although cocoa possesses many health benefits, high level intake could be deleterious, an effect believed to be caused by (−) epigallocatechin-3-gallate.

1.3.2.5 Conclusion

Unsweetened Natural Cocoa Powder showed renoprotective and cardioprotective potential during high-dose A/L administration [16]. It therefore suggests that

simultaneous ingestion of A/L and UNCP may be beneficial to the heart and kidney. However, regular intake of large quantities of UNCP could be deleterious to health because of the high content of copper.

1.3.3 Hematological changes and nitric oxide levels

Hematological parameters are one of the vital indices monitored during malaria treatment. Thus, in this study, the effect of UNCP on the hematological parameters and NO levels during high-dose (HD) A/L administration was investigated.

The nitrite concentration in the plasma was measured as an index of NO levels by Griess reagent system (South Africa) according to the manufacturer's instruction.

The results obtained from this study showed that A/L administration decreased the levels of white blood cell(WBC) count, lymphocyte count, hemoglobin (HGB), Red blood cell (RBC) count, and platelet counts in the group that received Coartem® (negative control group). Normally, these reduced indices imply bone marrow depression, autoimmune hemolytic anemia, systemic lupus, or severe hemorrhage.

Asiedu-Gyekye's group reported a reduction of 31.87% in the WBC count of the NCG (Coartem®) in comparison with the vehicle control group($p > 0.05$) [26]. Administration of 300, 900, and 1500 mg/kg body weight of UNCP restored the WBC levels during concomitant administration with A/L ($p = 0.1158$)[26].

Prophylactic administration of UNCP with A/L at doses of 300, 900, and 1500 mg/kg body weight restored the decreased levels of the RBC count by 4.17, 5.55, and 12.55%, respectively ($p > 0.05$).

Administration of UNCP at doses of 300, 900, and 1500 mg/kg body weight restored not just the WBC levels during concomitant administration with A/L but also other hematological parameters like the hemoglobin levels, red blood cell counts, platelet counts, and all other parameters measured.

Nitric oxide (NO) is known to have hepatoprotective and cardioprotective effects. Upon concomitant administration of A/L and UNCP at doses of 300, 900 and 1500 mg/kg, respectively. The NO observed by Asiedu-Gyekye's group was dose dependent, with 300 mg/kg of UNCP exhibiting the highest increase of 149.71% in NO ($p < 0.05$), 900 mg/kg gave a 34.25% ($p < 0.05$), and the 1500 mg/kg dose of UNCP showed 4.88% increment in NO ($p < 0.05$) [26]. The observed moderate nitric oxide increases that are beneficial could be attributed to the flavonoid content of the unsweetened natural cocoa.

Reference to this work is: [26].

1.3.3.1 Conclusion

UNCP restored some hematological disorders induced by high-dose A/L in guinea pigs by causing a significant increase in lymphocyte and platelet (PLT) levels at a dose of 1500 mg/kg. There was also an increase in NO with different doses of UNCP administration as a sequel to A/L dosing, which suggests that the combination (A/L and UNCP) is safe and advantageous. This study indicates the health benefits of daily ingestion of UNCP to prevent deleterious effects of A/L for the management of malaria.

1.3.3.2 Anti-asthma potential

Theobroma cacao has a lot of potential. UNCP was also investigated on its ability to help manage bronchial asthma. Anecdotal reports indicated that regular

consumption of UNCP was accompanied by prevention and reduction of asthmatic episodes. Bronchial asthma is prevalent in Ghana and West Africa. Due to its phytochemical composition and the presence of theobromine and theophylline, there could be a high possibility of a bronchodilatatory effect, the experiment was carried on in guinea pigs with prednisone and the drug for comparison. This study was a bronchial asthma model induced via the introduction of ovalbumin sensitization. The results showed a reduction in the brochoconstriction, inflammatory responses, and eosinophilia infiltration [9].

1.3.3.3 In vitro antimalarial activity of natural cocoa powder

Anecdotal reports from Ghana suggest that a daily intake of a beverage of natural unsweetened cocoa powder could protect an individual against *Plasmodium falciparum* malaria. However, as at then, there was no known scientific report linking the consumption of cocoa or its products to the reduction in malaria incidence. Thus, a research conducted to determine this antiplasmodial activity and elucidate possible mechanisms of this activity. An *in vitro* inhibitory studies of extracts and fractions of cocoa powder against *P. falciparum* revealed that the nonpolar extract (chloroform, ethyl acetate, and petroleum ether) had better antiplasmodial activity than the polar extract [2]. The chloroform extract was the most active, with 50 and 90% inhibition concentration at 48.3 ± 0.9 and 41.7 ± 7.8 μg/mL, respectively. The ring-stage of *P. falciparum* treated with chloroform of natural cocoa powder showed a decline in growth. These results suggested that natural cocoa powder has measurable direct inhibitory activity against *P. falciparum*.

These studies attest to the additional future health benefits of unsweetened natural cocoa powder when consumed on daily basis especially its organoprotective role and also in attenuating high-dose artemether-lumefantrine organ effects. The antimalarial potential of cocoa is very promising especially in Africa where malaria is endemic, thus could be very beneficial in the management of uncomplicated malaria with very limited adverse effects on the major organs and reproductive system.

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Chapter 5

Exploration of Cocoa (*Theobroma cacao*) By-Products as Valuable Potential Resources in Livestock Feeds and Feeding Systems

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Abstract

High cost of feeds and feeding management remain unresolved challenges facing livestock production globally specifically in developing countries. More than half of the production cost is associated with feeds and feeding alone; hence, it becomes imperative for livestock production science to explore lesser known or poorly exploited resources for use in animal feeds and feeding systems to reduce cost and increase productivity. One of such strategies is the use of alternative or nonconventional feed resources. Cocoa by-products have been reported as one of such nonconventional feed resources that can replace expensive and competitive conventional feed resources in livestock diets. Cocoa bean meal, cocoa bean shells, and cocoa pod husks are all potential but unexploited nutritive resources that can be considered as animal feed materials. Although their use is severely restricted by antinutritional factor (ANF) theobromine, which is toxic to livestock, there exist modern nutritional technologies capable of being applied to improve application of these resources in livestock feeding systems. Therefore, this chapter presents cocoa by-products as potential tropical feed resources in animal feeds and feeding systems with a view to providing solution to waste management problems associated with cocoa processing factories while increasing animal productivity and reducing cost of animal production.

Keywords: cocoa by-products, environmental pollution, feeds, livestock, utilization

1. Introduction

With current rising costs of conventional feed ingredients, animal nutritionists have advocated for the use of agro-industrial by-products as unconventional feedstuffs because they are cheaper and available in large quantities in producing countries. Cocoa pod husk, cocoa bean shell and cocoa bean meal form over 70% (w/w) of a whole matured fruit of cocoa (*Theobroma cacao* L.), and these are the major agro-industrial by-products from cocoa processing industries [1]. These by-products have continued to gain interest of researchers toward converting them to valuable uses such as in production of animal feeds—a critical contribution to improved food security.

Feed materials	Protein (% DM)	Fiber (% DM)	Fat (% DM)	Nonfiber carbohydrate	Gross energy (MJ/kg DM)
Dried cocoa pod husk	6.8–10	24.00–35.40	1.60–2.40	46.60	10.70
Cocoa bean cake	15.1–28.6	5.80–10.30	5.50–16.50	42.10	7.60
Cocoa beans shell	14.5–21.6	17.40–20.90	3.10–5.20	40.60	5.10
Bermuda grass	6–9	31.50	2.10	—	8.70
Soybean hull	12	34.20	2.20	—	8.40

Table 1.
Comparative chemical compositions of selected cocoa by-products, Bermuda grass, and soybean hull [7–9].

World production in 2017/2018 was downwardly revised to 4.59 million from a previous forecast of 4.64 million and now 3.3% below the prior season's 4.74 million [1]. Consequent upon this, large quantities of cocoa wastes or by-products are generated. Cocoa bean shell (CBS); a by-product of cocoa bean in chocolate, beverage and cocoa factories, which is crispy brown with pleasant smell is estimated at about 10,500 metric tons per annum and forms about 70% of the waste [2]. These wastes constitute environmental hazard to these factories and the immediate communities where they are sited. However, instead of this huge quantity of cocoa waste to constitute nuisance to the environment, they can be incorporated into ruminant feeding, thereby reducing the exorbitant cost of conventional feed ingredients.

One of the major qualities of alternative feed resources is their ability to provide adequate nutrients that meet up with nutrient requirement of the animal in question without compromising the animals' performance, reproduction, health as well as availability, acceptable forms, environmental friendly and reduction in the cost of feeding the animals [3]. This may be in their raw state or improved forms. Adamafio [4] compared chemical compositions of cocoa pod husk and Bermuda grass and soybean hull and found a favorable similarity. Alemawor et al. [5] added that cocoa pod husk could supply a substantial amount of energy requirements for ruminants. Cocoa pod husk nutrient constituents are also very similar to that of soybean hulls, which are routinely included in animal diets in North America [4]. It is evident from **Table 1** that both cocoa bean shell and bean cake are furnished with high crude protein (14–29% DM), a vital component of animal feed. Ruminant animals need 10% DM crude protein for maintenance [4] and 16% DM for growing ruminant animals [6].

Ozung et al. [10] reported that acid detergent fiber (ADF) and neutral detergent fiber (NDF) values were highest in the fermented cocoa pod hull meal (CPHM) (73.09 and 79.45%, respectively), followed by the raw CPHM (68.64 and 75.08%, respectively) and least in the hot-water treated CPHM (64.60 and 71.09%, respectively). Cocoa pod husk could be included at 20% DM as reported by researchers.

2. Cocoa by-products used in ruminant feeding

There are many by products obtainable from cocoa processing such as the husk of cocoa pod and the pulp, sweating surrounding the beans and the cocoa shells [11].

2.1 Cocoa pod husk

When the beans are removed from the pod after harvest, they are covered by the mucilage pulp. The parts of the cocoa pod left over, the cocoa husk, represents

between 2/3 and 3/4 of the total weight of the fruit (average fruit weight about 400 g) and is usually discarded by local farmers after harvest during cocoa bean processing after harvest.

Ashun [12] presents a calculation by Dittmar that cocoa plantations would produce around 4650 kg of dry cocoa pod per hectare. Typical values for the composition of cocoa husks are given in **Table 1**. More detailed information on protein quality is given by [13, 14]. The ash composition has been investigated in more detail by [15, 16], and the fatty acid composition by [12]. Vadiveloo and Fadel [17] freeze dried and milled the cocoa husk and analyzed the milled material for nutrient composition. These studies showed that cocoa husk in addition to the nutrients shown above contain high amounts of soluble phenolics and condensed tannins, and a high content of uronic acids. The theobromine level has been reported to be around 1.5–4.0 g/kg dry weight [18–20]. These data indicate that cocoa husk is rich in fiber but, poor in metabolizable energy and crude protein, in particular for non-ruminants.

2.2 Cocoa bean shell

Cocoa shell comprises of seed coat and embryo. The shell is a dry, crisp, slightly fibrous brown husk with a pleasant odor resembling that of chocolate. When the shell is removed, it may contain 2–3% of an unseparated cocoa nib. Cocoa shell is a good source of energy and minerals, P and Mg for ruminants [21]. Typical values for the composition of cocoa bean shells are given in **Table 1**. The fiber content is equivalent to medium quality grass hay in feeding value. More detailed information on protein and fatty acid quality is given by [22]. The phytase activity of cocoa shell has been reported to be low [23].

The chemical composition of cocoa bean shell indicates it might be a useful ingredient for ruminant feeding. Meffeja et al. [24] presented crude protein values of 5.9% and crude fiber of 21.3% which is comparable to the results reported by [25], while [14, 15] obtained much lower values of 32.5 and 45.9% respectively. Marcel et al. [26] asserted that cocoa bean shell contains 17.6% crude protein, 4.6% fat, 0.36% Ca, 0.61% P, 0.06% Na, U 61% Mg and 1.6% theobromine. [12] concluded in their feeding studies up to the 1960s that cocoa shell proved to be a useful ingredient in cattle feeding (for meat or milk production).

Flachowsky [27] concluded that cacao bean shells may be used as roughages in ruminant diets up to 5% of dry matter intake. The factor limiting the use of cocoa bean shell in feed is the theobromine level which is dependent on the way the cacao bean is prepared for the market. Originally the shell contains a limited amount of theobromine acquired from the nib during fermentation. The shell of most well-fermented commercial cacao beans contains over 1% theobromine—five samples contained between 0.80 and 1.69%. Abiola [18] reported a level of 1.9%. [28] presented a complete analysis of a commercial sample of roasted shells; the average theobromine content was 13 g/kg (8.0–16.9 g/kg), and the caffeine content around 1 g/kg. In another study [29] measured the theobromine and caffeine content of five different shell fractions collected over the whole growing season and observed 14.0 g/kg (7.5–21.0 g/kg) theobromine and 1.4 g/kg (0.8–2.3 g/kg) caffeine, respectively.

The nutritive value of cocoa shell was also studied in vitro and nylon bag technique and a feeding trial was carried out in growing cattle by [21]. The dry matter digestibility of cocoa shell was 63.5%. Approximately 30% of cocoa shell protein disappeared from the rumen after 12 h and there was a small increase after that time. Whereas for fat, there was an increasing amount that disappeared from the rumen after 12 h but reached the maximum value (73%) at 48 h.

2.3 Cocoa bean meal

Cocoa bean meal can be obtained from unsold cocoa beans or prepared from discarded cocoa beans, pressed cake of cocoa beans or residues from cocoa factories. The composition of the meal varies considerably depending on the amount of shell fragments incorporated in the meal and the degree of oil extraction. Reports of the proximate composition of the cocoa meal are summarized in **Table 1**. More detailed information on protein quality and mineral content is given by [22, 30], and the fiber and carbohydrates by [27]. The high fiber content and the content of the cell wall constituents (neutral and acidic detergent fiber and lignin) suggest that cacao bean shells are more suitable for ruminants than monogastrics [27]. A drawback of the cocoa meal is its high theobromine content, typically 20–33 g/kg. The caffeine content is lower, around 1–4 g/kg. Adegbola and Omole [31] studied the influence of treating ground cocoa meal with various concentrations of sodium hydroxide or warm water of various temperatures to improve the usefulness of cocoa meal as a grower-fattener ration for swine. Water treatments at a temperature slightly above 60°C for a few hours efficiently extracted theobromine. The hot water treatment retained nutritional quality of the product better than an alkali treatment.

3. Effects of cocoa by-products based diets on the performance of ruminant animals

Feeding lambs with cocoa shell at 9% inclusion level improved feed intake and growth. However, inclusion rates caused a reduction in feed intake and weight gain [32]. Others observed a reduction in body weight in sheep and goats when cocoa shell was included at 15% in the daily ration of sheep and goat. Alexander et al. [33] obtained a contrary result when they excluded cocoa shell from the diet of sheep. Tewe [34] when assessing the nutritive value of cocoa pod husk obtained increase in body weight of lambs when fed with 12–30% cocoa pod husk. Live weight gain was not affected by feeding <27% cocoa shell in the concentrate (or 11% in the ration). However, at 37% cocoa shell (or 15% the ration), live weight gain stated to decline. At this level, the ration contains approximately 0.24% theobromine which may be responsible for reduced utilization of metabolizable energy [21]. There was no evidence of toxicity in cattle when fed pod meal quantities of up to 7 kg per day [26]. Pod meal also has been reported to have similar nutritive value with corn-on-cob in dairy cattle ration. Rations containing cocoa pod meal have a somewhat lower feed efficiency for beef cattle, but this was compensated by the larger intake [2].

4. Effects on reproductive indices and health status of ruminant animals

Attempts to utilize cocoa waste materials as feed resources have shown that, often, when dietary concentrations exceed 10–15%, growth and reproductive indices are negatively affected [35, 36]. The consumption of organic mulch, composed of cocoa by-products, is reported to cause vomiting, central nervous system depression, restlessness, diarrhea, muscle tremor, ataxia, hematuria, tachycardia and seizures in animals [32, 37]. Cases of mortality have also been documented [33]. However, susceptibility to the detrimental effects induced by cocoa by-products appears to be species-dependent and age-dependent. Dried fresh CPH can be fed to cattle up to 7 kg per day without toxic effects and up to 2 kg per day to pigs without toxic symptoms. Up to 0.8 kg of cocoa shells (a good source of vitamin D)

is acceptable to cows. Cocoa products can only be safe for animal feeding when theobromine is drastically reduced or removed by cooking in water for 1½ h, filtering and drying. It should be noted that animals fed on a CPH diet tend to consume more water than normal due to high sodium (Na^+) content and the fact that the adsorption of water in the small intestines is proportional to the rate of sodium chloride (NaCl) adsorption. Additionally, animals fed on a CPH diet tend to have a leaner body for marketing.

