

**EFFECT OF TIME OF HARVESTING ON THE
DEVELOPMENT OF THE BLACK POD DISEASE OF
COCOA CAUSED BY *PHYTOPHTHORA*
PALMIVORA (BUTL.)**

BY

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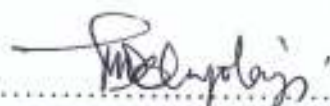
ABSTRACT

Research was conducted to study the effect of time of harvesting on the development of the black pod disease of cocoa caused by *Phytophthora palmivora*, ability to process and ferment the pods produced. Infected pods were harvested at one, two, three, four, five and six week after inoculation to determine the effect of time harvesting on the *mycellial* growth and *sporulation* of the fungus, the mineral contains of the dried beans as well as the suitability and ability of the pods and beans produced to undergo processing and fermentation. The results show that harvesting at two weeks after maturity was the most promising as it had the least *mycellial* growth, the highest number and weight of fresh pods and beans, dried pods and beans, fresh beans, dried pods and beans, fresh beans, dried beans, highest ether extract, moisture content, nitrogen free extract, crude fat and least crude fibre and ash contents coupled with a high processing and fermenting rate than harvesting at one, three, four, five and six weeks after maturity.



CERTIFICATION

This is to certify that this Research work was carried out by
ADEKAGUN J. K. of the Department of Crop Production, of the Federal
University of Technology, Akure under my supervision.



.....
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.....
Date:

DEDICATION

This work is dedicated to the Almighty God and to those people in developing countries who suffer and die because of the gross misuse and abuse of Agro-chemicals.



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My greatest acknowledgement is to the Almighty Father for granting me the opportunity and good health during the course of my studies. My sincere and unalloyed gratitude and thanks go to Prof. Ojeniyi who has been playing a fatherly role in the course of my academic pursuit and Prof. Olufolaji whose tenacity for hard work, thoroughness and doggedness saw me through this research work.. Dr Rex Aladesanwa whose useful suggestions and love were in evidence in the achievement of my set goal.

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1.0 INTRODUCTION

Cocoa (*Theobroma cacao*), a tree crop which is one of the major economic crops in the world is native to Brazil from where it was transported to other parts of the world including West Africa. Other countries of the world where cocoa is cultivated include Ghana, Ivory Coast, Nigeria Benin Republic, Ecuador, Venezuela, India (Opeke 1972).

Cocoa, palm produce, rubber, cotton and ground nut were the main stay of the Nigerian economy in the fifties to the early seventies before the discovery of oil in large quantities and of the Western Region in particular with cocoa alone accounting for over eighty percent of the region's earnings. The achievement of the then regional government (free education and health delivery system, industrial area development, good roads, infrastructural development etc) were all hinged on farming and especially on cocoa. Appendix II (Anon 2002).

Cocoa is used by many manufacturing industries as raw materials to produce beverages, dyes, drugs etc. The crop is highly valuable and this is why it is in high demand in food and drug industries in the developed world. (Akinwale - Ojo 1995). The demand for cocoa beans has not been met as a result of low productivity due to depletion in soil fertility, bad weather, effects of pests and diseases etc. Some of the most important fungal diseases of cocoa in Nigeria include: the swollen shoot disease caused by cocoa swollen shoot virus (CSSV), Brown pod disease induced by *Botryodiplodia theobromae* and black pod disease induced by *Phutophthora palmivora* (Adegbola 1972).

The Black pod disease constitutes a major production constraint in cacao (*Theobroma cacao* L) plantations. This disease occurs in almost all the countries where cocoa is grown (Filani 1975) sometimes inflicting heavy losses. Estimates of annual world cocoa production losses to black pod disease total 10% and in West Africa, 20-35%. The disease is caused by the fungus *Phytophthora palmivora* (Anon 1992). The fungus – *Phytophthora palmivora* belongs to the Division – *Mycota*, sub-division – *Eumycota*, class – *Phycomycetes*, order – *Peronosporales* and family – *Pythiaceae*. Its mycelia are hyaline and non-septate, producing asexual sporangia and sexual zoospores (Luz *et al* 1999). Primary infections of the hosts result from *sporangium* germination directly by germ tube or indirectly by producing motile zoospores. Infection of host tissue occurs by direct penetration of the epidermis through the stomata, (Opeke 1972). The fungus produces intercellular mycelium which ramify throughout the entire pod and enter the flower cushion through the peduncle causing local canker and proximal infection of new pods (Purdy *et al.*,1998). Heavily infected plants are characterized by pods having brown discoloration that eventually spreads over the whole pod which blackens and shrivels up at a later stage of the disease. (Schmidt *et al.*,1997). The attack of *P. palmivora* on young pods cherelles are not usually assessed because of interference with cherelle wilt and other pathogens attacking young cocoa fruits that are normally less than two months old and had not attained the maximum length of about 10cm (or two-thirds of the mature fruit size) hence the actual damage to the cocoa industry seems to have been under-estimated (Schmidt *et al* 1997, Purdy *et al.*, 1998).

Black pod disease can readily be controlled by the application of

fungicides such as Bordeaux, Ridomil plus, Perenox or other Copper compounds (Maddison, 1980). However the use of chemical has been regarded as unsafe and uneconomical for resource poor farmers in Africa who lack the knowledge about the use of chemicals and where land holding are usually small (Urquant 1961). Consequently, control based on phytosanitary and cultural measures such as regular removal and destruction of infected pods, removal of superfluous shade trees, regular weeding and prompt harvesting of ripe pods could be a viable control option for the disease.

The present study examines the effect of time of harvesting of cocoa pods on the incidence of black pod disease on cocoa and cocoa quality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diseases associated with Cocoa

Black pod disease of cocoa caused by the fungus – *Phytophthora palmivora* is the most important fungal disease of cocoa in West Africa and all over the world where cocoa is produced. It is so important to have warranted a research in Papua New Guinea in the early fifties (Adegbola, 1972) and by Thorold and Owens in both Ghana and Nigeria respectively at the same time (Darkwa 1973).

Field and laboratory studies shows that a lot of fungal disease pathogens are capable of causing rotting of cocoa pods however the following fungi are associated with rotting of crops generally and cocoa in particular, *Phytophthora capsitis*, *P. cititophthora* (Allforest 1993), *Fusarium solani* (Mart) Sac; *Fusarium decemcellure*, *Botryodiplodia theobroma* (Pat) (Rappaport 1993), *Monillia spp.*, *Thievelaviopsis spp.*, *Colletotrichum spp.*(Maddison1980); *Morasmus equiperimus*, *Corticium salmonicola*, Thread blight and the most devastating of these pathogens, *Phytophthora spp* (Williams 1977) as well as seven other species of *Phytophthora* have been identified as the causal agents of the black pod disease in different countries as follows:

P. megakarya and *P. infestans* are commonly found in Ghana, Nigeria and Cameroun while *P. palmivora* is prevalent in Ghana, Brazil, Ivory Coast and Nigeria. *P. capsici* is common in Trinidad, Venezuela and Malaysia. *P.*

citrophora, *p. nicotina*, and *P. megasporma* are equally devastating in Brazil, Venezuela and Malaysia.

Of all these, three are the most devastating and widely spread – *P. palmivora*, *P. capsici* and *P. infestans* (Anon 2000).

Phytophthora palmivora is of great concern for cocoa production as farmer are very familiar with the disease but control still remains evasive and it is devastating in many ways, it is therefore of great importance to cocoa and to many countries including Ivory Coast, Ghana, Nigeria, Brazil, Ecuador, Cameroun and Malaysia etc in which cocoa is the back bone of their economy and foreign exchange earnings (Engels *et al.* 2000).

2.2 Black Pod Disease Symptoms

The fungus is a widely distributed facultative parasite (Thorold 1977). A spore landing on a moist surface of cocoa pod sends out a slender hyphal filament into the pod through the epidermis. The fungus consists of an intercellular network (*Mycelium*) of hyphae which goes through the pod sending out side branches into the cells (Darkwa 1973).

The black pod disease exhibits a dark brown soft lesion on cocoa pods. Lessoned area is firm to the touch except when rotting has progressed for a considerable length of time. When secondary invaders like *Botryodiplodia theobroma* come in, white creamy sporulation appear which turn straw coloured in fairly dry conditions. Apart from cocoa the diseases is also found on coffee and rubber (McLaughlin, 1974). Adebayo (1977)

observed that the reaction of infected pods to different temperature and humidity levels can also be a determining factor of identifying the disease, for example the colour of *P. palmivora* lesions ranged from dark or brown in pods kept at 25-26°C and about 100% relative humidity to very high brown in those kept at high temperature and low humidity. *Fusarium solani* can easily be confused with those of *P. palmivora*. (Allforest 1993).

The two main important diseases of cocoa in Nigeria are the pod rot caused by *Botryodiplodia theobroma* and the black pod caused by *P. palmivora*. Of these two, the black pod disease takes the lead (appendix IV) causing heavy damage to the cocoa plants and un-estimated hardship to the cocoa farmer who suffers serious and untold economic losses. The virulence and fecundity of *P. palmivora* to survive is responsible for its ability to infect cocoa plantation within a short period of time and with devastating effect (Anon 1997).

Also the swollen shoot disease caused by the cocoa swollen shoot (CSSV) should not be underestimated in the cocoa plantation as the duo of black pod disease and the swollen shoot disease are capable of attacking various parts of the cocoa tree and ultimately reducing the yield in affected farm (Custracera 1964). (Appendix III & IV).

Phytophthora palmivora is endemic in Nigeria, Cameroon, Ghana, Brazil, Ecuador and the Ivory Coast, it is also very endemic in areas with prolonged high relative humidity periods during the cocoa cropping season. This fungus attacks several tropical crops apart from cocoa and such



tropical crops are cashew, coconut, areca palm, oil palm, Betel palms, rubber, citrus, coffee, coconuts, cotton etc. but cocoa is its major host (Turner 1972). However each of the crops are attacked by different strains of the fungus and more than one strain of the fungus attacks cocoa (Anon 1994). *P. palmivora* attacks all parts of the cocoa plant provided there is adequate soil moisture and temperature. Its infection could occur at any time (Adebayo 1975). Primary pod infection in cocoa may be as a result of rain splash, zoospore migration, contact with infected pods or through insects, animals and human agents. Reproduction can also be sexual or asexual. Fertilization in *P. palmivora* is *Amphigynous* and *Oospores* formed develop pathogenically. The fungus is widely distributed in the warmer parts of the world with the possible exception of French Guyana as over eighty hosts are known. Thorold (1977) discussed the factors affecting incidence of the black pod disease of cocoa as high relative humidity associated with a well distributed rainfall and low temperature. In Nigeria the peak season of the black pod disease infection was reported to be July to September and minimally in October to November (Adegbola 1972). In Ghana (Ahenkorah 1974) found out that it occurred in June to mid October. Asare-Nyako in 1973 also reported that studies of the cropping and disease patterns in Ghana revealed that cropping and disease incidence were earlier in certain parts of the country (Ghana) than in other parts and that the amount of rainfall was not necessarily correlated with the disease incidence, as disease curves reached peak earlier than yield curves. Cropping in

Ameleonado which was later than in non-Ameleonado i.e. Amazon. mixed Amazon Hybrids and the local Trinitario were less infected. Cherelles which were the most numerous were the least infected of all the varieties. Both Ameleonado and non-Ameleonado types which cropped earlier on the trunk than in the canopy were on the average as per disease incidence as incidence occurred earlier on the trunks while the canopy which cropped later had a significantly lower disease incidence than the trunk (Akinwale - Ojo 1995).

As the favourable disease infection periods are observed within specific periods of the year in the countries where the disease occurs it is reasonable to expect that cocoa that produce all or most of their pods at the epidemic period (May to October) are likely to suffer more from the disease than those bearing outside the epidemic period. Pod production and black pod disease are probably the most important mark determinants in cocoa. The two yield factors has consciously featured not only in the first and second but also in the third Nigerian cocoa Breeding Programmes. The aim of the Programmes were to identify from the cocoa population under study promising genotypes particularly in respect of high yield and low black pod disease incidence (Turner 1965, 1972).

Black pod disease attack which is higher on the matured pods than on the cherelles has its source of inoculum in the soil (Okaisabor 1975). Newhall (1971) observed that diseased pods, cushion and infected leaves are all important sources of primary inoculum for the *P. palmivora* induced

black pod disease but Blencove (1961) and Darkwa (1973) noted that the relative importance of each in initiating black pod *epiphytotic*s may differ from one geographical or geo-ecological area to another. The *epiphytotic* induction of cocoa black pod disease depends on the initial supply of inoculum and the innate susceptibility of the cocoa plants and the environmental condition under which the cocoa is growing which in turn are regulated by the local climate.

Black pod incidence is known to be favoured by low temperature of 22 -25°C high relative humidity of 80%, but adversely affected by high temperature and low relative humidity. It is known that climatic condition under the cocoa canopy are slightly different from those outside the cocoa stand (Macdonald 1932) and (Darkwa 1973) and as such the disease is favoured under the cocoa canopy.

Phytophthora palmivora can be found in the soil, old black pods hanging on the tree, flowers, pods and bud cushion and in pods left on the trees from the previous year's harvests. Pod infections are often associated with some predisposing factors. (Darkwa,1973) and (Wharton, 1974) analysed the infections on a number of trials and found out that 25% of the infections were due to contact with a diseased pod. A similar percentage followed injury to the pod a 5 - 27% was due to drip, resulting in great yield losses.

The first symptom of the disease is a brown water soaked spot which appears on the pods a few days after infection. This sport enlarges rapidly

and darkens until the whole pod is invaded and blackens. The fungus then forms its own fruiting bodies (*sporangia*) on the surface of such diseased pods. These contains the spores by which the fungus infects pods (Newhall 1968 and Filani 1975). The infestation later results into brown spot with irregular water soaked edges on the pods which later gets larger with whitish mould where new spores may have formed. Formed spots then coalesce and turn dark brown to form larger spots which turn black covering larger areas and in severe cases the entire pod.

Mydellia spores are spread to other pods both by invertebrates (ants, termites, flies etc) and also by vertebrates (man, apes, rodents, monkeys, etc). Water splash and drips from infected pods above the lower fresh pods is another source of spread of the disease. Thorold (1975) also observed that cultural operations is also another means of spread. Among the numerous effects of the pod rot disease on cocoa are immature pod production, deformed cocoa beans, immature ripening of pods, premature shedding of the pods, reduced cocoa beans sizes, Beans from infected pods have less sugar and with higher free fatty acids (FFA) and excess butter fats (Opeke 1972). Slaty beans which would fail to meet. Food and Agricultural Organisation (FAO) and World Health Organisation (WHO) minimum standard would be produced thereby causing reduction in the farmer's earnings.