5. Improvement of nutritive value of cocoa by-products

Agricultural by-products are usually characterized by their low nutritional quality; they contain highly fibrous materials and low protein content. Such characteristics often lead the by-products to be treated, either physically, chemically and/or biologically prior to feeding to animals [38]. A main obstacle of utilizing cocoa pod as an animal feed is its high fiber and low protein contents [39]. He further explained it contains a considerable amount of lignin, i.e., between 12 and 19% dry matter (DM), and such value is 2–3 times higher than that of rice straw. Studies using high inclusion levels of untreated cocoa pod in diets have resulted in lower digestibility and animal performance [40], confirming its low nutritional quality. Two main nutritional strategies have been proposed to overcome such limitation of cocoa pod, i.e., either by mixing with a more fermentable or digestible feedstuff [15] or by treating the pod with certain chemical or biological agents to improve its digestibility [5, 41]. Several methods have been adopted in the treatment and processing of cocoa pod husk meal for the purpose of animal feed formulation. Some of these methods include hot-water treatment [31]; alkali treatment [42]; enzyme (mannanase) treatment [43]; urea treatment [25]; fungal treatment [44] and microbial detheobromination [45]. These treatment procedures are somehow expensive and complex for the local farmers to adopt, hence the need to devise cheaper and less cumbersome methods like fermentation and hot-water treatment of cocoa pod husk meal and further ascertaining their nutrient/chemical compositions vis-a-vis their suitability for in animal feeding trials.

6. Cocoa by-products and its application in monogastric nutrition

In the developing countries where cocoa is a major cash crop, there are huge quantities of by-products that are discarded, causing enormous economic problems by polluting the environment. These by-products are usually considered as "waste" and left to rot on the cocoa plantation, which can cause environmental problems, such as producing foul odors or propagate diseases, e.g., pod rot, because they are not composted [46]. Considering the growing world population and disappearing raw materials, and a real threat of reduced food sources, it is not surprising that awareness about the needs of preservation and re-usage of materials that are treated as a waste is rising [47]. The main raw material for the production of all kinds of cocoa products is dried and fermented cocoa beans, and cocoa shells are one of the by-products of cocoa beans obtained in the chocolate industry. When cocoa is processed, there are three types of co-products: cocoa pod husk, cocoa bean shells, and cocoa mucilage. It is possible to use milled cocoa shells, without any modifications, as well as to alkalize cocoa shells, and then use them as food additive [48]. However, the most common use is still for feedstuff. A number of studies explored the potential of cocoa shells to replace a part of a usual animal diet and investigated their influence on animals, because it contains theobromine, which may have a negative

effect on some species [49]. Specifically, cocoa beans contain approximately 2–3% of theobromine, which crosses from seed into shell during fermentation [50]. The toxicity of a cocoa shell meal to broilers was examined by [51], and the authors added cocoa shell in amounts of 1, 2, 4, and 6% to the meal and concluded that 4 and 6% had a significant influence on the decrease of body weight of broilers.

In a subsequent experiment, they added exactly the same amount of pure theobromine as there was in cocoa shells that were in the previous meals, but the broilers' weight was drastically decreased. Pure theobromine was more toxic than that furnished by the cocoa shell meal. [52] confirmed that increasing the intake of sun-dried cocoa shells from 0 to 30% resulted in decreasing average daily feed intake and egg production, together with decreased weight of spleen, kidney, and ovary in hens fed with a diet containing 25 and 30% cocoa shell, because of increased theobromine intake. Olubamiwa et al. [53] however, claimed that cocoa shells that were boiled for 15 min could be used in laying hen feeds up to 20% without an influence on egg production and feed conversion. Recent studies were oriented on growing pigs. Magistrelli et al. [54] have shown that the use of cocoa shells in pig nutrition may have a positive effect on the balance of intestinal microbial ecosystem. Cocoa shell feeding for 3 weeks increased microbial populations of the Bacteroides-Prevotella group and *Faecalibacterium prausnitzii*, which produce short chain fatty acids, in particular butyrate, which positively influences growth and differentiation of enterocytes, and exerts anti-inflammatory effects, thereby reducing the incidence of a wide range of intestinal inflammatory diseases. Despite a reduction of *Lactobacilli*, cocoa shell feeding improved the proportion between the main phyla of the intestinal ecosystem, which may help to reduce the risk of excessive fattening, which is considered to be detrimental to the quality of the end products [55]. Ogunsipe et al. [56] also examined the addition of cocoa shells into pigs' meals and found that 20% was the optimal biological level of cocoa shells as an energy substitute for maize in a pig diet.

A number of researchers have reported poor performance following the ingestion of cocoa materials by chickens. Egg production was adversely affected by the consumption of cocoa bean shell in a study conducted by [53]. Teguia et al. [57] also observed detrimental effects on growth when the level of cocoa pod husk incorporated in the diet of broiler chickens exceeded 10%. According to [58], broiler chickens fed 15% untreated cocoa bean meal exhibited negative effects including an increase in creatinine levels and a reduction in feed intake, weight gain and hemoglobin level.

These adverse effects were absent in chickens fed a similar amount of cocoa bean meal which had been treated with alkali or hot water to reduce theobromine content. However, detrimental effects were observed at an inclusion rate 30% of alkali-treated or hot water-treated cocoa bean meal. These observations are in conformity with the findings of earlier studies, in which [59, 60] observed depressed weight gain and feed intake as well as increased mortality when chickens were fed 10–30% cocoa bean meal. Similar observations have been made following the inclusion of cocoa shell meal in the diets of broiler chickens [51].

The effect of replacement of maize by cocoa husks in a grower-finisher ration was determined in 180 broiler chickens. Cocoa husks were substituted for the maize component in the ration (65 g maize/100 g of diet) at levels of either 0, 10, 20 or 30% of the maize. The birds fed the diet with the 10% substitution level showed significantly faster growth than the control animals whose growth rates were not significantly different from the birds fed the diet with 20% maize replacement. When compared with the control birds, low body weight and poor efficiency of feed utilization were observed for the birds fed the diet with 30% maize replacement [57]. It was concluded that cocoa husk might be used as an ingredient for

poultry grower finisher diets [57]. A series of studies was conducted by [53] with the aim of finding commercial usage for cocoa bean shell in poultry (layer) diets. The results of this experiment indicated very strongly that the 15-minutes boiling duration is the best for optimal and profitable utilization of cocoa bean shell in layers mash [53]. Hamzat et al. [61] concluded that cocoa bean shell can be included up to 15% in the diet of rabbit. In their work, five experimental diets were formulated such that diet 1(control) was maize based while diets 2, 3, 4 and 5 had 5, 10, 15 and 20% cocoa bean shell respectively. Measurements taken were live weight gain, final live weight, feed intake, feed conversion ratio and cost per kg weight gain. Results showed that cocoa bean shell was useful in feeding weaned rabbits. Rabbit fed 15% cocoa bean shell were significantly ($p > 0.05$) different from the 20% cocoa bean shell diet in final live weight and daily weight gain, feed conversion ratio and cost per kg gain in weight [61].

Besides the nutritional interest, economic analysis of using cocoa bean shell as feed supplement in rabbit production was studied by [62]. Data used for this study was collected from an experimental study of performance of rabbits fed graded levels of various treatments of cocoa bean shell as feed supplement. Gross margin and dominance analysis were used to analyze the data. The study showed that untreated cocoa bean shell can be used economically at 100 g/kg inclusion in rabbit feed while hot-water treated cocoa bean shell can be included up to 200 g/kg in rabbit feed. The study recommends the use of hot water treatment of cocoa bean shell at 200 g/kg inclusion for optimum profitability of rabbit production [62].

Hot-water treated cocoa bean shell based diet was evaluated in respect of performance and physiological response of weaned rabbits. The treatment reduced the theobromine content of cocoa bean shell [36]. Feed intake and weight gain were significantly ($p < 0.05$) high in rabbits fed hot-water treated cocoa bean shell up to 200 g/kg. Water intake was highest in rabbits fed 400 g hot-water treated cocoa bean shell/kg. Rectal temperature and pulse rate also increased with increase in hot-water treated cocoa bean shell inclusion [36].

7. Enzyme addition improves utilization of cocoa by-products in poultry feed

Addition of enzyme has been reported to improve utilization of cocoa by-products in poultry feed. This is due to the fact that studies have shown that cocoa by-products contained high fiber. Alemawor et al. [5] reported that cocoa pod husk has high levels of lignin (14%), non-starch polysaccharides (NSP)-like hemicelluloses (11%), cellulose (35%), and pectin (6%). It is important to note that these nutrients are not readily available to monogastrics (poultry and pigs) because this class of animals lack fiber-degrading enzymes needed to hydrolyze NSP [63]. Undigested NSP can influence intestinal transit time and increase digesta viscosity. All these result in inefficient nutrient absorption which ultimately affects growth performance of animals. A good example is phosphorus (P) in plants and plant products such as cocoa pod husk which is available as phytate-phosphorus [64] and is not readily available to monogastric because they lack the enzyme phytase which is responsible for phytate hydrolysis. Even if they do, the quantities are insufficient [65].

Hence, for efficient use of CPH in monogastric diets, it is important to include exogenous fiber-degrading and phytase enzymes in such diets. These enzymes are able to hydrolyze fiber and phytate, improve nutrient utilization, and improve performance [66–68]. Phytase has also been shown to improve amino acid and energy utilization [69]. In order to determine the effect of enzyme

supplementation on poultry utilization of cocoa pod husk, an *in vitro* enzyme treatment study was conducted by [70] to test the effect of various combinations of selected exogenous fibrolytic enzymes on the digestibility of CPH feedstuff. Concentrations of 0.8, 0.6 and 0.8% w/w respectively for Pentopan®MonoBG, Viscozyme®L and Pectinex®5XL were observed as appropriate levels for supplementing CPH feedstuff. Among the enzyme combinations tested, the Pentopan®MonoBG + Viscozyme®L, Viscozyme®L + Pectinex®5XL and Pento pan®MonoBG + Viscozyme®L + Pectinex®5XL formulae were most effective in maximizing sugar release from CPH feedstuff by 42–53% increase with a corresponding reduction (7–14%) in crude fiber and non-starch polysaccharide fractions ($p < 0.05$). The authors concluded that supplementation with multi-enzymes or blends of exogenous NSP-degrading enzymes may enhance the capacity of poultry to efficiently digest and utilize dietary CPH.

Similarly, [71] carried out a study to determine if inclusion of cocoa pod husks (CPH) in layer diets will affect laying performance and egg characteristics. Two hundred and sixteen (216) Bovan Brown (BB) layers (92 weeks old) were randomly assigned to 12 experimental diets for 12 weeks in a completely randomized design. There were three levels of CPH inclusion: 0, 10 and 15%. For each level of CPH, diets were further sub-divided into four and each portion treated with, (i) no enzyme, (ii) phytase only, (iii) a commercial enzyme cocktail only and (iv) a combination of both phytase and cocktail. The enzyme cocktail was added at a rate of 200 g per ton of complete feed. The phytase was added at the rate of 250 g per ton of complete feed to give a phytase activity of 500 FTU (Phytase Units)/kg of complete feed. The authors reported that adding CPH did not affect average daily feed intake (ADFI). Hen day egg production for layers on diets with 0, 10 and 15% CPH, with a combination of phytase plus an enzyme cocktail (76.19, 73.81 and 66.34 respectively), was better than that of hens on diets without enzymes. Adding phytase, a cocktail enzyme, or a combination of the two improved egg weight. There were no effects of CPH or enzyme addition on egg quality characteristics. The authors concluded that cocoa pod husk (up to 15%) plus exogenous enzymes can effectively be used in layer diets without adversely affecting production performance or egg quality characteristics.

8. Conclusion

There is an increasing demand on food and feed resources by man due to rising global population. In order to prevent future food crisis and loss of animal protein, animal nutritionists have continued to explore alternative feed resources to meet the needs of both man and farm animals. Several crops and their by-products have potential as possible alternatives for livestock feed industry. One such crop is cocoa, a very abundant crop in tropical regions of Africa. Its by-products have been successfully used as alternative feedstuff in livestock production. Cocoa by-products show great potential as an alternative feed resource that can replace conventional feed ingredients used in animal nutrition.

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Section 4

Cocoa Breeding and Genetic Resources Utilisation

Chapter 6

Cacao Genetic Resources Conservation and Utilization for Sustainable Production in Nigeria

Festus Olakunle Olasupo and Peter O. Aikpokpodion

Abstract

Theobroma cacao, the source of chocolate, is one of the most important tree-crop that serves the purpose of sustaining the economy of millions of households and the largest non-oil foreign exchange earnings in Nigeria. The management of cacao genetic resources as it affects sustainable production of cocoa in Nigeria is reviewed. These include details of the diversity present in the germplasm collections, their utilization in varietal development and current status of the field genebanks as revealed by DNA fingerprinting using single nucleotide polymorphisms (SNPs) markers. Factors contributing to continuing backlash in the annual outputs of cocoa in Nigeria are also highlighted. The prospects of advances in the science of cacao genomics for up-scaling production and its impacts on the improvement of the industry in the country are discussed.

Keywords: germplasm introduction, genetic diversity, cacao breeding, cacao genomics, cacao science, sustainable production

1. Introduction

Theobroma cacao L., a member of Malvaceae family [1] is a small under-story tree that produces cocoa beans used in the manufacture of chocolate, cosmetics, confectioneries and other cocoa products. Recent evident from restricted fragment length polymorphisms (RFLP) and microsatellite analyses of ancient Criollo trees, consolidated proposition of the humid tropics South American Amazon regions as its center of origin [2]. Cacao is now grown as a tree crop in the tropical regions of the world between latitudes 20° north and 20° south of the equator [3]. Cocoa is one of the most economically important agricultural commodities in Africa and has been contributing to gross domestic product (GDP), national income (NI) and foreign exchange earnings of many African producing nations. It is marketed in The United States of America, Europe, United Kingdom and Asia where its butter and solids are used by processing industries. The global exports value of dried beans is between USD 8–10 billion per annum and there has been increasing demand for chocolate in the developing economies of Brazil, China, Eastern Europe and India [4]. In West and Central Africa, more than 96% of cocoa production is carried out by smallholder farmers who rely on proceeds from cocoa beans as a major source of family income [5, 6]. The world produced 4.744 million metric tons of dried beans in 2017 and 76% of the world production comes from Africa with about 7% of the continent exports from Nigeria, ranking the country 6th in the world [4]. In Nigeria,

cocoa provides means of livelihood to more than 5 million people and serves the purpose of sustaining the economy of millions of households to live above poverty and hunger. Although most of the country's budgetary revenue comes from sale of crude oil, agriculture contributes significantly to the economy with about 70% of the population engaged in agriculture. Income obtained from cocoa exports accounts for up to 27% of the 41.48% of Nigeria's GDP attributed to agriculture [7] and it is the largest non-oil foreign exchange earnings. However, cocoa production in Nigeria has not been sustainable as it reflected in the decline of the country's export data observed for almost two decades. Many factors are responsible for the low farm productivity which in turn is contributing to continuing instability and negative downturn in cocoa production in Nigeria [8]. These include but not limited to climate variability, diseases and pests infestations and poor access to improved planting materials. Development of improved hybrid varieties with good yield quality potentials is a necessity for a sustainable cocoa production system and this is subject to the amount of genetic variability available within germplasm collection. Thus, determined and consistent efforts are required in the acquisition of the needed genetic materials/resources for their conservation, evaluation and efficient use to develop well-adapted cultivars for combating the cocoa production challenges.