Chemicals that are available in the market for the control of the disease are Perenox, Cupavate, Bordeaux mixture, Kocide, Brestan,

Kokobre Sandoz, Ridomil, Benlate etc (Adegbola 1975). The use of resistant varieties which is an alternative control measures has been reported (Adegbola 1972), and this has been in the use of LAFI No. 7 in Western Samoa which is a prominent cocoa producing areas of the World and it was found to be resistant to the disease (Sounko 2000). In Nigeria, the Cocoa Research Institute of Nigeria and researches are still on to prove them to be so by the use of their products (cocoa beans) and rooted cuttings (Wrigley, 1962; Adebayo, 1975). The result of this investigation would go a long way in helping the resource poor farmers in Nigeria and other part of the world where cocoa is grown.

Other varieties that are resistant to black pod disease had been reported in Brazil and Cameroon. Such varieties should be of great economic value to both the farmers and to the consumers who would have a healthier product.

2.3 Control of Black Pod Disease of Cocoa

The first attempt at working on *P. palmivora* was made by a German Scientist called Glenditech in 1953. Since then black pod has been investigated by several workers during the late twenties, after the first researcher (Dade, 1928) and Adebayo (1975), reported that the reductions in the number of insect pests and the attendant inoculation which led to the reduction in the number of wounds through which the parasite can infect pods may account for reduction in pod loss due to black pod disease. He

further indicated that ability to thrive under different conditions can be used to advantage in the control of the disease. Thus control measures used in Ibadan Oyo State could be different from those used at Ikom in Cross River State.

In order to control this disease, the Cocoa Research Institute of Nigeria (CRN) and the Cocoa Research Institute of Ghana (CRG) were saddled with the problem of finding possible control measures even though preference has been place on the non chemical control measures, the use of chemical (fungicides) cannot be overlooked or ignored since it is still the most effective and fastest means of controlling the black pod disease of cocoa (Mathews, 1978; Anon 1980). In the light of this, some fungicides have been used over the years, but with the evolution of biotypes of the fungus, resistance to the fungicides by the fungus has been developed; Continuous research is needed to cater for the variation in the concentration and composition of the fungicide. Chemical control is thus burdensome and expensive.

Newhall (1971), recommended spraying trees with twelve or more pods after every 15-20mm of rainfall in a year in Costa Rica. Asare-Nyako (1975) was able to control black pod disease and obtained economic yield in Ghana by spraying with Bordeaux at weekly interval. However, clean propagating materials, effective cultivation practices, adequate pest and disease management strategies continued to make a good cultural control measures for the black pod disease (Anon CTA,1998). Wharton (1955)

found that control with Bordeaux mixture and Perenox could be uneconomical in Ghana and both of them recommended good sanitation and cultural practices. Anon (1997) indicated that crop could yield well without the use of chemicals such as fungicides, insecticides and herbicides and that the yield may even be better where little or no chemicals are used but with adequate cultural methods and good farm management. (Anon 1998 and Anon 1998)

Chemicals used to control and prevent fungal attack on crops are classified according to their mode of action such as contact, systemic or both combined together. Some are known for the potency of their active ingredient Perenox a cuprous oxide sprayed at 4.5g/litre and at an interval of 14-21 days, Kokobre Sandoz also a cuprous oxide sprayed at 14.5g/litre and at an interval of 14-21 days, Procida BBS 1% copper sulphate sprayed at 10 + 4g/litre and at an interval of 14 days, Kocide having copper hydroxide sprayed at 4.5g./litre and at an interval of 14-21 days, Brestan 60% copper hydroxide sprayed at 1.5g/litre at an interval of 14-21 days, Bordeaux mixture with copper sulphate sprayed 10g and lime 4g/litre and at an interval of 14-21 days, Ridomil plus 50wp with metalaxyl + cuprous oxide and applied at 3.3g/litre at an interval of 28-30 days. All the aforementioned chemicals have been used for the control of the black pod disease and have about equal effective properties but with Copper sulphate plus lime (Bordeaux) and Ridomil plus 50wp having the best result because of their preventive power while Ridomil and Bordeaux with

its curative power on regular spraying in the Fernando Po Island, losses has been reduced to as low as 10% (Are, 1974). Cocoa die back disease caused by *Ouchobasidium* (common in the Pacific) was controlled by the use of Procida BBS 1% (Wharton, 1955). Ridomil plus 50wp is a systemic fungicide penetrating effect and can be used for the control of all fungi especially the *P. palmivora* and the downy mildew of maize (Zarks, 1975).

Other methods that can be adopted in the control of the black pod disease of cocoa involves application of systemic fungicides and depends on the seasonal variation in the population of sources of inoculum. Outbreak of *P. palmivora* vary with time and according to the regions and even from one country to another. Generally, *P. palmivora* appears in May to October in African countries. The incidence increase to reach a peak in outbreak in July- September with this type of development there is need to commence control measures (spraying, sanitation of plots etc) as early as May with the on set of the rains, (End *et al.* 2000).

Luz *et al.* (1999) also stated that constant and periodic removal of parasitic organisms such as mistletoes, regular weeding and pruning are also some of the methods of control that could be practiced.

Apart from the direct economic reasons why cocoa should be improved, the following are the other reasons why cocoa production should be improved through disease control especially, the pod rot disease.

- Retention of traditional flavour and aroma
- Quality of beans

- Processing ability of the seeds
- For food production and industrial materials (Anon 2002).

3.0 MATERIALS AND METHODS

3.1 The Study Site

Identical but independent trials, were conducted in 1998/1999, 1999/2000 and 2000/2001 at the Ibule Seed Garden, Tree Crop Unit of the Ondo State Ministry of Agriculture and Rural Development, Akure. The study site was located in the rainforest zone of south Western Nigeria. The physical and chemical properties have been described by Smyth and Montgomery (1962) and that the *alfisols* and *mollisols* with p^H of 3.8-6.7, clay content of 16-67%, CEC of 5.63-16.20 and iron oxides of 0.23-8.72% are more suitable than the ultisols and oxisols. Rainfall in the area averages 1300mm per annum in a bimodal pattern with a long season from April to August and a short one from September to early November. The two seasons are separated by a short dry spell, the August dry spell (Appendix V).

3.2 EXPERIMENTAL DESIGN

The trials each consisted of six treatment comprising five randomized single tree replicates of nine (9) years old c20xc64 hybrid cocoa trees planted in 1990 from rooted cuttings. Treatments were hand pollinated cocoa flowers which were harvested at one, two, three, four, five and six weeks after maturity (12345and6 WAM) following artificial inoculation with *Phytophthora palmivora* pathogen at full maturity i.e twenty weeks after pollination.

3.3 Pollination

Prior to artificial pollination, (Adebayo 1977) all the pods and already fertilized flowers were removed with a sharp harvesting knife, leaving only matured but unopened flowers on each tree. To prevent pollination by insects, the unopened flowers were covered with hoods made of mosquito nets that were firmly secured with safety pins. After twenty four hours when the flowers had opened, pollen grains were collected from the male trees (c69) and transferred on to the stigmas of the female trees (c20) with the aid of forceps. The artificially pollinated flowers were now covered with the hoods for seventy two hours to preclude any extraneous pollination by insects. Successful pollination and was considered to have taken place if after seventy-two hours the flowers would be in swollen ovary state which is an indication that fertilization has been effected on the ovules while dried and shrunken (dead) flowers were indication of unsuccessful pollination and thus no fertilization, such flowers were pruned off.

3.4 Inoculation

At maturity i.e, twenty weeks after pollination, (Adenikinju 1989) all the healthy pods were tagged and surface disinfected with 20% solution of sodium *hypochlorite* (NaOCl) in order to prevent any surface contaminations that may interfere with proper identification of the pathogenic fungus used for the inoculation exercise. A cut surface of 1cm in area was then made with a sharp pocket knife on the healthy pods with

similar cuts being made on the advancing edges of pods naturally infected with the black pod disease. The diseased part was then inoculated onto the healthy pods. The diseased pods which acted as sources of inoculum together with ant tents on trees and mummified pods were all removed and burnt or buried. Infection of healthy pods by black pod disease was later confirmed through isolation of fungi from the inoculated spot and viewing them under the microscope.

3.5 Data collection and analysis

Data collected include weekly measurements of radial growth of the fungus on the pods over a period of six weeks after maturity, fresh weights and dry weights of beans upon extraction.

3.5.1 Fresh weights

Five pods from each of the treatment were harvested broken by knocking them on a log of wood, their beans extracted separately on treatment basis and weighed with the aid of a 50kg capacity pocket balance at the experimental site.

3.5.2 Dry weights

The extracted beans were dried at 135^{0c} for 2 hours in a moisture extraction oven. Dry weights of the beans were determined with sensitive electronic weighing balance.

3.5.3 Proximate analysis of the beans

Analysis of the moisture content, crude fat, crude fibre, ether extract, ash and nitrogen free extract were carried out and tested for by using Soxhlet Method of extraction at the Coop Cocoa Factory at their Ilesha Owo Express Road Akure, by a qualified biochemist according to the method described by Association of Analytical Chemist (AOAC) 1990 as follows:

Five pods from each of the treatment (one, two, three, four, five and six weeks after maturity) were randomly selected, processed as described by Adenikinju 1989 and kept separately in a closed air tight container from which samples were taken for analysis.

3.5.3.1 Moisture content determination

The moisture content of the beans were determined using the oven method which is based on weight loss of water due to evaporation.

Clean and dried porcelain dishes were weighed using Mettler balance and their respective weights were recorded (W1) 5g of each wet sample were weighed (W2) out from the prepared samples and later dried at 135°C for 2 hours to a constant weight (W3) and allowed to cool in a desiccator before weighing. The loss in weight during drying was expressed in percentages in accordance with AOAC 1990.

$$\begin{aligned} \%MC &= \frac{\text{Loss in weight}}{\text{Original weight of sample}} \times \frac{100}{1} \\ &= \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} = \%MC = A \end{aligned}$$

3.5.3.2 Determination of crude fat (butter fat)

10g. of each sample were put in extraction thimble. Water was removed by the oven drying method at 135⁰C for 2hours to permit penetration of the samples by ether. The samples were extracted with Soxhlet extractor for 7 hours. The ether was then evaporated from the samples and what remains were the crude fat which were weighed and the percentage crude fat in each sample calculated in line with AOAC 1990 thus:

$$\frac{\text{wt of crude fat}}{\text{wt of sample used}} \times \frac{100}{1} = \% \text{ crude fat} = B$$

3.5.3.3 Determination of crude fibre (dry matter)

After the removal of the crude fat, the remaining residue was boiled in sulphuric acid (1.25%) for 30minutes, filtered and allowed to cool for 30minutes in 1.25% sodium hydroxide. It was again filtered to remove proteins, sugars, minerals etc. present and the residues now which is dry matter (crude fibre) were dried and weighed. Percentage crude fibre was then calculated in line with AOAC 1990 thus:

$$\frac{\text{wt of crude fibre}}{\text{wt of original sample}} \times \frac{100}{1} = \% \text{ crude fibre} = C$$

3.5.3.4 Determination of protein content

5g of each sample were weighed into a nitrogen free filter paper. The samples were then digested in concentrated sulphuric acid with sodium sulphate and a catalyst to convert or remove all ammonical nitrogen present. Excess sodium hydroxide was added to make the solution strongly alkaline. Water and distilled ammonia were added and the nitrogen in the samples

were determined by titration. The amount of protein in the samples was then calculated by multiplying the amount of nitrogen by 6.25 (as protein is said to contain 16% nitrogen). The percentage protein (ether extract) was thus calculated according to AOAC 1990:

$$\frac{\text{Amount of protein in sample}}{\text{D}} \times \frac{100}{\text{Wt of sample}} = \text{Protein content of the sample} = \text{I}$$

3.5.3.5 Determination of ash content

10g of each sample was weighed into small crucible, it was then reheated in furnace at 600°C for 7 hours. It was later weighed after it has cooled down to room temperature the ash content was then calculated thus:

$$\frac{\text{wt of ash}}{\text{wt of original sample}} \times \frac{100}{\text{I}} = \% \text{ ash} = \text{I}$$

3.5.3.6 Determination of the sugar and starch content of the samples

The sugar and starch content of the samples' value was determined by the difference, that is all analysed properties were summed up and subtracted from 100 in line with AOAC 1990.

$$100\% - (\% \text{ water} + \% \text{ crude fat} + \% \text{ crude fibre} + \% \text{ ash}) = \% \text{ sugars and starch.}$$

3.5.3.7 Ability to process and ferment the cocoa beans

Five cocoa pods which were randomly selected from each of the

treatments (1,2,3,4,5 and 6 WAM) were harvested and processed according to Adenikinju 1989 and physical observations were carried out on them as follows:

1. the ease with which the beans were extracted from the pods,
2. the number of pods that were easily processed,
3. the state of the beans (wetness and dryness),
4. the position of the beans inside the pods (the adhereness to the pods, firmness and looseness),
5. Fermentability of the beans produced.

The pods were dehydrated as a result of heavy infection, the beans were loose dried up giving room for weevils to attack them and they cannot be fermented.

3.5.3.8 Statistical Analysis

The data collected were subjected to statistical analysis of variance (ANOVA). And means were compared using Least Significant Difference (LSD) at 5% according to Little and Hills (1978).

4.0 RESULTS

The mean of the results obtained in 1998/1999 and 2000/2001 were used as there were no significant differences between the results of the two seasons trials.

4.1 Comparative effect of time of harvesting on the mycelial growth of *Phytophthora palmivora* when artificially inoculated on cocoa pods

It was observed that pods at one, two, three, and four weeks after maturity has the least *mycelial* growth in that order with 1.00, 1.30, 1.40 and 2.00cm² area coverage. The least of 1.00cm² was recorded for one week after maturity and the highest of 3.20cm² was recorded for six weeks after maturity and showed no significant difference from one another. However, *mycelial* growth on pods at five and six weeks after maturity differed significantly from one to four weeks different groups were observed, fig. 1.