2. Germplasm introduction

Cacao was first introduced into Africa from Bahia, the Amazonian Region in Brazil by the Portuguese to Principe in 1822, with the establishment of 30 cacao plants of the Amelonado type (Lower Amazon Forastero) in Principe and later expanded to the neighboring island of Sao Tome [3]. Toxopeus [9] also reported that the two cacao trees of the Amelonado variety which were successfully established in Sao Tome from a batch of plants brought in from Bahia, Brazil in 1822, became the parents of the subsequent cacao trees of the island. Further movement of cacao from Sao Tome Principe islands to Fernando Po (the present-day Bioko in Equatorial Guinea) took place in 1855 by the Spanish seafarers [9, 10]. Other introductions were also made by Swiss missionaries from Suriname, and the first set of cocoa seeds were sown on Africa mainland in 1857 [11]. However, the variety which was originally brought into the island from Brazil was in its adopted home known as 'Sao Tome Creolou,' to distinguish it from other varieties that had been introduced subsequently. Following the introduction of the variety into West Africa and its wide cultivation in the region, it became popularly referred to as 'West African Amelonado.'

The history of cacao genetic materials introduction into Africa and subsequently into Nigeria is simply illustrated in (**Figure 1**). Cacao germplasm introduction to Nigeria can be presented as a sub-set of its introduction to Africa using the two phases partitioning ideology (Exploratory Colonial Period and Expansionary Experimental Pre- and Post-Independence Period) as reviewed by Aikpokpodion [7].

2.1 Exploratory colonial period (1874–1909)

The first era of cacao introduction into Nigeria started in 1874 by Chief Squiss Ibaningo, a trader who transported pods of Amelonado cocoa from Fernando Po into Bonny (now Rivers State of Nigeria) [1]. Many of the liberated slaves who were settled on the Fernando Po Island who had worked as contract labourers in the local cocoa plantations also contributed to cacao introduction into Nigeria. In 1880, there was an evidence of cacao plantation near Agege, owned by J.P.L Davies, most probably a

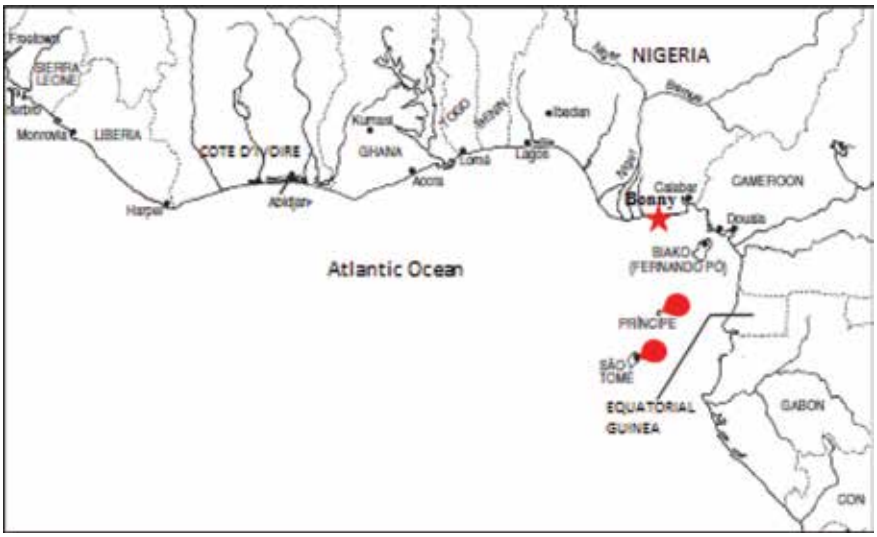

Figure 1.
Points of cacao introduction into Africa (Sao Tome and Principe) and Nigeria (Bonny Island). ● Place of first introduction to Africa in 1822. ★ Place of first introduction to Nigeria in 1857.

liberated slave [13], who had been the captain of a freighter plying the West African coast and could have obtained the seed from Fernando Po. The first cacao seedlings were planted somewhere near Ibadan prior to 1890 [14]. Berry [15] also reported that Ogunwale was the first to carry about 200 cacao pods to Ibadan for planting from Agege, which must have been from Davies' plantation. Furthermore, around 1900, the Colonial administration also introduced some red-podded cacao (Trinitario) materials to the botanical garden established in Agege Lagos in 1888 from British West Indies, presumably by the Jamaican curator of the garden. Several cacao germplasm materials were also introduced into Nigeria by the missionaries and slave traders. As a result of these various introduction efforts, a fair range of variability was presumably present on farmers' plantations with self-compatible 'West African Amelonado' types dominating the complex mixture of cacao of diverse origin. Towards the end of nineteenth century, cocoa cultivation and industry had started with 95% of national export of 21 tons in 1895 coming from the Western Region [12].

2.2 Expansionary experimental pre- and post-independence period (1910–2018)

At the beginning of twentieth century, cocoa was already a valuable commodity crop in Nigeria. Therefore, further introductions of cacao genetic materials during the second era were for economic reasons with the aims of obtaining better income and premium due to greater yields and higher bean and chocolate quality. However, during the last decade, the "People, Planet and Profit" concept of sustainability has become a significant factor in cacao germplasm introduction. This has bearing with the concept of "Preventive Breeding" where clones showing resistance to regionally important diseases of cocoa growing regions could be introduced through international intermediate quarantine centers [7]. This was to ensure that there is present in the germplasm collections, adequate sources of resistance to cope with, in case of new disease spread in order to prevent local cocoa economy crash that is associated with any epidemics.

Series of additional germplasm introductions following the initial successful introduction of the Brazilian Amelonado of the Lower Amazon Forastero type have

been reviewed by authors [3, 7, 16, 17]. The 'Trinitario' and 'Criollo' types were introduced to Nigeria in 1920 which formed hybrids with the original Amelonado type [18]. The former Nigeria Department of Agriculture was established in 1910 at Moor plantation, Ibadan. Nevertheless, the formal germplasm conservation and selection programs started around 1931 by O.J. Voelckler at the Nigerian Department of Agriculture in Moor Plantation, Ibadan under the Colonial Administration [17] with the sole aim of development and release of improved planting materials. In 1933, further germplasm introductions of some Trinitario and Criollo selections were made respectively, from Trinidad and Ceylon (the present day Sri Lanka) [19]. However, the outbreak of cocoa swollen shoot virus disease in the 1930s in Ghana, Togo and Nigeria almost destroyed the cocoa industry due to narrow genetic base in the population. This led to the establishment of West Africa Cocoa Research Institute (WACRI) in 1944 with the headquarters in Tafo, Ghana and a sub-station in Ibadan by an inter-territorial research thrust under the British West African Colonial Administration. Following the institute's establishment, several accessions from Upper Amazon Forastero and 'Trinitario' populations collected by Pound [20, 21] in Trinidad were introduced in 1944 into Tafo in Ghana by WACRI to widen the genetic base of the germplasm [22, 23] and subsequently into Nigeria from Ghana. Open pollinated pods from 11 selected accessions of the Upper Amazon Trinidad introduction that were established in Ghana were also introduced into Ibadan, Nigeria to form the F_2 Amazon population and these form the source of the open pollinated "F_3 Amazon" or "Mixed Amazon" materials in Nigeria up till present time.

The Nigeria sub-station of WACRI was upgraded to national research station, the Cocoa Research Institute of Nigeria (CRIN) in 1964 to focus on research that will facilitate improved production of cocoa. Then cacao seeds from 10 different crosses were introduced from Wageningen (The Netherlands) between 1964 and 1965 to raise 390 hybrid seedlings established in CRIN. Between 1965 and 1967, a large-scale introduction of Upper Amazon cacao materials was made from Trinidad as part of the Trinidad-Nigeria Cacao Introduction Scheme sponsored by the Cocoa Alliance [24]. This consisted of 313 clones and 701 seedling progenies of intra-Nanay, intra-Parinari, intra-Iquitos and inter-P (Pound's selections) crosses derived from a total of 350 crosses [25]. These clones and hybrids introduced from Trinidad constituted the "T clones" of CRIN germplasm collections. Materials were also acquired from Costa Rica, Indonesia, Fernando Po, Kew Gardens (United Kingdom) and Miami (USA) [19]. Between 1998 and 2004, 43 clones were introduced into Nigeria through an international initiative known as "Cocoa Germplasm Utilization and Utilization: A Global Approach," a project sponsored by the United Nations Common Fund for Commodity (CFC), through the supervision of the International Plant Genetic Resources Institute (IPGRI) as the executing agency [26]. Many other genetic materials introductions have not been reported adequately purposely because it is considered of hardly any use to publish introductions made if most of them did not survive due to the difficulties in their establishment. In the late 2017 and early 2018, some clones were introduced from International Cocoa Quarantine Center, University of Reading, of which 32 clones were established in a new germplasm plot at CRIN, Ibadan.

3. Status of Nigeria cacao field genebank

The genetic resources of cacao are composed of genetic variability that serve as the raw materials for the development of new varieties and cultivar improvement to enhance the production system for sustaining cocoa industry. These materials are conserved *ex situ* in field genebanks (germplasm plots of CRIN) and various

seed garden plots in major producing state of the country as well as *in situ*, that is in farmers' fields across the growing ecology. Appreciable diversity among these materials is of fundamental importance in the sustainability of cocoa production as it provides the necessary adaptation to the prevailing biotic and abiotic environmental challenges and enables changes in the genetic composition to cope with changes in the environment. The set of materials introduced during the Colonial era and the widely cultivated cacao in Nigeria in the early twentieth century (Amelonado and Trinitario varieties) were known to have narrow genetic base. However, the incidence of CSSV epidemics in the 1930s led to the introduction of Upper Amazon materials into the country's cacao genepool. Furthermore, several targeted germplasm collection efforts were made from mid-1960s up till recent times by CRIN breeding programme to broaden the diversity of Nigeria cacao genetic base. Research reports from the studies of genebanks collections and cacao accessions collected from farmers' fields in growing ecologies using 12 microsatellite markers showed that there is an appreciable improvement in Nigeria cacao genetic diversity [7, 16, 17, 27]. Of the 574 accessions studied, a total of 144 alleles were detected with a mean allelic richness of 4.39 alleles per locus. Upper Amazon parent population was observed to have largest genetic diversity (H_{nb} = 0.730), followed by the Posnette's Introduction (H_{nb} = 0.704) while the least (H_{nb} = 0.471) was recorded in the local parent population. Population structure analysis of cacao types grown in farmer's fields showed that the Upper Amazon Forastero, Amelonado, Trinitario and others constitute 66, 24, 6 and 4% of cacao grown, respectively (**Figure 2**).

More recent diversity studies using simple nucleotide polymorphism (SNP) markers [28], revealed the presence of cacao genetic groups that cut across the major primary populations in Nigeria germplasm collection (**Figure 3**) thus indicating appreciable improvement in the genetic base. This progress has been attributed to the international clones recently introduced into the country's cacao collection. However, there is paucity of report on the evaluation of recently introduce germplasm materials to enhance their utility in varietal development. This may have been responsible for authors' reports that a small proportion of the genetic diversity available in field genebanks at CRIN had been used to develop improved varieties supplied to farmers as planting materials [7, 17, 28]. In addition to this, the impact of mislabeling and off-type among the parental clones in the seed gardens and germplasm accessions of Nigerian field genebanks have been

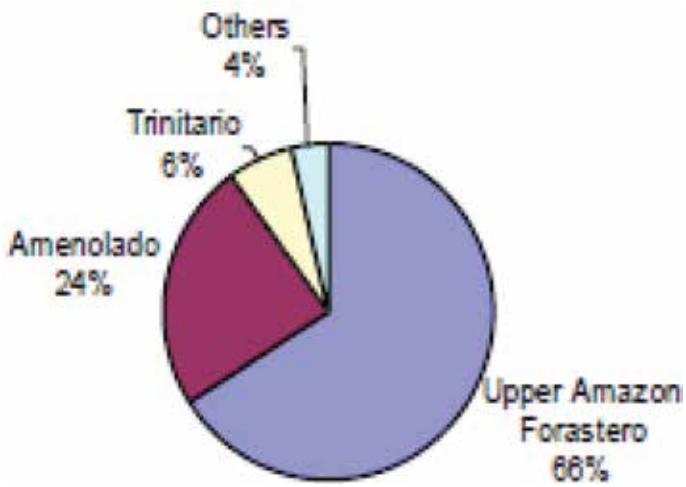

Figure 2.
Population structure indicating cacao types grown on farmers' fields in Nigeria.

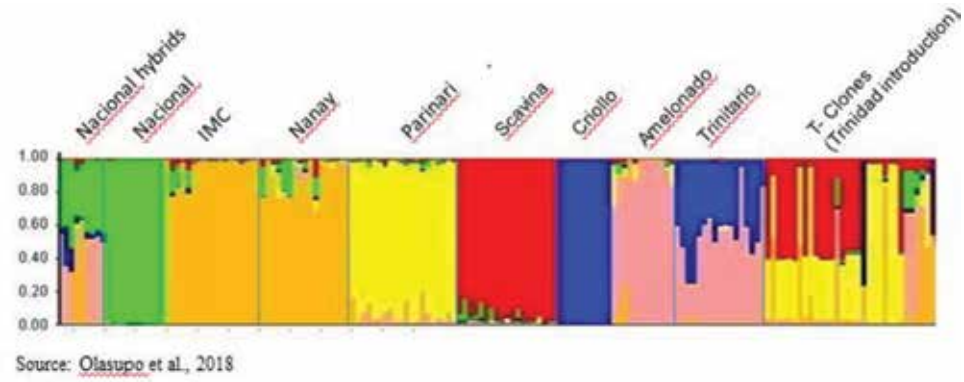

Figure 3.
Population structure of Nigeria cacao germplasm collections.