4.2 Effect of time of harvesting on fresh pods with beans

The results showed that fresh pods and beans harvested at one, two, and three weeks after maturity were significantly different from one another as they gave 8, 10 and 9g respectively. However, they were equally significantly different from those of four, five and six weeks after maturity which yielded 6, 3 and 2.3g respectively. The results also showed that harvesting at two weeks after maturity was more than at one week and thereafter the weights were decreasing with increase in age (i.e. delay in the time of harvesting). With pods harvested at one, two and three weeks after maturity weighing 8, 10 and 9g respectively and pods harvested at four

weeks after maturity with 6g while pods harvested at 5 and 6 weeks after maturity had 3 and 2.3g, which were significantly different from one another (fig. 2).

4.3 Effect of time of harvesting on the fresh cocoa beans

The results obtained were similar to what was obtained when the fresh pods with beans weighed together. On comparison, it was observed that the weight of the fresh beans were decreasing with increase in age. At two weeks after maturity the weight was the larger (10g) while it was smaller (1.8g) at six weeks after maturity. However, harvesting at one and three weeks after maturity were not significantly different in weight from one another with 8 and 9g respectively, but were significantly different from those of five and six weeks after maturity which weighed 2.2 and 1.8g respectively. There was no significant difference between them. Thus the fresh beans weights were in four significantly different groups as follows:

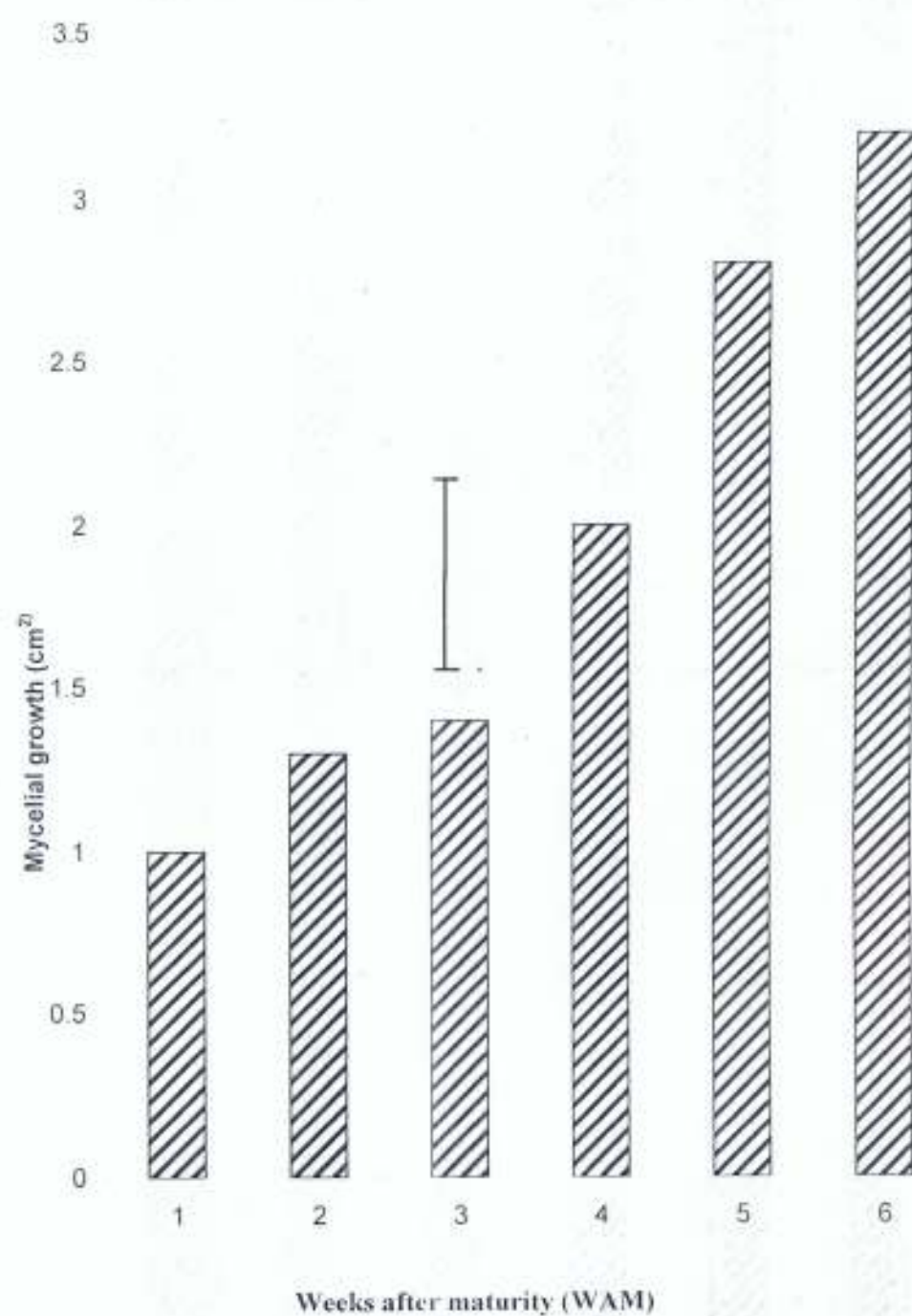
Group I with beans of pods harvested at one and three weeks after maturity.

Group II made up of harvesting at five and six weeks.

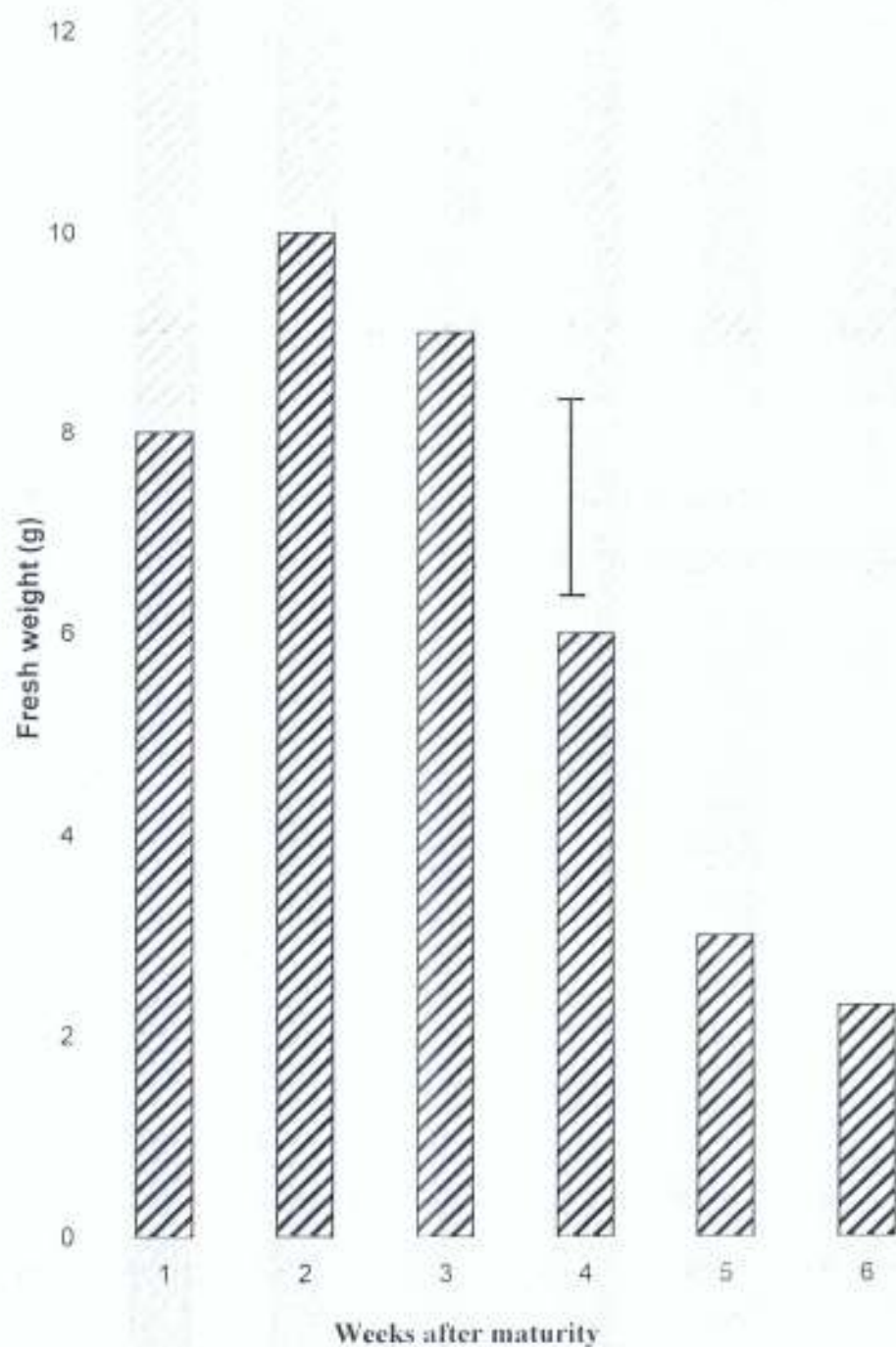
Group III consisting of two weeks after maturity and