Genotype	Sample ID	Assessment	Tc32	Tc144	Tc193	Tc230	Tc242	Tc372	Tc529	Tc560	Tc591	Tc619	Tc645	Tc723	Tc872	Tc917	Tc929	Tc994	Tc998	Tc1060	Tc1442
AMAZ 15-15	ICGT, Trinidad	Reference	TT	AC	AC	AA	CT	AA	AC	GG	AA	CT	GG	TT	CG	CC	GG	CC	AA	CT	TT
AMAZ 15-15	IBAM1515-04_NIG132	True to type	TT	AC	AC	AA	CT	AA	AC	GG	AA	CT	AA	00	CG	CC	GG	CC	AA	CT	TT
AMAZ 15-15	IBAM1515-02_NIG1918	Offtype	TT	AC	AA	AA	CC	AA	AC	GG	AC	TT	AG	GG	CG	00	CG	CC	AG	CT	TT
AMAZ 15-15	IBAM1515-01_NIG1901	Offtype	TT	CC	AA	AG	CC	AA	CC	GT	AA	CC	AA	GT	CG	CT	GG	CC	AG	CC	CC
AMAZ 15-15	IBAM1515-03_NIG1876	Offtype	AT	CC	AA	AG	TT	AA	AC	GT	AA	CC	AA	GG	GG	CT	GG	CT	AA	CT	CC
PA 150	ICGT, Trinidad	Reference	AA	AC	AA	GG	CC	AA	AC	TT	AC	TT	AA	GG	GG	CT	CC	CC	AG	CC	CC
PA 150	IBPA150010_NIG1947	True to type	AA	AC	AA	GG	CC	AA	AC	TT	AC	TT	AA	GG	GG	CT	CC	CC	AG	CC	CC
PA 150	IBPA15005_NIG387	True to type	AA	AC	AA	GG	CC	AA	AC	TT	AC	TT	AA	GG	GG	CT	CC	CC	AG	CC	CC
PA 150	IBPA15009_NIG891	True to type	AA	AC	AA	GG	CC	AA	AC	TT	AC	TT	AA	GG	GG	CT	CC	CC	AG	CC	CC
PA 150	IBPA150-12B_NIG208	Offtype	AA	AC	AA	GG	TT	AT	CC	GT	AC	CT	AA	GG	CC	CT	GG	TT	AG	CC	TT
PA 150	IBPA150228_NIG1955	Offtype	TT	AC	AA	AG	CC	AA	CC	TT	CC	TT	AG	GG	CG	00	GG	CT	AG	CC	CT
PA 150	ADPA15013_NIG723	Offtype	AA	AC	00	AG	CT	AT	AC	GG	AA	TT	AG	GG	CG	CC	GG	CT	AG	CT	TT
Playa Alta	CATIE, Costa Rica	Reference	TT	CC	AA	AG	CC	AT	CC	TT	AC	TT	GG	GG	CG	TT	GG	CC	AA	CT	CC
Playa Alta	IBPLA04_NIG392	True to type	TT	CC	AA	AG	CC	AT	CC	TT	AC	TT	GG	GG	CG	TT	GG	CC	AA	CT	CC
Playa Alta	IBPLA03_NIG393	True to type	TT	CC	AA	AG	CC	AT	CC	TT	AC	TT	GG	GG	CG	TT	GG	CC	AA	CT	CC
Playa Alta	IBPLAV-12_NIG450	True to type	TT	CC	AA	AG	CC	AT	CC	TT	AC	TT	GG	GG	CG	TT	GG	CC	AA	CT	CC
Playa Alta	IBPLA11_NIG1873	Offtype	AT	AA	AA	AA	CC	AA	AA	TT	AC	TT	GG	GG	CG	CT	CG	CT	AA	CC	CT
Playa Alta	IBPLA099_NIG1897	Offtype	AT	CC	AA	AG	TT	AA	AC	GT	AC	CC	AA	GG	GG	CT	GG	CT	AA	CT	CC
Playa Alta	IBPLA09_NIG1898	Offtype	AT	AA	AC	AG	CT	AT	AC	GT	CC	TT	GG	GT	CG	CT	GG	00	AA	CC	TT
SCA 6	ICGT, Trinidad	Reference	AA	AC	CC	AA	CT	AA	AA	GG	CC	TT	AG	GG	CC	CC	CC	CC	AA	CT	CT
SCA 6	IBSCA605_NIG383	True to type	AA	AC	CC	AA	CT	AA	AA	GG	CC	TT	AA	GG	CC	CC	CC	CC	AA	CT	CT
SCA 6	IBSCA604_NIG388	True to type	AA	AC	CC	AA	CT	AA	AA	GG	CC	TT	AA	GG	CC	CC	CC	CC	AA	CT	CT
SCA 6	IBSCA602_NIG458	True to type	AA	AC	CC	AA	CT	AA	AA	GG	CC	TT	AA	GG	CC	CC	CC	CC	AA	CT	CT
SCA 6	IBSCA6-08_NIG096	Offtype	AA	AC	CC	AA	CT	TT	AC	TT	AC	TT	GG	GT	GG	TT	GG	TT	AA	CT	TT
SCA 6	IBSCA6-11_NIG882	Offtype	TT	CC	AA	AG	CC	AA	CC	GT	AA	CC	AA	GT	CG	CT	GG	CC	AG	CC	CC
SCA 6	IBSCA607_NIG891	Offtype	TT	CC	AA	AG	TT	AA	AA	GG	CC	TT	AA	GG	CC	CT	GG	CT	AA	CC	CT
SCA 6	IBSCA6-06_NIG1964	Offtype	TT	CC	AA	AG	CT	AA	AA	GT	AC	CT	AA	GG	CC	CC	GG	TT	AA	CC	TT

Table 1.
Examples of DNA fingerprints based on multi-locus matching of 28 SNPs between original references and Nigerian cacao collection (showing truncated profiles).

a significant problem hindering their efficient conservation and use for breeding programs. The occurrence of mislabeling and offtypes was first observed in the Nigerian cocoa germplasm collection by the application of SSR markers for genotyping [17]. This was recently consolidated by Olasupo et al. [28] through the application of SNPs for DNA fingerprinting of field genebank collections in which high level of mislabeling was detected in the recently introduced international germplasm materials (**Table 1**). Mislabeling has been identified as one of the key factors contributing to the high rate of unwanted/unproductive progenies produced in seed gardens because this can seriously compromise the quality of seedlings that would be distributed to farmers [17, 29]. Labeling errors in cacao field genebanks have been attributed to error from the source of germplasm introduction [30], human mislabeling errors in the nursery [31], sprouted rootstocks overtaken the scions due to poor germplasm management and multiplied error effect of using wrongly identified clones for new seed garden establishment [28].

In addition, the genetic diversity of cacao that constitute valuable resources in field genebanks useful to sustain cocoa production in Nigeria are further threatened with the following challenges:

1. Lack of funding for long-term management of collections, for research on diversity, evaluation and use in breeding

2. The field genebank collections are predisposed to the challenge of climate variability and its associated diseases and pests outbreak

3. Insufficient well trained personnel (expertise) for effective collection, conservation and management of germplasm materials

4. Urbanization and scarcity of land have led to the loss of many germplasm plots thereby resulting into extinction of some valuable genetic resources.

5. Problem of old age germplasm plots and the need to rejuvenate the old trees

6. Loss of germplasm to natural disaster (such as fire outbreak). There is the need for duplication of most of the field ***genebanks to serve as back-up (preferably *in vitro* or cryo-preservation)

7. Insufficient genetic variation in the collections to enhance effective selection of some specific traits.

Therefore, there is the need for all the stakeholders to protect the diversity of cacao germplasm collections with the aims of continuous supply of planting materials that have great potentials for high yield, disease and pest resistance, good architecture, drought tolerance and excellent flavor quality in the future.

4. Breeding efforts and impacts on national output

Cacao breeding research in Nigeria can be partitioned into four phase:

First Breeding Phase (1931–1956): The first phase of Nigeria cacao breeding started in 1931 [19]. Some of the trials conducted include the progeny trials of 1942 and 1945 by Voelcker which was the first of its kind in cacao research worldwide. Other trials conducted within this period include clonal trial in 1953 and the double cross hybrid vigour trial in 1954. The major breeding challenge during this period was low level of genetic variability among the parental accession. The main output of the programme was the identification of some materials with hybrid vigour and clonal propagation of the materials. These were N38 and other clonal selections—NT (Nigerian-Trinidad hybrid) 39, 114, 164, 215, 216, 284, 310, and 655. Others selections from the local clones and West African Amelonado population were HH268 and IS36.

In 1938, the West African Cocoa Research Institute (WACRI) started a *Cocoa Improvement and Varietal Development Programme* with the main objective of dealing with the threat posed by the cocoa swollen shoot virus (CSSV) disease in the West African sub region, particularly Ghana and Nigeria. The two main outputs were obtained from this programme.

i. First, introduction of the Upper Amazon genetic materials into Ghana and Nigeria cacao genepool which was very efficient in combatting the CSSV menace.

ii. Two general purpose varieties were also obtained from the programme. The F_3-Amazon—a third generation progenies resulting from open pollination

of the eleven approved Amazon T (Trinidad) types having broad parental background and have been shown to be superior to West African Amelonado in establishment ability, vegetative vigor, precocity (earliness in bearing) and yield. F_3-Amazon variety is also tolerant of CSSV and capsid attack [23, 32]. The Mixed Series II Hybrids (WACRI Series II) varieties were produced from crosses of Upper Amazon cacao with local selections. The hybrids showed better establishment ability (adaptability), precocity and higher yields than F_3-Amazon [33, 34]. In Nigeria, the yield of Series II Hybrids could be higher than F_3-Amazon yield by as much as 30% (165 kg/ha) [34].

Second Breeding Phase (1961–1970): The main focus during this period was on breeding of cocoa variety for specific ecological needs [35] with four primary objectives:

i. Select superior genotypes with high yield and desirable commercial qualities.

ii. Drought resistance or establishment ability

iii. *Phytophthora* pod rot resistance

iv. Cocoa swollen shoot virus (CSSV) resistance or tolerance

Two major outputs of this programme include selection of 12 "CRIN Establishment Ability" Elites which had a significant and prolonged experience in the south western part of Nigeria due to increased deforestation. A large number of germplasm was also introduced from Trinidad through a programme sponsored by the Cocoa Alliance, London [19].

Third Breeding Phase (1971–1980): The objective of this programme among others was to develop varieties that are resistant to or tolerant to pod rot disease caused by *Phytophthora megakarya* and *P. palmivora*. This programme was not fully implemented due to inadequate experts in the Breeding unit coupled with the challenge of self-incompatibility, a limitation to selfing of the Upper Amazon cacao trees [24]. Inadequate funding of breeding research was anther limiting factor.

Fourth Breeding Phase (1998–2008): This was a 10-year CFC/ICCO/IPGRI project with primary objective to develop new cacao varieties that will be high yielding, early bearing, resistant to Phytophthora pod rot, resistant to mirids and also have good and acceptable physical and chocolate flavor quality. The on-station and on-farm evaluations in this programme by CRIN resulted in the selection and consequent registration and release of eight new varieties (CRIN Tc-1, CRIN Tc-2, CRIN Tc-3, CRIN Tc-4, CRIN Tc-5, CRIN Tc-6, CRIN Tc-7 and CRIN Tc-8) in 2010. These hybrid varieties have diverse genetic base, they are early bearing, high yielding, with very low input, resistant to major pests and diseases of cacao, highly adaptable to cacao ecologies of Nigeria. The low input for high productivity attributes of these varieties is of great benefit for sustainable production of cocoa in Nigeria.

5. Current production challenges

Although remarkable breeding efforts have been invested through germplasm acquisition and development of improved varieties with high yielding potentials, cocoa yield at the farm level in Nigeria is still low when compare with the yield

potentials of improved varieties and there is continuous decline in the national output over the past three decades. Sustainable production of cocoa is achievable when prior attention is given to all factors involved in the production line. The following key constraints have been reported by [8] to be responsible for the low yield and production of cocoa in the country.

i. In adequate supply of improved planting material

ii. Poor access to improved planting materials

iii. Old age of trees

iv. Black pod disease

v. Poor price of cocoa beans

vi. Stem borer

vii. Mirid

viii. High cost of labour

ix. High price of chemicals and inputs

x. Loss of soil fertility/poor soil

xi. Adulterated chemicals

xii. Termites

xiii. Bryophyte

The solutions to most of these problems require intensive and focused breeding. Therefore funding cacao breeding research aimed at addressing these challenges should be given prompt attention. The role of the government in proffering solution to some of these problems cannot be over emphasized. Government would need to subsidize farm inputs needed for cocoa production. In addition to this there is the need for public private partnership efforts and intervention of stakeholders in cocoa industry to address these challenges.

6. Advances in science for cocoa production sustainability

Recent advancement in the fields of genetics, breeding and biotechnology has been used to the benefit of cacao improvement worldwide. The first molecular markers used for cacao genetic diversity study were isozymes [36], but these have limited numbers of loci and low polymorphisms. Significant progress has been made in the past two decades in cacao genomic mapping and germplasm characterization as reviewed by Guiltinan et al. [5]. More attention needs to be given to genomic sciences of cacao since these tools could be used in addressing many of the unanswered questions in the area of yield, pests and diseases, drought, architecture and flavor quality for sustainability of cocoa production. One of the major challenges of cacao

Figure 4.
Cacao establishment on well irrigated land without the use of plantain shade.

breeding is its long gestation period which takes a minimum of 2–3 years (from seed to seed). Cacao breeding is yet to tap from the advantage of marker assisted selection in reducing the breeding cycle as it is applicable in many other crops.

Tissue culture technique is useful not only for cryo preservation of germplasm materials. Application of somatic embryogenesis through temporary immersion technology will enhance mass clonal production of improved seedlings for large scale distribution to farmers to solve the problem of insufficient planting materials. Recent advancement in cacao science have been reported [37] that makes it possible for cacao planted on well irrigated land to survive without the conventional use of plantain as shade crop (**Figure 4**). This technology has the potential to solve the challenge of deforestation associated with new cacao establishment. This will also help to extend Nigeria cacao growing ecology to savannah region for increased productivity.

7. Conclusions

The sustainability and future of Nigeria cocoa production is hinged on the amount of diversity of genetic resources conserved and utilized in development of planting materials for farmer. Funding of cacao germplasm collections and research on its conservation, evaluation and use for breeding should be a top priority and collective efforts of public private partnership. There is the need for targeted exploitation of useful underutilized genetic resources available in the germplasm collections for varietal development in future breeding program. Conservation of cacao genetic materials in Nigeria needs re-organization and efficient re-hauling by establishment of correctly identified clones in new breeders' core collection germplasm plot using technologies of barcoding labeling and drip irrigation systems. Cocoa production in Nigeria will be revived to attain sustainability if the sector could tap from the great potentials of scientific innovations and technological advancement to the advantage of the industry.

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Conflict of interest

The authors declared that they have no conflict of interest.

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Chapter 7

Cocoa Genetic Resources and Their Utilization in Palm-Based Cropping Systems of India

Subbian Elain Apshara

Abstract

Cocoa (*Theobroma cacao* L.) became an integral part of palm-based cropping systems of India. It is being grown profitably as a mixed crop under arecanut (*Areca catechu* L.), coconut (*Cocos nucifera* L.), and oil palm (*Elaeis guineensis* Jacq.) gardens of the southern states Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh. It is well adapted to the humid tropics with high rainfall and short dry spells as well as irrigated gardens of tropical belts, utilizing the shade provided by the palms. Research efforts of more than four decades at the ICAR-CPCRI (Indian Council of Agricultural Research-Central Plantation Crops Research Institute) and KAU (Kerala Agriculture University) have allowed efficient utilization of conserved cocoa genetic resources by farmers to provide additional income through multispecies cropping systems in the spices and plantation sector. National Horticulture Mission of Govt. of India identified cocoa as a potential crop for area expansion and development to meet both internal and export demands. Improved varieties were developed with high pod yield, bean quality, suitable to different agro-climatic zones and to tackle major biotic and abiotic stress. This chapter outlines the contributions of cocoa breeding efforts at the research institutes and State Agricultural Universities; developmental programs of Ministry of Agriculture and procurement and processing facilities to the growth of the cocoa sector in India.

Keywords: cacao, cocoa, dry beans, genetic resources, germplasm, breeding

1. Introduction

Cocoa was introduced into India way back in 1798 at Courtallam in Tirunelveli District of the Old Madras State (Tamil Nadu) by East India Company [1]. Cocoa was distributed then in the agro-climatic region covering Western Ghats Hills and Plains of Malabar (Kerala) and Mysore (Karnataka) states, having more rainy days and short dry periods [2]. Commercial cultivation of cocoa as a plantation crop under palms started in early 1970s, and the current area under cocoa is 82,940 ha with a production of 18,920 tonnes of cocoa beans [3]. From the traditional hilly regions, cocoa production has shifted and expanded to coconut gardens of non-traditional areas of Tamil Nadu and Andhra Pradesh states utilizing the 50% shade available in the gardens and irrigation. Safe conservation of genetic resources of this introduced crop and its utilization in breeding program is a top priority considering its perennial nature, adaptability, and compatibility with palms. Cocoa tree has a

typical growth habit and a distinct morphology highly responsive to climate change and growing environments, which necessitates long-term conservation of genetic resources and dynamic breeding programs [4] as systematically adopted in India.

2. History of cocoa improvement in India

Cocoa breeding is one of the oldest improvement programs in the world. In India, the oldest plantations with Criollo type cocoa were established and evaluated for their performance in Kallar and Burliar Fruit Stations in the Nilgiris between 1930 and 1935. Those found with high yield potentials were distributed wherever the agro-climatic conditions suited the crop. In 1962, Indian Council of Agricultural Research (ICAR) decreed to grow Criollo type in South India and Forastero type in North East India. In 1964, few Malaysian clones of Forastero and Trinitario types were imported, and research on arecanut + cocoa and coconut + cocoa mixed cropping systems were conducted at Central Plantation Crops Research Institute (CPCRI), Regional Station, Vittal, Karnataka and other centers, Peechi and Palode in Kerala and Kahikuchi in Assam and proved as profitable cropping models. In 1965, a research-cum-demonstration unit of Cadbury India Pvt. Ltd. was established in Chundale in Kerala [5]. In 1969, systematic research was started in CPCRI with additional introductions of germplasm, followed by Kerala Agricultural University (KAU) in 1979 and then continued at KAU in 1987 with Cadbury India Pvt. Ltd. funding. In 2008, Tamil Nadu Agricultural University (TNAU) initiated cocoa research with funding from Mondelez International.

3. Breeding strategies in India

Systematic and long-term cocoa improvement programs are being taken up with the following strategies: (1) germplasm collection, conservation, cataloging, characterization, and evaluation; (2) breeding through selection and hybridization; (3) standardization of vegetative multiplication, establishment of seed gardens/clonal orchards; (4) comparative yield trials (CYT); (5) multi-locational trials (MLT); (6) screening and resistance breeding for biotic and abiotic stress; (7) biotechnology and bioinformatics approaches; and (8) demonstration gardens [6, 7].