Group IV comprising of harvesting at four weeks after maturity.

Significant differences occur with the groups.



g.1: Mycelial growth of *Phytophthora palmivora* on artificially inoculated cocoa pods



2: Effect of the black pod disease of cocoa on fresh weight of cocoa pods with beans



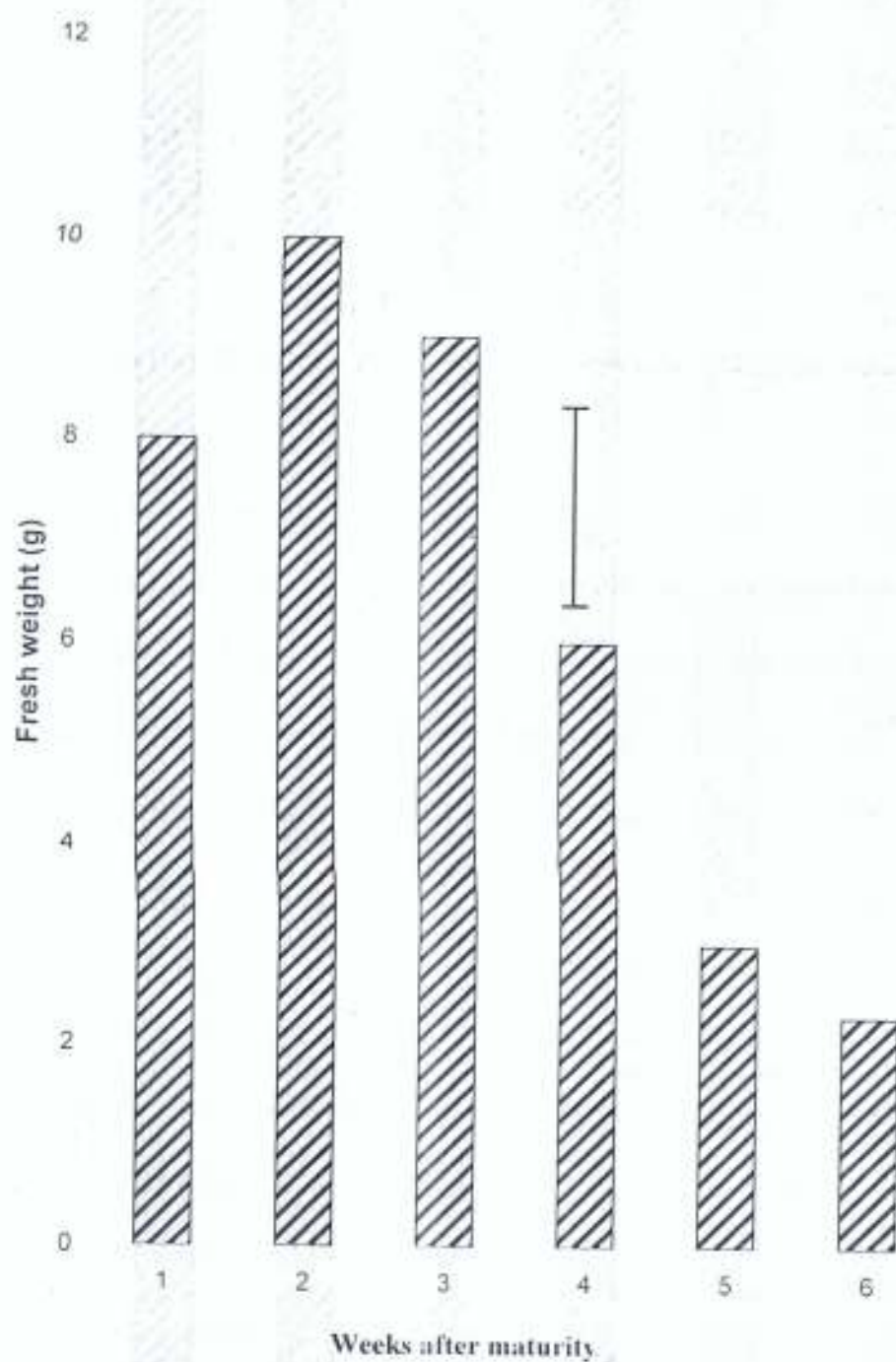


Fig.3: Effect of the black pod disease on fresh weight of cocoa beans

4.4 Effect of time of harvesting on weight of dried pods with beans

The result obtained showed similar trend with those obtained when fresh pods with beans were weighed as they were decreasing in weight with increase or delay in time of harvesting after maturity.

Pods harvested at two, three and four weeks after maturity were not significantly different from one another with 5, 4.60 and 4.40g weight respectively but were significantly different from those of 1.40 and 1.20g weights recorded for weeks five and six after maturity which were not significantly different from one another but from those of 1 week after maturity with 6g.

The effect of time of harvesting on the weight of the dried pods with beans where in three significantly different groups thus: Group I.... with two, three and four weeks after maturity with 5, 4.6 and 4.4g weight and Group II.... Having five and six weeks after maturity with 1.4 and 1.2g weight and Group III – one week after maturity with 6g (Fig 4).

4.5 Effect of time of harvesting on weight of dried beans

The result were similar to those obtained for those of fresh pods with beans, as they equally showed decreasing weights with increase in age.

However, beans harvested at two, three and four weeks after maturity with 15, 14 and 13.84g weights were not significantly different from one another but when compared with those of five and six weeks after maturity which had .4 and .3g weights were significantly different from one another. Those of weeks five and six after maturity were not significantly different from one another but significantly different from those of

one, two, three and four weeks after maturity, so also harvesting at one week is significantly different from those of two, three, four, five and six weeks after maturity (Fig 5).