3.1 Introduction of germplasm

The basic step in any breeding program is the introduction of germplasm from both the primary and secondary centers of origin and distribution. Cocoa in its native zones of South and Central America and other major producing countries of Africa is suffering with many debilitating diseases caused by both viral and fungal pathogens and serious pests. To safe guard the germplasm exchange program, Intermediate Cocoa Quarantine Centre (ICQC, R) was established in Europe. The University of Reading took over the responsibility for cocoa quarantine in 1987 [8]. The center routinely collects important clones from international and national gene banks, wild collections and conducts virus indexing. It conducts strict quarantine for other major diseases and pests using the guidelines for safe movement of germplasm formulated by FAO/Bioversity International [9, 10]. Clones that have been cleared at the quarantine are supplied as bud sticks, with proper import permits and sanitary certificates to cocoa researchers over 20 countries. It also maintains the International Cocoa Germplasm Database to know about the gene banks, clones, traits, and even SSR/SNP profiles [11]. CPCRI also utilizes facilities offered by

ICQC, R and collected exotic clones with desirable traits for specific research purpose, for which ICAR-NBPGR (National Bureau of Plant Genetic Resources) is the nodal agency in India. Around 500 collections are being maintained in the National Active Germplasm Site (NAGS) for cocoa at CPCRI, Regional Station, Vittal, Karnataka and also at KAU, Kerala. The germplasm collections include clones from The Amazon, Brazil, Ecuador, Ghana, Kew, Jamaica, Mexico, Nigeria, Peru, and local collections from Kallar in Tamil Nadu, Wayanad, Idukki in Kerala, and Shiradi Ghats of Karnataka. All these are being conserved and evaluated for their adaptability, precocity, compatibility, stability of yield, productivity, and quality of produce in the introduced environment.

Diversity among the genetic resources is important for improvement program, and Bartley [12] explained the existence of diversity based on the degree of human involvement in establishment of cocoa groups. The three basic types of cocoa, Criollo, Forastero, and Trinitario, which have specific pod and bean characteristics [13] are also among the collections. Expression of diversity is estimated from different indicators of variability, especially, morphological traits that are important for cataloging and characterization of germplasm. Bioversity International has standardized the descriptor status for cocoa, which comprises of 60 characteristics. Turnbull and Eskes [14] developed visual aid to identify widely distributed cocoa accessions with a minimal descriptor of 20 characters. Morphological variability with regard to tree architecture, leaves, flowers, fruit shapes, apex form, pod rugosity, prominence of ridges and furrows, husk thickness, pod size, color, bean size, shape, and color are characterized, and passport data documentation has been undertaken in 30-year old cocoa collections [15]. National identity numbers (Indigenous/Exotic Collection, IC/EC No.) were obtained from NBPGR, New Delhi for further utilization in the breeding program. Assessment of distinctness, uniformity and stability (DUS) of traits is currently underway.

3.2 Breeding through selection

The presence of genetic variability offers wide scope in selection of potential types. Yield is the main selection criterion. An easy approach in yield improvement is to select superior plants and subsequently developing them into clones. The major selection criteria followed in cocoa are, trees yielding 100 pods/tree/year, pods weighing 350–400 g or more with 35–40 beans having a fermented single dry bean weight of 1 g. Dry bean production is in general considered as a combination of three yield components: bean weight per pod, number of pods per tree, and number of trees per hectare. It is expected to be 1 kg and above per tree in a year, and productivity is usually assessed over 6 years in varietal trials after stabilization. At CPCRI, seven high yielding clones VTLC-1, VTLC-5, VTLC-7, VTLC-8, VTLC-9, VTLC-11, and VTLC-30 were selected and utilized as parents in the breeding programs as well as in establishment of seed gardens [16]. KAU identified seven clones CCRP 1 to 7 and released these selections for cultivation. Though individual tree selections are being made from seedling progenies, they have to be further evaluated in clonal trials for confirmation. Heritability for yield is low or average when based on single tree harvests but higher when based on yields from plots containing several trees, and so clonal varieties are gaining importance [17]. To assess the phenotypic value of genotype even in hybrid selection programs, clonal trials are advised [18]. From the clonal trials, three varieties VTLCC-1, VTLCS-1, and VTLCS-2 have been released for commercial cultivation in different agro-climatic zones in the countryn (**Table 1**). Genetic analysis on 17 plant, pod, and bean characters in 44 exotic cocoa clones resulted in the selection of superior genotypes for higher performance with traits of high heritability and genetic advance. Based

Characters	VTLCC 1	VTLCS 1	VTLCS 2
Canopy area (m^2)	12	12	15
No. of pods/tree/year	55	55	55
No. of beans/pod	35	42	41
Single dry bean weight (g)	0.9–1.05	1.3	1.21
Dry bean yield kg/tree/year	1.3	2.5	2.7
Dry bean yield kg/ha	890	1700	1840
Shelling %	12	11	15
Nib recovery %	88	88	85
Fat content %	50	52	53
Features	Self compatible line suitable for North Eastern Zones	High yielder withstands both biotic and abiotic stress	High bean index, stable yielder withstands both biotic and abiotic stress

VTLCC—Vittal Cocoa Clone.

VTLCS—Vittal Cocoa Selection.

Table 1.
CPCRI cocoa varieties developed through selection.

on the pod yield, VTLC-25, VTLC-15, VTLC-18, VTLC-36, VTLC-13, VTLC-37, and VTLC-17 have been identified as heavy bearers with an optimal canopy. These clones recorded single dry bean weight of more than 1 g, 10–15% shell, high nib recovery 85–90%, and more than 50% butter fat content making them suitable for industries as well [19, 20].

In the palm-based inter cropping systems, the pod yield in general is expressed with respect to the canopy area which is mainly maintained as cone/umbrella shaped single tier architecture. In the evaluation trials of Trinidad cocoa and Wayanad collections, 5 clones each are selected for high pod and dry bean yield ranging from 2.2 to 3.3 kg/tree/year [21, 22] with an optimal canopy of 15–20 m^2. Trait specific improvements are being taken up in the current breeding programs. A bean index of 100 beans/100 g, that is, dry bean weight of 1 g is considered as a standard for grade I beans [23], and so selections are aimed at a larger bean size of 1 g and above, which will have high butter content suitable for chocolate industry. The bean size is influenced by genotype, environment, and also the processing methods. Box, basket, and tray methods are being examined by research institutes as well as by farmers. Variation in bean indices has been observed across collections and the single dry bean weight ranged from 0.7 to 2.5 g. To improve the qualitative parameters, Criollo cocoa is used in hybridization programs [24], and white bean genotypes are being evaluated for quality parameters. Cocoa is considered as functional food, and so dark chocolates are consumed for their health benefits. Catechin, epicatechin, and procyanidines are the main polyphenols present in cocoa contributing to bitterness, astringency, and the organoleptic quality of cocoa. Cocoa beans of different clones evaluated for polyphenols and antioxidant activity exhibited distinct differences. Polyphenols ranged from 82 to 136 mg/g, procyanidin 49 to 64 mg/g, fat content of 24–55%, and antioxidant activity of 77–98% among cocoa clones studied. In general, cocoa beans with high polyphenol and procyanidin contents exhibited high antioxidant activities which are utilized for qualitative improvement [25].

Cocoa butter with free fatty acid (FFA) content of 1% or less together with acceptable flavor is the best indication of good quality beans. The type of fatty acids in 18 hybrids and 10 elite clones has been assessed, and from the profile, it is now known that 11 fatty acids are involved in the quality of cocoa beans. The fatty acids palmitic, stearic, oleic, linoleic, and arachidic acids present in all the genotypes invariably. The percentage of stearic acid is the highest in a range of 30.5% in VTLCP-7 to 44.2% in VTLCP-1. The other fatty acids differed among the genotypes in the percentage of expressio.

3.3 Breeding through hybridization

Hybrid vigor between parents showing good combining ability is readily exploited in cocoa improvement programs along with inter-population heterosis. Most commonly adopted method is developing hybrids between two distant genotypes.

3.3.1 Method of hand pollination and fruit set

For production of true hybrids with specific objectives and to confirm the compatibility reaction, hand pollination is routinely practiced. In artificial pollination, a flower bud, which will open the following day, recognized by its whitish color and swollen appearance, is selected. The bud is covered with a hood of plastic tube of 5 × 1.5–2.0 cm size, which is sealed to the bark using materials like plasticine/glaze putty. The tube is covered with muslin cloth at the top, kept in place with a rubber band. Opened flowers are collected from the desired male parent, and stamens are carefully taken out by pushing the corresponding petal. One entire anther with a part of the filament is deposited on the stigma. The pollinated flowers are labeled using tin foil pieces fixed in the cushion using ball pins. The hoods are removed 24 hours after pollination, and in 3–5 days, fertilization is confirmed by the visual swelling of the ovary [26].

3.3.2 Progeny trials

Different cross combinations have been tried with specific objectives for development of hybrids. At CPCRI, five progeny trials have been evaluated with 76 cross combinations during 1983–1994 at Vittal, Kidu, and Kasaragod centers with objectives of more number of pods, high dry bean yield, big bean size, and drought tolerance. Of these, 20 hybrids were identified as best hybrids and further evaluated as clones. Among them, 17 progenies exhibited high vigor and cropping efficiency even at early years of development [27, 28]. From the progeny trials, four hybrids VTLCH-1, VTLCH-2, VTLCH-3, and VTLCH-4 have been released as improved varieties for cultivation in the country; in 2006, the Golden Jubilee year of CPCRI, RS, Vittal and VTLCH-5 is released as Nethra Centura for the centenary year of CPCRI, Kasaragod (**Tables 2** and **3**).

Hybridization program at KAU was initiated during 1983 and continued up to 1993. During this period, a total of 159 parents were included to produce 239 crosses, 187 pods, and 21,819 hybrid seedlings. Nursery evaluation of these hybrids is done with HD^2 (H—height and D—diameter) value. A total of 3126 superior seedlings were field planted in series I, II, III, and IV and progeny trials I, II, III, and IV, and 163 superior hybrids were selected, utilized in various breeding program for release of hybrids, and used as better combiners in clonal gardens. Three hybrids CCRP 8, CCRP 9, and CCRP 10 with high yield and good level of tolerance to

Trial	Progenies tested	Progenies selected	Dry bean yield (kg/tree/year)
Progeny I (1983)	5	VTLCP-1	1.01
Progeny II (1984)	25	VTLCP-7	1.48
		VTLCP-49	1.47
		VTLCP-50	1.42
		VTLCP-11	1.39
Progeny III (1987)	13	VTLCP-18	1.08
Progeny IV (1992)	15	VTLCP-29	1.25
		VTLCP-30	1.52
Progeny V (1994)	18	VTLCP-26	1.33
		VTLCP-27	1.62

VTLCP—Vittal Cocoa Progeny [27].

Table 2.
Progeny trials of CPCRI.

Characters	VTLCH 1	VTLCH 2	VTLCH 3	VTLCH 4	VTLCH 5
Canopy area (m^2)	16	15	18	18	16
No. of pods/tree/year	50	50	41	40	66
No. of beans/pod	40	40	41	40	43
Single dry bean weight (g)	1–1.11	1–1.5	1–1.05	1–1.07	1–1.11
Dry bean yield kg/tree/year	1.4	1.5	1.7	1.6	2.5–3.0
Dry bean yield kg/ha	959	1030	1150	1090	1500–1800
Shelling %	13	11	15	15	11
Nib recovery %	87	89	87	87	88
Fat content %	54	54	51	51	52
Features	Early stable heavy bearer	Heavy bearer, tolerant to black pod rot	Suitable for water limited conditions	Suitable for water limited conditions	Suitable for high density planting both under arecanut and coconut

VTLCH—Vittal Cocoa Hybrid [29].

Table 3.
CPCRI varieties developed through hybridization.

Vascular Streak Dieback (VSD) have been released as varieties for cultivation in the country (**Table 4**) [24].

3.3.3 Seed gardens/clonal orchards

Based on the compatibility reactions, self-incompatible but cross-compatible high yielding parents are selected and multiplied as clones through soft wood grafting and established as clonal orchards or seed gardens. Bi-clonal and poly-clonal gardens were

Variety	Breeding method	Important traits
CCRP-1	Single tree selection from local population	Heavy bearer, self incompatible Vascular Streak Dieback (VSD) tolerant No. of pods—56.2/tree/year No. of beans/pod—46.2 Single dry bean weight—0.8 g
CCRP-2	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—53.9/tree/year No. of beans/pod—45.5 Single dry bean weight—1.0 g
CCRP-3	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—68.5/tree/year No. of beans/pod—42.3 Single dry bean weight—1.0 g
CCRP-4	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—66.2/tree/year No. of beans/pod—45.4 Single dry bean weight—1.1 g
CCRP-5	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—37.9/tree/year No. of beans/pod—45.25 Single dry bean weight—0.8 g
CCRP-6	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—50.1/tree/year No. of beans/pod—48 Single dry bean weight—1.9 g
CCRP-7	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—78.1/tree/year No. of beans/pod—46.9 Single dry bean weight—0.9 g
CCRP-8	Hybridization	High yielder and VSD tolerant No. of pods—90.4/tree/year No. of beans/pod—48.8 Single dry bean weight—0.9 g
CCRP-9	Hybridization	High yielder and VSD tolerant No. of pods—106.7/tree/year No. of beans/pod—36.7 Single dry bean weight—0.8 g
CCRP-10	Hybridization	High yielder and VSD tolerant No. of pods—79.6/tree/year No. of beans/pod—41.6 Single dry bean weight—1.1 g

Ref. [26].

Table 4.
Cocoa varieties of KAU.

established for production of hybrid seeds with known parentage and performance. These clonal orchards are established and maintained at CPCRI, Research Centre, Kidu, Nettana, Karnataka [30] and at College of Horticulture, KAU, Vellanikkara, Thrissur. In cocoa seed pods, seedlings and clones (grafts/budded plants) are being used as planting materials. CPCRI nurseries are accredited by National Horticulture Board (NHB), with four star rating for quality planting material supply and acting as model nursery on cocoa. Sixteen regional nurseries were established in different states for the area expansion programs of Govt. of India.

Generation	Genotypes	Plants selfed	Flowers selfed	Pods obtained
S0	102	102	25,052	147
S1	51	178	6263	163
S2	9	41	693	24
S3	5	55	1720	48
S4	2	17	428	9
S5	1	1	132	0
Total	75	394	34,288	391

Table 5.
Inbred population field established (1989–2010) at KAU.

3.3.4 Inbreeding

Inbreeding forms a part of the breeding activities, not only to breed parents with some degree of homozygosity for the production of hybrids but also to breed materials homozygous for desirable traits like disease resistance. Existence of self-incompatibility makes inbreeding efforts in cocoa difficult. Since few self-compatible trees are also identified in the populations, selfing is possible but it should be continued up to six to seven generations to attain homozygosity and thereafter to be utilized for crossing to exploit the hybrid vigor. KAU has taken up this task of selfing self-compatible plants and with over 20 years of continuous effort, maintains two genotypes of S4 generation, 5 of S3, 9 of S2, and 51 genotypes of S1 (**Table 5**) [24, 26, 31, 32].

3.4 Comparative yield trial (CYT)

The clones and progenies developed through selection and hybridization programs are multiplied as clones and evaluated under comparative yield trials in different situations. Under high density planting in arecanut garden, five hybrids VTLCP-6, VTLCP-20, VTLCP-15, VTLCP-1, and VTLCP-19 have been identified as best performers even in their initial years of growth [28]. Comparative study of parents and progenies as clones resulted in identification of VTLCP-6, VTLCP-2, and VTLCP-20 and parents VTLC-1 and VTLC-56 as potential high yielders [33]. In another trial, clones suitable for both arecanut and coconut canopies have been identified [34] and released as varieties. Under coconut in double hedge system of planting, hybrids VTLCP-22, VTLCP-18, and VTLCP-1 showed the best performance with an optimal canopy and high yield [35]. Evaluation of clonal and seedling progenies of selected genotypes has resulted in identification of four hybrids and two clones for multiplication both as clones and seedlings for utilization in the area expansion program [36].

3.5 Multi location trial (MLT) and demonstration plots

To assess the survival and stability of hybrids and clones in different agro-climatic conditions, multi-location trials are important. Elite clones of cocoa are under evaluation in both traditional and nontraditional states, namely Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra, West Bengal, and Assam, for studying genotype × location × environment interactions. Further, 115 front line demonstration plots were established under participatory research cum

demonstration plot scheme in farmers plots as well as in five AICRPP (All India Co-ordinated Research Project on Palms) centers funded by Directorate of Cashewnut and Cocoa Development (DCCD), Kochi and Directorate of Arecanut and Spices Development (DASD), Calicut, for identification of location specific varieties, common varieties, and to tackle the climate change effects.

3.6 Resistance breeding

Chemical control can be effective against fungal diseases but will pollute the environment and make the cultivation expensive. Integrated disease and pest management by the use of resistant materials and cultural and biological methods is probably the best way to contain pathogens and pests in the long run for sustainable crop protection. Breeding for resistance, therefore, became the primary objective for cocoa breeders worldwide. Sources of resistance have been identified for major diseases, and since cocoa genome is sequenced, it is expected to provide models for plant pathogen interactions and also facilitate identification of resistance genes. Many cocoa pests such as mealy bugs, aphids, caterpillars, and borers are also reported in India [37] are currently being managed with chemical control measures. Screening of available germplasm for prevailing diseases, existing and emerging pests will therefore be very important in light of seasonal weather variations.

3.6.1 Black pod rot

India is free of most of the debilitating viral and fungal diseases known in cocoa. Since the current cocoa growing area comes under high rainfall zone and the main harvest season coincides with monsoon, incidence of black pod rot caused by *Phytophthora palmivora* is comparatively higher. Though the main harvest is safe guarded with systematic annual pruning, in the post monsoon period, the second season crop is still affected by pod rot. On field screening, clones have been categorized into having <10, 10–25, and >25% damage levels. *In-vitro* screening using isolates of *P. palmivora*, *P. capsici*, and *P. citrophthora* indicated that collections of Nigerian origin exhibit certain degree of tolerance [38]. Few Wayanad collections have also expressed field tolerance to pod rot when tested over three seasons. Further, 21 exotic clones collected exclusively for *Phytophthora* pod rot resistance was identified for utilization in cocoa hybridization program. The variety VTLCH-2, a combination of ICS 6 × SCA 6 is found to be tolerant to black pod rot in India as well. KAU has taken up screening and hybridization program for combining desirable traits of CCRP released varieties and black pod resistance in cocoa.

3.6.2 Vascular streak die back

In India, vascular streak die back (VSD) caused by *Oncobasidium theobromae* was first reported from Kottayam, Kerala, and it began to spread to adjoining cocoa growing areas of the state. As this disease cannot be controlled effectively by the use of fungicides, KAU breeding program concentrated mainly on production of VSD resistant varieties. Hybrid seedlings were screened in the nursery by subjecting them to high inoculum load by keeping them in the midst of infected seedlings. The tolerant and vigorous seedlings were selected and established in field for evaluation. CCRP varieties have been especially released for VSD resistance and also have been utilized for establishment of clonal gardens for seedling supply [31].

3.6.3 Tea mosquito bug

Tea mosquito bug (TMB) (*Helopeltis* sp.) incidence became severe in the recent years in summer and post monsoon seasons. *Helopeltis antonii, H. theivora*, and *H. bradyi* are reported on cocoa in South India. Insect population is influenced by many factors like temperature, humidity, water stress, condition of cocoa tree, etc. The development and use of mirid resistant cocoa varieties is one of the alternatives to chemical control and resistance studies in cocoa have mostly concentrated on assessment of field damage [39]. Damage on flushes, cherelles, and pods of individual trees and different grade levels of infection on cherelles and pods are assessed to work out the TMB tolerance among genotypes. Penetrometer readings for determining the hardness of sclerotic layer, thickness at primary and secondary furrows of pod husk have been recorded in 100 cocoa genotypes and interpreted with reference to insect resistance [40]. Mechanism of plant resistance to insects is a complex phenomenon. Plant attractiveness to some extent affects the level of infestation, antixenosis prevents feeding, while antibiosis disturbs the pest development, and finally cocoa tolerance is assessed from the ability of a tree to contain damage and recover from it. Red colored pods with the smooth surface have been identified as tolerant to TMB damage among Wayanad collections.

3.6.4 Low moisture stress

Cocoa plants are susceptible to environmental conditions especially temperature and drought and considerably influences the pod yield [41]. Cocoa is very sensitive to water scarcity and undergoes a period of low moisture stress for 5–6 months in its current growing condition in India. Detailed study on climate change and weather variability over 43 years (1970–2012) at Vittal, which is located between 12°15′N latitude and 75°25′E longitude, showed 38% yield variability in cocoa [42]. The trends of temperature increase are +0.4°C for mean maximum ($P < 0.001$) and +0.4°C for mean minimum during the last decade. Breeding for drought tolerance is unique to our country and is taken up with systematic screening of available germplasm as well as hybridization programs. Screening of accessions is conducted for physiological parameters like stomatal resistance, chlorophyll fluorescence, proline accumulation under stress and by studies on seed germination under low osmotic potential, etc. A total of 216 cocoa genotypes have so far been screened for physiological and biochemical parameters under different trials [43]. In all these studies, field measurements were taken during unstressed (October) and stressed (March) conditions. Few Nigerian collections have been identified as drought tolerant and used for hybridization with high yielding Malaysian collections under two progeny trials. Two hybrids VTLCH-3 and VTLCH-4 have been released as varieties suitable for cultivation under water limited conditions in the country. Studies on leaf morphology, stomatal behavior, water relation components, and biochemical factors indicated that thick leaf, higher wax content, efficient stomatal closure, and high tissue elasticity are responsible for better adaptation of cocoa plants to drought conditions. The application of chlorophyll fluorescence as a tool to screen cocoa for drought tolerance has been confirmed with a series of genotypes. Recently, photosynthesis, chlorophyll fluorescence, and water potential under stress and nonstress conditions were estimated in 11 genotypes from different geographical origins, Columbia, Brazil, Peru, Mexico and Ecuador [44]. Seasonal and varietal differences were found, and transpirational water loss was found to be reduced with increased stomatal closure, which is considered as a favorable drought trait in any crop. Among the 52 new introductions, five Amazon and Pound collections have been found to be adaptable to water limited conditions [45] with high yields, which will

be further utilized in the breeding program. Genotypic differences for morpho-physiological criteria, potential antioxidant enzymes, and biochemical components depicting drought tolerance in young seedlings were determined with cocoa clones and hybrids under controlled low moisture stress conditions [46–48]. From these trials, standards and thresholds for several physiological parameters related to cocoa were established.

Hadley [49] detailed the visual estimates of physiological traits in cocoa, and the morpho-physiological parameters include measurements of flowering, fruiting, cherelle wilting, leaf flushing, branching and pruning intensity, canopy shape, density, and light transmission on different point scales. In order to understand and elucidate the optimum canopy shape and structure of cocoa, different spacing and canopy sizes have been studied at CPCRI [50], which showed significant differences in crop yield. In an experiment with grafts, the photosynthetically active radiation (PAR) and light interception varied significantly over the years with two spacings (2.7 × 2.7 m and 2.7 × 5.4 m) and three canopy sizes (small, medium, and large), and similar results were noticed with transpiration rate and stomatal conductance [51]. It is important to note that the maximum leaf area should be maintained, self shading of leaves should be avoided, and pruning should be done to the extent of retaining 20–30 leaves/developing pod to ensure the yielding potential of the genotype. With an annual pruning of single tier canopy, fertilizer dose of 100:40:140 g NPK in two splits with 20 L of water as drip is being practiced in maintenance of field gene bank under arecanut and coconut-based cropping systems.

3.7 Biotechnology and bioinformatics

DNA fingerprinting with RAPD markers has been done earlier on 76 collections, and the clones VTLC-11, 67, and 83 and VTLC-93 were identified as highly divergent. DNA extraction protocol of cocoa with fully expanded but soft leaves is standardized with the modified SDS method. Recently, 16 SSR primers specific to cocoa were used to assess diversity in 44 exotic clones, and both morphological and molecular diversity were assessed in detail [52]. An attempt has been made to identify the markers for drought sensitivity by utilizing susceptible and tolerant parents and progenies of cocoa [53]. About 75% of the genomic data of cocoa is available in the public domain which has paved the way for analyzing genes related to specific needs. CPCRI hosts one of the Agri Bioinformatics center under the Department of Information Technology and through bioinformatics tools, proteins involved in drought tolerance, *Phytophthora* resistance, and carotenoid biosynthetic pathways have been analyzed, and databases, CocoaESTdb, CocoaSTRESSdb, and a Standalone EST microsatellite mining and analysis tool (SEMAT) have been developed [54–60].

4. Future prospects

Cocoa improvement has attained a positive phase with the sequencing of its genome. Identifying genes responsible for incompatibility and disease resistance is the main concern of geneticists and molecular biologists. Expression of genes for resistance and quality parameters and their validation with trait specific germplasm is very important for future cocoa improvement program. Possible use of inbred lines will be taken up. Development of early selection, detection, and diagnostic methods for resistance will enable rapid screening of plant material and permit pre-selection activities. Because of the health benefits of dark chocolates, biochemical

constituents and antioxidant properties of cocoa are to be given greater attention in the breeding programs. Farmers participatory plant breeding, *in-situ* conservation of land races, exploitation of flavor components from genotypes belonging to specific geographic region, varieties for changing climatic conditions, and environment-friendly management strategies will be considered. Adaptability of cocoa genotypes in traditional and nontraditional zones should be verified, and location specific varieties should be developed [61]. At the national level, expansion of cocoa cultivation with the quality planting material of elite clones, collaborative approach between research institutes, universities, state horticulture departments, and developmental agencies are required. At the international level, participation of India in cocoa genetic resources networking and regional breeding groups of both developed and developing countries is important. Collaboration of India with Asia Pacific regional countries, Malaysia, Indonesia, Philippines, Vietnam, and Papua New Guinea is essential with their common coconut-based cropping systems with known pest and disease problems. This will enhance region specific sustainability of cocoa cultivation.

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Section 5

Cocoa and Political Economy

Chapter 8

Cocoa Plant, People and Profit in Ghana

Kenneth Peprah

Abstract

Cocoa production assumed commercial dimension by the beginning of the nineteenth century in Ghana. Soon after that the country became the world's leading producer of cocoa. Since then the ecological system of the cocoa plant, people involved in its management and their profit motives have been interwoven. The cocoa plant ecosystem in Ghana has been performing well under variable soil and climatic conditions. In addition, the different cocoa actors have unequal powers which affect sharing of the incentives from the sale of cocoa beans. Hence, sustainability of the cocoa industry in Ghana depends on ethical unity amongst the multistakeholders. By using the conceptual 3Ps approach to analysis of sustainability (environment, sociocultural and economic), benefits accruing and problems will become evident to elicit appropriate remedy.

Keywords: cocoa plant, people, profit, sustainability, Ghana

1. Introduction

Cocoa, *Theobroma cacao*, is a very important crop because it provides food, income, employment, industrial raw material and resources for poverty reduction [1]. Besides the provision of livelihood for millions of smallholder farmers, cocoa also provides raw material for the multibillion global chocolate industry. Hence, reduction in cocoa production is immediately felt by the smallholder farmers and the chocolate industry. As of 2013/2014 cocoa crop year, Africa contributed the majority of production (72%), Latin America (16%) and Asia together with Oceania (12%). With regards to dried cocoa beans as industrial raw material, Europe and Russia process/grind (39%), North and South America (21%), Asia and Oceania (21%) and Africa (19%). In terms of cocoa consumption, the European Union (EU) accounted for 36%, North America (24%), Asia and Oceania (16%), Europe outside the EU (10%), Latin America (10%) and Africa (4%) [2]. Therefore, the maintenance of cocoa to ensure continuous production and supply is of international concern. An analysis of world cocoa economy between 2011/2012 and 2022/2023 found steady decline in cocoa stock [2]. The media recently started announcing the threat faced by the cocoa industry. "We'll run out of cocoa [beans for chocolate production] in just SEVEN years-the world in just SEVEN years—the world will officially run out [of cocoa] on October 2, 2020" Star Sunday 6 October 2013, "no more chocolate by 2020!—Chocolate bars may get replaced by slabs of palm oil and vegetable fats packed with raisins and nougats by 2020" The Times of India 13 April 2014, "manufacturers warn that the world may soon run out of chocolate!" The Week 17 November 2014, "don't panic, but we could be running out of

chocolate"—by 2020, "the world could see chocolate deficit of 1 million tonnes" the Telegraph 17 November 2017, "enjoy today's Easter eggs: they could become a luxury" the Observer 4 April 2015 [2].

An analysis on the feared extinction of cocoa indicated a number of debilitating factors such as low cocoa productivity, pests and diseases, declining soil fertility, unavailability of inputs, high cost of inputs, outdated production systems, poor farm management practices, adverse effects of weather and climate change on environment, inefficient marketing systems, low uptake of innovations, technology and knowledge transfer as well as inadequate extension and advisory services [2]. The commercial cultivation of cocoa has always been affected by pests and diseases. The diseases include black pod (*Phytophthora* spp.), witches' broom, frosty pod rot (*Moniliophthora roreri*) and the cacao swollen shoot virus. The pests which infest cocoa include insect and vertebrate pests. Examples of insect pests include tea mosquito bug (*Helopeltis theivora*) in the family Miridae (capsid bug), cocoa mirid (*Distantiella theobroma*/*Sahlbergella singularis*/*Helopeltis* spp./*Monalonion* spp.), cocoa mealy bugs (*Planococcus* spp./*Pseudococcus* spp.), aphids (*Toxoptera aurantii*), leaf-eating caterpillar, ring bark borer (*Phassus hosei*) and cocoa pod borer (*Conopomorpha cramerella*). Examples of vertebrate pests are rats and squirrels [3]. In Ghana, mirids (capsids) are the most aggressive black pod (*Phytophthora* pod rot/ *Phytophthora palmivora*/*Phytophthora megakarya*/*Phytophthora capsici*) and cocoa swollen shoot virus disease (CSSVD) is the main indigenous pests and diseases. Pests and diseases have coevolved to threaten cocoa production as well as sustainability to the farmer, environment and industry [4, 5]. These diseases could reduce crop yield from 20 to 86% in West Africa [6]. Recently, impacts of extreme weather events on cocoa, particularly, drought associated with the global climate change have been reported [7]. In Brazil, 2015–2016 drought resulting from El Nino Southern Oscillation (ENSO) led to cocoa tree mortality of 15% and reduced cocoa yield by 89% [7]. Consequently, several scientific methods have been developed to manage and control these cocoa pests and diseases. For instance, integrated pest management has been employed to deal with pests. Various sociocultural, mechanical, physical, biological/agronomic and chemical measures are available to control and manage cocoa pests and diseases [3]. Research in cocoa varieties tolerable to changing global and local climates and adherence of research to stringent cocoa quality criteria are ongoing. Also, whether cocoa biotechnology research is a plus or minus to cocoa sustainability agenda needs careful consideration [8].

The existing literature has concentrated much on the cocoa plant (cocoa natural environment) by discussing cocoa diseases. Also, other authors have studied cocoa farmers' perception on environmental issues, particularly, climate change [9–11]. In the context of cocoa farmers (cocoa people), the focus has been cocoa farmer livelihood which is often tied to cocoa profit (economic aspect of sustainability) [1, 12, 13]. The linkages between cocoa natural environment regarding yield/production and cocoa purchasing as well as the multiple effects on poverty and carbon reduction have also been researched [14, 15]. A literature gap still exists regarding the consideration of the natural environment of the cocoa plant together with all stakeholders (people) and the economic sustainability (cocoa profit/incentive).