4.6 Effect of time of harvesting on Proximate Analysis of the dried beans

The following parameters were determined for the various samples: moisture content, ether extract (protein) crude fat, Nitrogen free extract, Ash and Crude fibre. The difference obtained were in two major groups thus:

- * Those minerals (nutrients) that were decreasing with increase in age (time of harvesting)
- * Those that were increasing with increase in time of harvesting

The mineral or nutrient contents in the first group that are decreasing with increase in age are Ether Extracts (E/E) otherwise known as Protein, Moisture Content (M/C) or Water Content (W/C) Crude fat (C/F) and Nitrogen Free Extract (NFE) Ash Content (A/C) the decrease was gradual when analysed in the six treatments that is one, two, three, four, five and six weeks after maturity as shown in Figures 6, 7, 8, 9 and 10.

The only mineral or nutrient that was progressively increasing with increase in time of harvesting as contained or shown in figure 11 is the dry matter or crude fibre content of the dried beans.

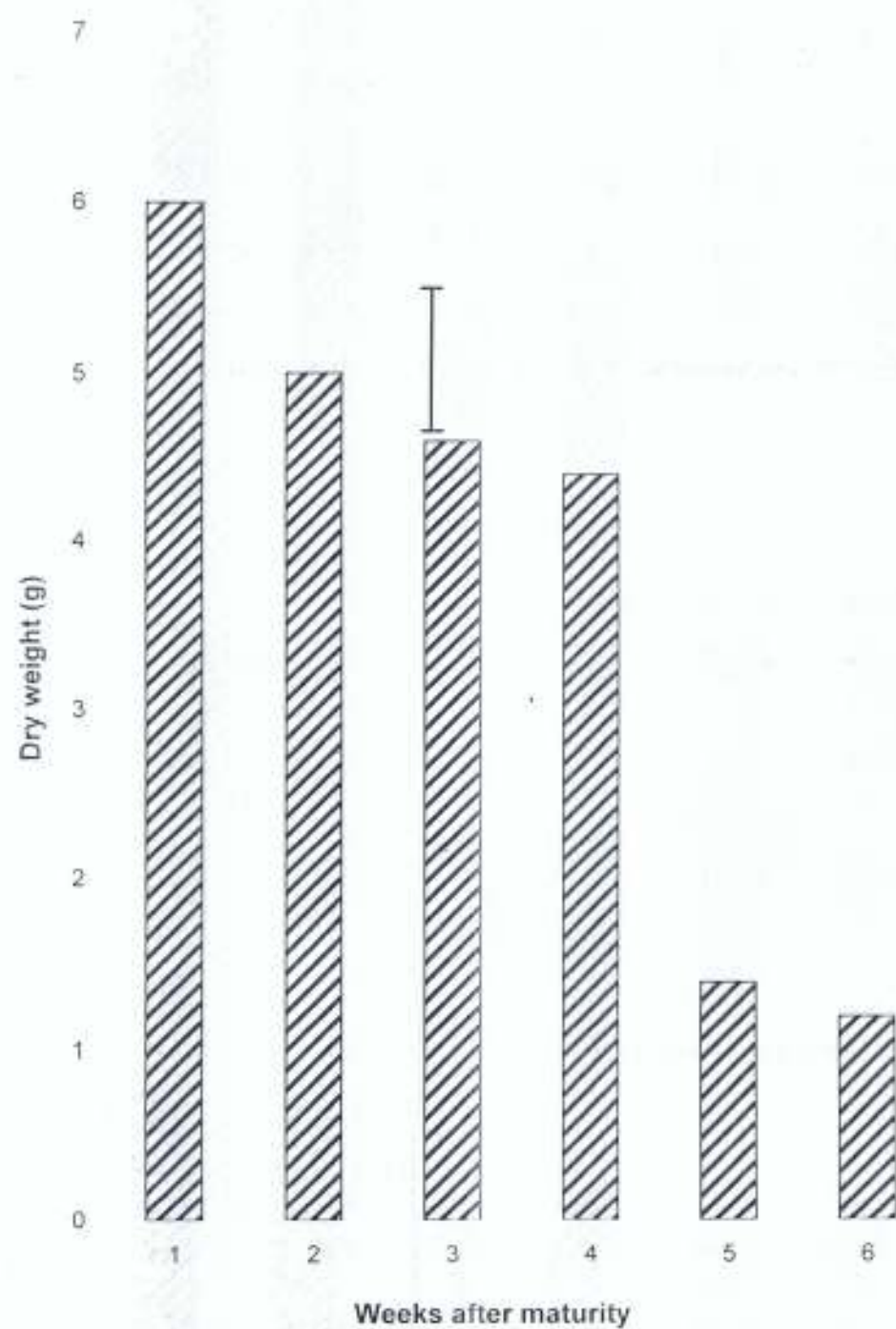


Fig. 4: Effect of the black pod disease on cocoa dry pods with beans

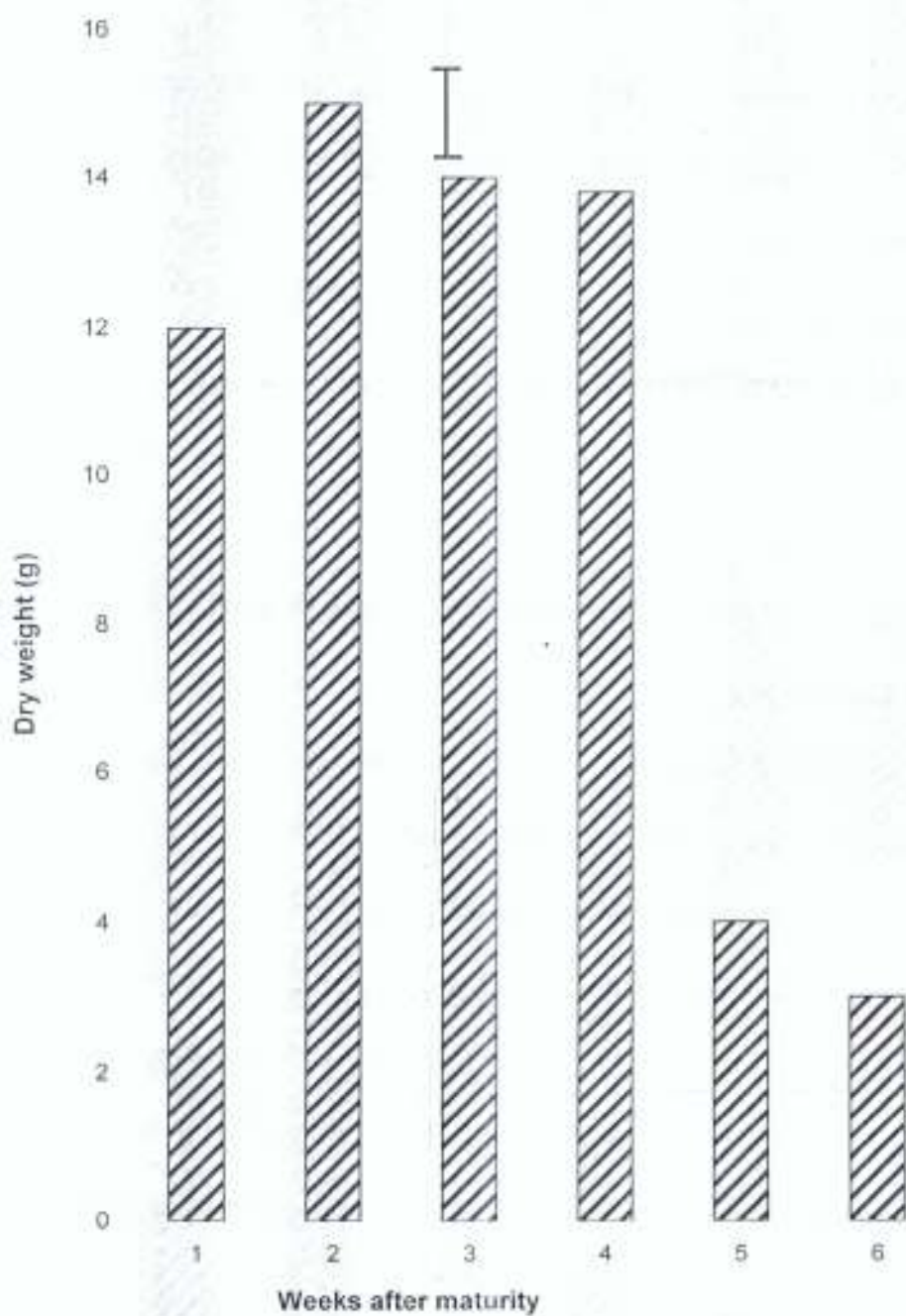


Fig. 5: Effect of black pod disease on dry cocoa beans

4.7 Effect of time of harvesting on ether extract (E/E) of the dried beans

The ether extract content of the six treatments decrease with increase in age, the result showed four significantly different groups with one and two weeks after maturity and having 100mg each in group 1, weeks three and four after maturity had 80mg and 60mg respectively while the fourth group of weeks five and six after maturity had 20mg each. Ether extract in all the groups were not significantly different within the groups but significantly different from one another. Fig. 6.

4.8 Effect of time of harvesting on the (M/C) of dried beans