This chapter contributes to the discourse by arguing that sustainability of *Theobroma cacao* goes beyond the cocoa plant/crop to include the people involved in its management and sharing of incentives and profits thereof. Hence, the traditional "3Ps" usually used in the analysis of sustainable development applies to this study. The "3Ps" refer to profit (economy), people (society) and planet (environment) as the three main pillars of sustainability, otherwise, referred to as the triple bottom line [16]. Instead of planet representing environment, this chapter uses the natural environment of cocoa plant/crop. The people include primary producers of cocoa

(cocoa farmers) and secondary cocoa workers such as cocoa farmers' labourers working in the cocoa farms for wages, workers of Ghana Cocoa Board (COCOBOD) as well as employees of private and public cocoa-purchasing companies. The profit accruing from the sale of dry cocoa beans is depended on by all the cocoa people, in particular, and the country of Ghana as a whole.

2. Methodology

The study area is Ghana, the world's second largest cocoa producer after Cote D'Ivoire and located in West Africa. Cocoa is grown in the deciduous and rain forest as shown in **Figure 1**. Data were drawn from one district in the Volta Region (Ho

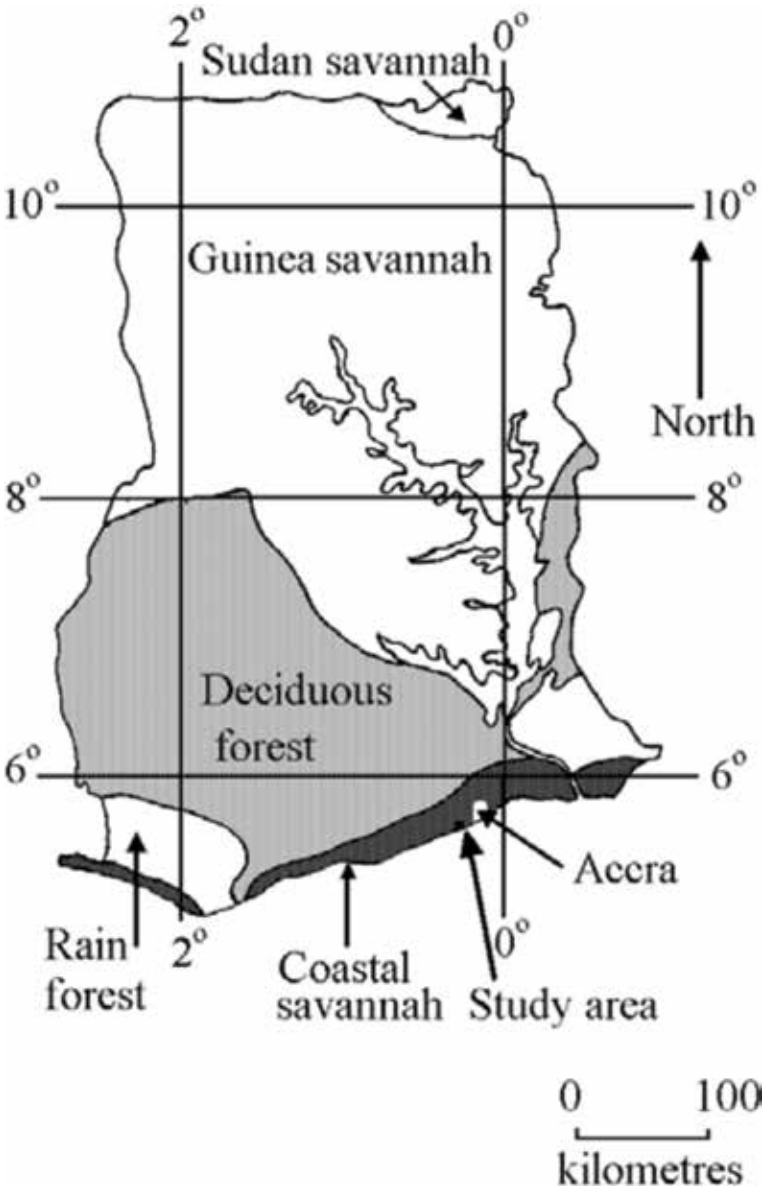

Figure 1.
Rain and deciduous forest of Ghana. Source: Adopted from ([17], p. 226).

West District) and two districts in the Brong-Ahafo Region (Asunafo North Municipal and Asunafo South District). This was a qualitative study in which data were sourced through the use of key informant interviews. Data were triangulated with community farmer meetings and focus group discussion. These were supported with information from the literature. The theoretical underpinnings were based on elaboration on the "3Ps" (the triple bottom line of sustainability). The assumption that sustainability of cocoa (SC) is the function of the "3Ps" (cocoa plant, people and profit) indicated by Eq. (1) provided the base from which subsequent equations were developed.

$$SC = f(Cocoa\ Plant + People + Profit) \tag{1}$$

$$\begin{aligned} Cocoa\ Plant = {} & Natural\ Environment\ Sustainability\ Only \\ & + \{Cocoa\ Plant \cap Profit\} + \{Cocoa\ Plant \cap People\} + \{3Ps\} \end{aligned} \tag{2}$$

where

$$3Ps = \{Cocoa\ Plant \cap People \cap Profit\} \tag{3}$$

$$\begin{aligned} People = {} & SocioCultural\ Sustainability\ Only + \{People \cap Profit\} \\ & + \{Cocoa\ Plant \cap People\} + \{3Ps\} \end{aligned} \tag{4}$$

$$\begin{aligned} Profit = {} & Economic\ Sustainability\ Only + \{Cocoa\ Plant \cap Profit\} \\ & + \{People \cap Profit\} + \{3Ps\} \end{aligned} \tag{5}$$

Also,

$$\{Cocoa\ Plant \cap Profit\} \tag{6}$$

refers to as conservation of cocoa

$$\{Cocoa\ Plant \cap People\} \tag{7}$$

refers to as deep ecology of cocoa

$$\{People \cap Profit\} \tag{8}$$

refers to as political economy of cocoa

$$\{Cocoa\ Plant \cap People \cap Profit\} \tag{9}$$

refers to as sustainability of cocoa.

In this context, conservation of cocoa implies sustainable maintenance in its natural environment either *in situ* or *ex situ*. During the conservation process, evolutionary changes in cocoa are permitted provided such changes engender present and future benefits. Also, deep ecology of cocoa extends the right to live (freedom) to cocoa plant/crop. Therefore, this right to survival does not depend on the expectations of cocoa people. Again, political economy of cocoa refers to the linkages between economic, social and political considerations, in which economic stands for profit whilst social and political refer to the cocoa people.

Following from the "3Ps" assumption, it is argued that sustainability of the cocoa industry in Ghana depends on ethical unity between the multi-stakeholders. This argument is based on the theory of ethical economy pivoted upon conflicts and compromises. Ethics as applied hereafter does not build on the meaning of general

rules of morality but rather as intrinsic value in economics. *Ethics is not to be conceived as an extrinsic limitation to economic pursuit but, on the contrary, as an intrinsic source of economic value. This is an approach that conceives of ethics as the practice of creating values and norms of action (a nomos) that is particular to a specific situation at hand* [18]. This brings in Eq. (10) which states that sustainability of cocoa is a function of ethical economy. Then, ethical economy refers to the intersection of multi-stakeholders, their interests, responsibilities and linkages that confer value to the multi-stakeholders and the country in the management of the cocoa industry.

$$\{Cocoa\ Plant \cap People \cap Profit\} = f(ethical\ economy) \tag{10}$$

$$Ethical\ economy = multistakeholders\ interests\ responsibilities \cap linkages \tag{11}$$

3. Results

The results of the study are presented under cocoa plant, people and profit, respectively.

3.1 Cocoa plant

Cocoa plants produce cocoa beans which are converted into dry cocoa beans, paste/liquor, cake, butter, powder, chocolate, chocolate products and waste as by-products. **Table 1** shows Ghana's cocoa production for three consecutive crop seasons as compared with that of Cote D'Ivoire.

Globally, 2016/2017–2017/2018 crop years (in 1000 tonnes) show that cocoa production has decreased from 4739 to 4645 tonnes (−2%) [20]. The 2017/2018 crop year in 1000 tonnes indicates La Cote D'Ivoire as the leading producers with 2000 tonnes and Ghana follows with 900 tonnes. The rest of Africa contributed 618 tonnes, the rest of Latin America 317 tonnes, Indonesia (280 tonnes), Ecuador (270 tonnes), Brazil (165 tonnes) and the rest of Asia (88 tonnes) [21]. There is a slight difference in the global cocoa production estimates of ICCO (4645 tonnes) and 2018 Cocoa Barometer (4638 tonnes) for 2017/2018 crop year [20, 21]. Ghana made a gain in cocoa production from 850 to 900 tonnes (in 1000 tonnes), about 6% increase in production from 2016/2017 to 2017/2018.

Generally, there are two cocoa populations, Criollo and Forastero. Trinitario, a hybrid of the other two, is considered as the third population. The type planted in Ghana is of the Forastero group. It was first introduced in 1815 and 1843 by the Dutch and Swiss, respectively, but failed to survive. Later in 1879, a Ghanaian, Tetteh Quarshie, successfully started a cocoa farm and raised about 300 trees by 1890.

Country	2014/2015 (tons)	Global supply (%)	2015/2016 (tons)	Global supply (%)	2016/2017 (tons)	Global supply (%)
Cote D'Ivoire	1796	42.1	1581	39.9	1900	41.7
Ghana	740	17.4	778	19.6	850	18.7
Africa (total)	3074	72.1	2911	73.4	3365	73.9

Source: Adopted from [19].

Table 1.
Ghana's cocoa production from 2014 to 2017.

However, due to efforts put into the cocoa industry by the British colonial administration and the independent government of Ghana after 1957, Ghana became the leading producer and exporter of dried cocoa beans from 1939 to mid-1960 [22]. Presently, Ghana is the second in the world's cocoa production and export after Cote D'Ivoire.

Whereas the soil on which the cocoa farms are found, the weather, climate and other environmental effects keep changing, the cocoa trees often term Tetteh Quarshie have kept their original physiology unchanged in many farms in Ghana. The stems of the Tetteh Quarshie cocoa trees remain very large and their heights compare with the medium layer tropical forest trees. Their productivity is very low indeed. Over the years, Cocoa Research Institute of Ghana (CRIG) has developed cocoa seedlings with shorter gestation period (5 years) than the Tetteh Quarshie (7 years). Farmers called it "agric" as the seedlings were introduced by the Ghana Agricultural Extension Service. These seedlings were modelled along the lines of the Tetteh Quarshie in terms of environment requirement (shade loving), height and stem development. However, the yield is by far superior. The soil remains heavily leached, and soil nutrients are mined for nearly 100 years of cocoa cultivation in addition to changing climate and other environmental factors. The soil is treated with doses of cocoa chemical fertilisers, and the cocoa trees are sprayed with various agrochemicals to prevent or control pests and diseases.

In the context of sustainable agriculture and sustainability of Ghana's cocoa, the third cocoa seedling has been introduced in Ghana (Tetteh Quarshie seeds is the first; "agric" cocoa seedling is the second). A subsidiary of Ghana COCOBOD, Cocoa Health and Extension Division (CHED), is tasked to assist cocoa farmers to cut down and remove old cocoa trees and replant with the new cocoa seedlings. The work is tedious for the cocoa farmers; many of whom are old people. The new cocoa seedlings at maturity are relatively shorter in height, have smaller stems and are sun loving rather than shade loving. Hence, deforested areas have become qualified areas for cocoa farming. The gestation period has been further reduced from 5 to 3 years. Therefore, cocoa farming has become attractive once again in Ghana's forest areas. One farmer intimated: *regarding the new cocoa farm one can use the manual spraying machine to spray the farm, but my cocoa farm with its tall trees I still use the machine powered by motor engine, that is the only way I can send the agrochemicals to the branches and leafs at that height*. Another farmer expressed the fear that: *some of the old cocoa trees are as old as 70 years, yet they keep bearing fruits. Whether this third new cocoa seedling will be able to bear fruits/pods after 20 years is my fear*. The farmers are still using their knowledge of the Tetteh Quarshie and "agric" to manage the new cocoa seedlings. For instance, a farmer said: *one cannot clear a piece of land, prepare it and plant only the new cocoa seedlings on the farm. First of all, we plant plantain (Musa ABB), cassava (*Manihot esculenta*), cocoyam (*Colocasia esculenta*) and "cocoa farm" yam (Dioscorea), major food staples of farmers to provide shade for the young seedling and food for farmers*. In about 2 years when fruiting of the new cocoa trees begins, we start to remove the food crops from the cocoa farm. Even trees are permitted in the newly started cocoa farm for the first 2 years to provide shade for the young cocoa seedlings from the scorching sun. Then, the gradual removal of the trees will begin. Hence, cocoa agroforestry as was the case with Tetteh Quarshie and "agric" cocoa trees would not be the case with the newly introduced cocoa seedlings. Farmers alluded to the fact that yield of the new cocoa

[1] A farmer claimed that: *even though the seedlings are the same for the smallholder farmers and the agricultural demonstration farms, the Ghana COCOBOD cocoa demonstration farms produce more fruits/pods than the farms of the smallholder cocoa farmers.*

breed is far superior than the two earlier breeds.[1] Hence, farmers are yet to bring their cocoa management practices closer to the expectation of the Ghana COCOBOD in order to enjoy full benefits of the new cocoa seedlings. These developments are taking place under the "Cocoa Rehabilitation and Intensification Programme" (CORIP) which is funded by the Dutch government. To achieve the same end is "The Next Generation Cocoa Programme" (MASO) funded by MasterCard Foundation is collaborating with COCOBOD on cocoa farms rehabilitation and job provision for the youth aged 18-25 years. These are implemented by COCOBOD.

Another issue bordering on sustainability of Ghana's cocoa is agricultural land use conflict between cocoa farming and gold mining. The latter has taken precedence over cocoa farming due to the higher and quick returns to gold mining as land use [1].[2] The threat posed to the sustainability of cocoa farming in Ghana by gold mining is real. The fear is heightened by the fact that many of the gold miners operate illegally as "galamsey," meaning "gather them (gold) and sell." The land tenure arrangement in the country places tenant cocoa farmers at a disadvantage. The customary land owners have greater control on land than the tenant cocoa farmers. The "galamseyers" deal directly with the land owners who do not benefit directly from the proceeds of the cocoa farm. They gave the land out to the tenant cocoa farmers sometime in the past. Ghana's cocoa frontier has moved from Tetteh Quarshie's cocoa farm in the Eastern Region to Ashanti, Brong-Ahafo plus Volta and Central Region to the Western Region which share boundary with La Cote D'Ivoire [23]. Irrespective of which region the farmers come from Ghana, they move along with the cocoa frontier changes. This has given rise to customary land owners and tenant cocoa farmers (local cocoa farmer investors). The protection of the investments of these farmers hold sway for the sustainability of Ghana's cocoa industry, with particular reference to land tenure and land use conflict with gold mining.

3.2 Cocoa people

In Ghana, the top-down blueprint approach is more prominent than the bottom-up approach in decision-making. The need to reverse this trend and put the cocoa farmers first rather than Ghana COCOBOD is necessary for the sustainability of Ghana's cocoa industry. The cocoa farmers are the fulcrum which sits the rest of the cocoa people. The multi-stakeholders of the cocoa industry include the farmers, COCOBOD staff and many private sector players (e.g., employees of the licenced cocoa-purchasing companies, transporters and logistics handling companies and assistants of private transport companies).

Cocoa farmers on the average cultivate about 4 ha of land. The farms are held under various tenure arrangements such as customary land owners' cocoa farms and tenant cocoa farms. There are shared croppers and monetary cash rental of land tenure arrangements. However, the cocoa farmer is the one who possesses passbook and has used it to sell dry cocoa bean. Cocoa farms are considered as big assets no matter the size. They are inheritable assets often passed on from parents, uncles, aunties and other relatives to their loved ones or the person next in succession.

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[2] A cocoa farmer has this to say: *the Chief Executive Officer (CEO) of Ghana COCOBOD came to Asummura and I asked him that, CEO, if the chair of Ghana Chamber of Mines comes to your office to tell, Isaac, I have found gold under your office (referring to the Ghana Cocoa House in Accra), take away all your files, furniture and computers and let me pull down the Ghana Cocoa House and mine the gold under it; and, you the CEO of Ghana COCOBOD will fold your two arms on your chest and look into her eyes and say to her, Joyce, you know I am a gentleman, please go ahead with your request. I asked, is that how you will behave?*

Hence, there are cocoa farmers who do not cultivate the farms they own. Some farmers also bought already cultivated and fully matured farms.

The COCOBOD is made up of its chief executive officer and two deputies, one for cocoa agronomy and the other for cocoa operations. There is the board of directors appointed by the government to oversee the entire activities. The COCOBOD operates under the Ministry of Finance. It is subdivided into eight directorates, seven departments and five subsidiaries. The directorates are human resource, research, audit, finance, medical, legal, special services—security and intelligence and general services—estates, civil works and transport. The departments include public affairs, security, procurement, scholarships, information service, estate and transport. The subsidiaries work to achieve cocoa production and marketing. They include Cocoa Research Institute of Ghana (CRIG), Seed Production Division of COCOBOD (SPD), Cocoa Health and Extension Division, Quality Control Company and Cocoa Marketing Company.

The cocoa private sector players are non-governmental firms licenced to buy cocoa and their employees. There are several non-salary workers who do various paid jobs in the cocoa farms on contract, at the marketing depots and at the harbours during shipment. These private labour providers are very important cocoa people in the production and supply chain.

With regard to sustainability of cocoa in Ghana, the COCOBOD is rather the pivot that carries the other cocoa people including the farmers. So far not much has been done to bring all the cocoa people together by the COCOBOD, even in terms of representatives to discuss their various roles in the industry. There is the association for cash crop farmers such as Cocoa, Coffee and Shea Nuts Pickers Association (COCOSHE) which interacts with the COCOBOD. Hence, national cocoa farmers association is not in existence, although cocoa buying firms have established various farmers associations to increase their share of the dry cocoa beans from the farmers. The lack of national common front for cocoa farmers has an effect on farmers' share of cocoa incentives which then becomes a function of the benevolence of the ruling government. Often, cocoa people who do office work at the COCOBOD and at the private cocoa marketing firms enjoy better living standards than the cocoa farmers who produce the beans. Gone are the days when cocoa farmers were better off than most salaried workers in Ghana. The notion of rich cocoa farmers belongs to a few farmers owning over 50 acres of mature cocoa farm. The need to improve on the standard of living of cocoa farmers is very critical to the sustainability of the cocoa industry. The issue is not about merely living above the poverty line. A much better standard of living would provide incentive for farmers to increase cocoa production through intensification and where land is still available by cultivating addition land. We have cocoa farmers who do not drink or eat chocolate. The reason is simple; income from the sale of dry cocoa beans is used for essential items on the farmers' scale of preference. Chocolate drink or bar is a luxury which is unaffordable. A farmer retorted: *do not come empty handed to interview cocoa farmers for data for your thesis or other research work. At least, you could come with a bar of chocolate to encourage us. I do not remember the last time I ate chocolate*. The same may apply to branded chocolate drinks in Ghana. In a family of six, when asked how many times you feed your family in a day, the father—a cocoa farmer—said: *we eat three times in a day. The main food for the day is done as supper/dinner. The reminder of that food is used for breakfast (first meal in the day) and lunch (second meal in the day). The only exception is when the supper/dinner is fufu and soup. Sometime, I ask my wife to prepare fufu and soup for the family as first meal in the day*. Fruits were not a usual part of their meal. The farmer said: *fruits do not do well in my cocoa farm and that avocado pear, citrus and mango disturb the cocoa trees. Sometimes, I get banana from my backyard garden*.

Another issue is the unequal power relationship amongst the cocoa people as often shown in the functions of the various stakeholders. The COCOBOD is tasked by the state to market cocoa beans and products, ensure quality control, evacuate/transport cocoa, undertake research and training, manage and control cocoa pests and diseases, carry out cocoa rehabilitation and maintenance projects and perform cocoa farmer extension services. The licenced private cocoa buying firms are to provide extra funds to purchase cocoa under the directives of the COCOBOD. Cocoa farmers are to produce and supply dry cocoa beans. The secondary employees (from COCOBOD, licenced private cocoa buying firms and others) supply supplementary labour. Although all the cocoa people revolve around the dry cocoa beans, COCOBOD by the nature of its functions weld considerable power than any other stakeholder. Hence, decision-making resides in the COCOBOD.

3.3 Cocoa profit

Normally, cocoa year begins on October 1 and ends on September 30 of the following year. Presently, the COCOBOD has sourced US$ 1.3 billion loan from the Dutch government to purchase cocoa for 2018/2019 (TV3 News360, September 19, 2018). Whatever incentives accrue to cocoa farmers depend on the magnanimity of the COCOBOD to encourage cocoa farmers. This approach is mostly informed by the target of government revenue from cocoa which is indicated in Ghana's budget by the Ministry of Finance. Other considerations include disparity between farmers' incentives in Ghana and their counterparts in Cote D'Ivoire and Togo. Also, the COCOBOD is mindful that sustainability of cocoa depends on the supply of dry beans by farmers. It is guided by the history of cocoa purchases which includes the 1924 as well as 1937–1938 cocoa "hold ups" by farmers and other Africans against expatriate buyers [24]. Hence, farmer incentives are determined by these factors rather than what farmers rightly deserve.

Farmers bear all the risks in cocoa price volatility as the COCOBOD can hedge cocoa at the stock exchange and reduce the risk. COCOBOD uses forward contract and by so doing transfer price and exchange rate risks from cocoa buyers to farmers [19, 21]. Again, the COCOBOD has not succeeded in stabilising farm-gate price. In addition to the motive of the country's cocoa revenue, cocoa farmers do not receive decent incentives [19]. **Table 2** shows cocoa farm-gate price in Ghana and Cote

Items	Cocoa farm-gate prices for countries	
	La Cote D'Ivoire	Ghana
Yield (t/ha)	0.49	0.42
Size of the farm (ha)	3.5	2.6
Total output (ton)	1.7	1.1
Farm-gate price ($/ton)	1487	1630
Total cocoa income ($)	2528	1793
Input cost ($/ton)	872	393
Net income ($)	1656	1400
FOB price ($/ton)	3120	3120
Producer price as % of FOB	47.7	52.2

Source: Adopted from ([19], p. 26).

Table 2.
Cocoa farm-gate price in Ghana and La Cote D'Ivoire.

D'Ivoire. Once the farm-gate price in Ghana is higher than that of Cote D'Ivoire, then, incentives to farmers become less a concern. The net income per farm in Ghana is lower than that of Cote D'Ivoire due to lower yield in Ghana. Hence, the attention of the COCOBOD is placed on yield/production increases rather than farmers' incentives. In such a situation, a farmer wishing to increase his/her incentive should think of increasing crop yield/production as price increase is no go area for farmers.

Figure 2 shows annual cocoa purchases in Ghana since 1959/1960 cocoa crop year. The trend line displays increases in purchases. The implication is that cocoa production is increasing as well as the availability of dry cocoa beans for purchase in Ghana. Furthermore, it implies that illegal cocoa trade from Ghana to its neighbours of La Cote D'Ivoire and Togo is reducing.

Table 3 presents information in a passbook of a farmer with his express permission. The data on inflation is to help in analysing the changes in the farmers' income, the lowest inflation (0.04% in May 1999) and the all-time highest (63% March 2001). It is clear that at the beginning of cocoa production, farmers' income comes in trickles (December 11, 2011 = GHS19.37, December 30, 2011 = GHS16.14, February 13, 2012 = GHS6.46 and September 24, 2012 = 64.55). The income is spent as it comes. Often, the dry cocoa beans are sold to cater for immediate family expenditure. The farmer intimated that no amount of income is left to be plucked back to improve the cocoa farm. Also, it is evident that price changes did not happen often. For instance, in 2011/2012 cocoa year, 5 kg brought to the farmer GHS16.14, but in 2012/2013, it increased to GHS16.69, about 3% increase in income. As to how come the same 5 kg in 2013/2014 was rewarded with GHS10.62 is difficult to understand.

With regard to COCOBOD scholarship available for secondary school education, the farmer said: *I got cocoa scholarship for my son during his 3 years in the senior secondary school. He has completed the school but I could not afford the fees at the university. He has moved to Accra to look for work*.

Another concern on cocoa profit is the issue of illegal cocoa trade. It includes traders who purchase cocoa illegally from farmers and transport them to Cote D'Ivoire or Togo. Presently, illegal cocoa trade is discouraged by the higher Ghanaian price than that of the two neighbouring countries. The only problem is that sometimes the purchased cocoa in Ghana does not come with immediate cash returns (farmers are not paid promptly, after weighing the beans). It takes between 2 and 3 weeks before the farmers are reimbursed with their monies. In such situations, farmers who are in dire need of money release their cocoa beans at the lower price to the illegal cocoa buyers because of prompt payment.

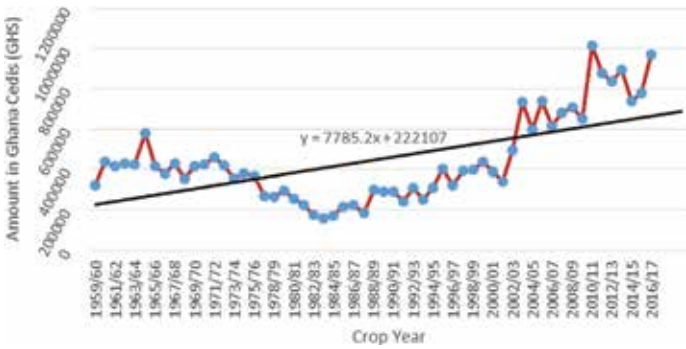

Figure 2.
Cocoa purchases in Ghana (1959/1960–2016/2017). Source: [25].

Crop year	Date	Cocoa beans output (kg)	Value (GHS)	Ghana inflation rate (%)
2011/2012	11 December 2011	6	19.37	8.6 (December 2011)
	30 December 2011	5	16.14	8.6 (December 2011)
	13 February 2012	2	6.46	8.6 (February 2012)
	24 September 2012	20	64.55	9.4 (September 2012)
Subtotal		33	106.52	
2012/2013	12 November 2012	20	66.79	9.3 (November 2012)
	03 December 2012	6	20.03	8.8 (December 2012)
	07 February 2013	3	10.01	10.4 (February 2013)
	09 May 2013	11	36.72	11.0 (May 2013)
	29 June 2013	5	16.69	11.6 (June 2013)
	20 September 2013	35	116.82	11.9 (September 2013)
Subtotal		80	267.06	
2013/2014	17 November 2013	43	143.52	13.2 (November 2013)
	25 December 2013	15	50.17	13.5 (December 2013)
	01 May 2014	5	16.69	14.8 (May 2014)
	27 July 2014	10	33.73	15.3 (July 2014)
	21 August 2014	5	10.62	15.9 (August 2014)
Subtotal		78	254.73	
2014/2015	28 October 2014	37	207.20	16.9 (October 2014)
	22 December 2014	13	72.80	17.0 (December 2014)
Subtotal		50	280.00	
2015/2016	23 October 2015	19	265.20	17.4 (October 2015)
Subtotal		19	265.20	
2016/2017	21 October 2016	37	281.20	15.8 (October 2016)
	04 November 2016	6	45.60	15.5 (November 2016)
Subtotal		42	326.80	
2017/2018	15 September 2017	28	212.80	12.2 (September 2017)
	08 December 2017	25	190.00	11.8 (December 2017)
	28 December 2017	20	144.00	11.8 (December 2017)
	19 January 2018	5	38.40	09.9 (August 2018)
Subtotal		78	575.20	

Source: Author's field work (2018).
Source of inflation data: GSS (www.statsghana.gov.gh>CPI) 25 September 2018.

Table 3.
Details of a smallholder farmer passbook.

4. Discussion

The discussion is organised along conservation of cocoa, deep ecology of cocoa, political economy of cocoa and sustainability of cocoa as summarised in the equations, respectively.

4.1 Cocoa conservation

The conservation of cocoa is the primary concern of both the farmer and the COCOBOD. Whereas the farmers are conserving all the three tree types of cocoa (Tetteh Quarshie, "agric" and the new type), the COCOBOD wants the farmers to switch to the newly introduced type. There are farmers who still prefer Tetteh Quarshie due to its resistance to pest and disease as well as to the changing environmental conditions. Some of the 1920 cocoa farms are still available in Ghana; no wonder, yield per hectare is low. Conservative cocoa farmers slowly adopt new cocoa seedlings. A farmer said: *Tetteh Quarshie paid my school fees. My father died and left the cocoa farm to me. I am using it to pay my children's school fees. I do not see the need to cut the trees down and replace with "Agric*." In the same cocoa farm, one can find a mixture of Tetteh Quarshie and "agric." However, there are many farms which contain only the "agric."

4.2 Deep ecology of cocoa

The cocoa farmers are more attached to their cocoa trees. Every single cocoa tree is very important to the farmer. Hence, the right to live as enjoyed by the cocoa trees will continue due to their significance to the farmers. To bring grid electricity to Asummura, the cocoa trees along the Aboum, Asummura road, were cut down to make path for the grid lines. One of the affected farmers said: *We need electricity so some cocoa tree have to be destroyed. We cannot say we do not want the electricity. So I agreed to the cutting down of some trees*. The grief of this farmer could be seen on his face and be heard in his voice. Farmers are willing to protect their cocoa trees, and they enjoy the support of the COCOBOD. The problem is the land use conflict with gold mining. The problem becomes aggravated when the cocoa farmer is a tenant, and the land is owned by another customary group of people.

4.3 Political economy of cocoa

The government of Ghana inherited the cocoa industry from the British colonial regime. The paternalistic attitude of owning the industry and farmers as children of the government continues at the detriment of the farmers. Government revenue is the primary factor in the cocoa industry. Cocoa farmers' incentive is of secondary concern. As farmers receive a lot more incentives from their cocoa farm, the more they will protect the trees in order to continue to enjoy such incentives. Hence, the politics, the economics and the social concerns of cocoa farmers are interlinked in the cocoa industry. Increase in cocoa farmer incentives means reduction in government revenue and vice versa.

4.4 Sustainability of cocoa

The importance of cocoa plant either as Tetteh Quarshie, "agric" or the new cocoa seedlings will continue to be conserved by cocoa farmers. The survival of cocoa trees now and in the near future is in the hands of cocoa farmers who attached so much significance to their farms no matter how small the size. The government of Ghana and its COCOBOD's interest in the cocoa industry will continue now and into the future. Improvement in the cocoa industry is therefore assured by the COCOBOD. Unless there is a catastrophe, extinction of cocoa does not appear to be imminent, at least, in Ghana's case.

5. Conclusion and recommendations

The study concludes that the sustainability of the cocoa industry depends on the three major aspects of the industry: cocoa plant, people and profit. Equity in the distribution of the profit will encourage all the people involved, particularly, the primary producers—cocoa farmers. The farmers will continue to attach great importance to their farms and sustain them. The plants will enjoy better management from the farmers with the support of the COCOBOD.

It is recommended that as the cocoa farmers increase, COCOBOD should categorise them into new and old farmers. Then, targeted incentives should flow from the COCOBOD to these farmers based on their needs. The new farmers have trickle income, whilst the old farmers have stabilised a more reliable income. COCOBOD should go beyond the prices of cocoa and see to the raising of cocoa farmers' living standards. Cocoa farmers should be made to enjoy some cocoa products like chocolate bars and chocolate drinks.

Regarding scholarships, the COCOBOD should step up the scheme to the university level in the face of the recent government policy of free education for senior secondary school.

It is strongly suggested to the government and its COCOBOD to adopt the ethical economy approach to ensure cocoa sustainability in Ghana. In this case, corporate social responsibility (CSR) is not a moral obligation to cocoa farmers, their communities and other cocoa workers or compliance to international standard; rather, CSR becomes an economic pursuit with an economic value and return to all the multi-stakeholders including the government and the country.

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Conflict of interest

I declare that there is no conflict of interest.

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Almost five million tonnes of cocoa produced annually drives the US$100 billion global chocolate industry. To sustain the industry, cacao planting materials (seeds and clones) have been successfully moved from the Amazon forests in America to the humid tropical forests of Africa, Asia, and Australia. In more than 150 years of commercial cacao cultivation, smallholder farmers that supply the bulk of cocoa beans still face several production constraints that impede their efficiency. Scientific technologies have therefore been deployed to remove these constraints by ensuring a continuous supply of good quality cocoa beans to meet growing global demand. This book provides insight into these scientific advances to address these current and emerging problems and to assure the sustainability of the global cocoa industry.

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