Harvesting at six weeks after maturity has the least moisture content of 8mg while harvesting at one, two and three weeks after maturity has the highest of 14.80mg each. However, the result obtained were in four significantly different groups whose members were not significantly different from one another but different from those of other groups with one, two and three weeks after maturity having 14.80mg each in the first group and four, five and six weeks after maturity and having 12, 10 and 8mg in the other three groups respectively as shown in fig 7.

4.9 Effect of time of harvesting on the crude fat content (CFC) of the dried beans

The result obtained for the crude fat content of the dried beans was shown in figure 8 which followed the same trend with moisture content for the six treatments. It was observed that weeks one and two after maturity

has 1.80 and 1.60g being the highest were significantly difference from one another in group I and II respectively, while weeks three and four after maturity with 1.28 and 1.24g respectively, showed no significant different from one another and were in group III which were significantly different from the members of group I. Weeks five and six after maturity showed 0.96 and 0.92mg respectively giving the least crude fat content and as shown are in group IV whose members were not significantly different from one another but different from those of the other groups (Fig. 8).

4.10 Effect of time of harvesting on the nitrogen free extract (NFE) of the dried beans

The result also showed the same trend with what was obtained for the effect on moisture and crude fat contents as shown in fig. 9. It was observed that high NFE was observed from one to three weeks after maturity, which significantly decreased as the harvesting period increased up to 6 weeks after maturity as observed in this study. The NFE ranged between 2.50 – 0.5mg for 1-6 weeks after maturity (fig. 9).

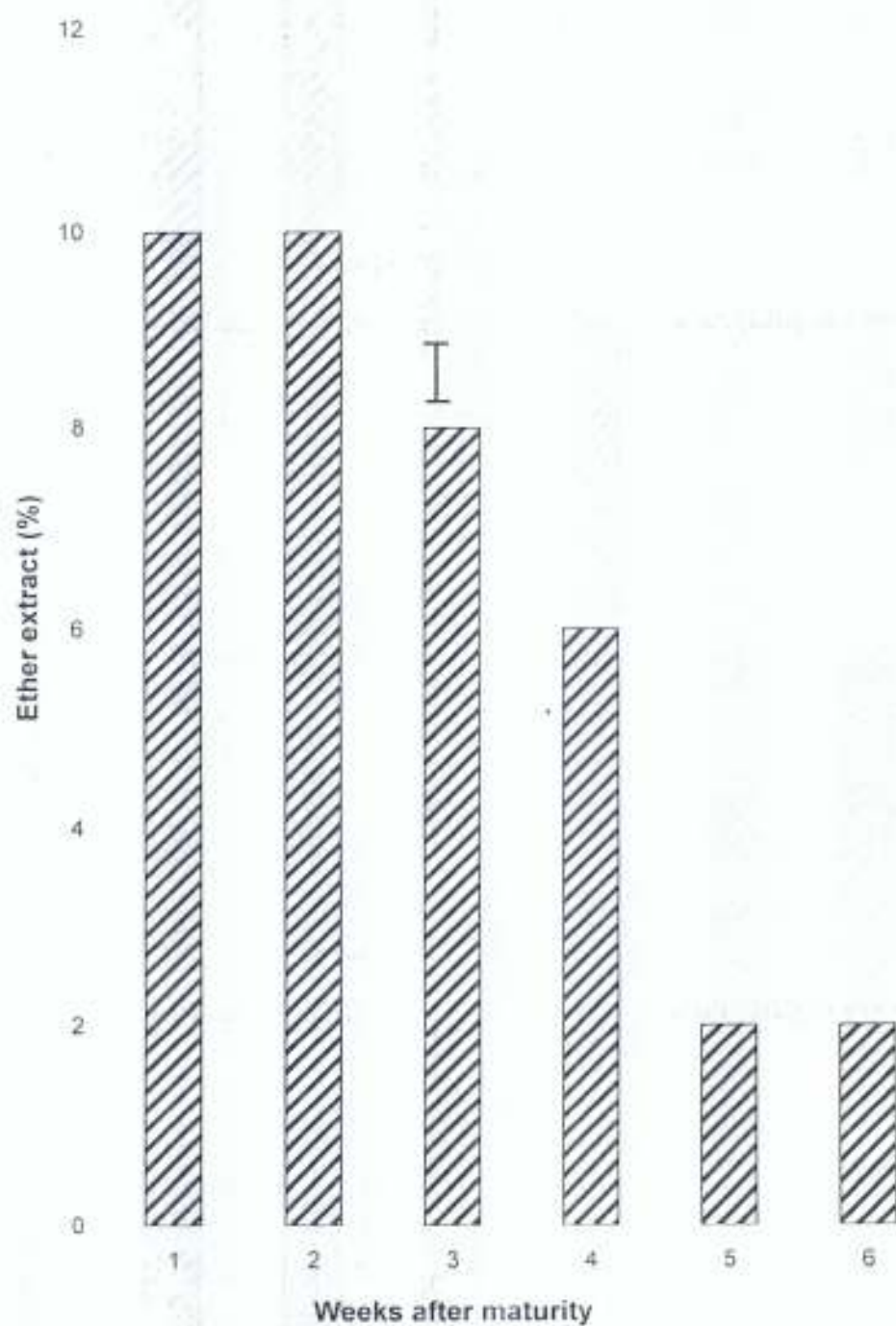


Fig. 6: Effect of black pod disease on ether extract (E/E) of dry cocoa beans

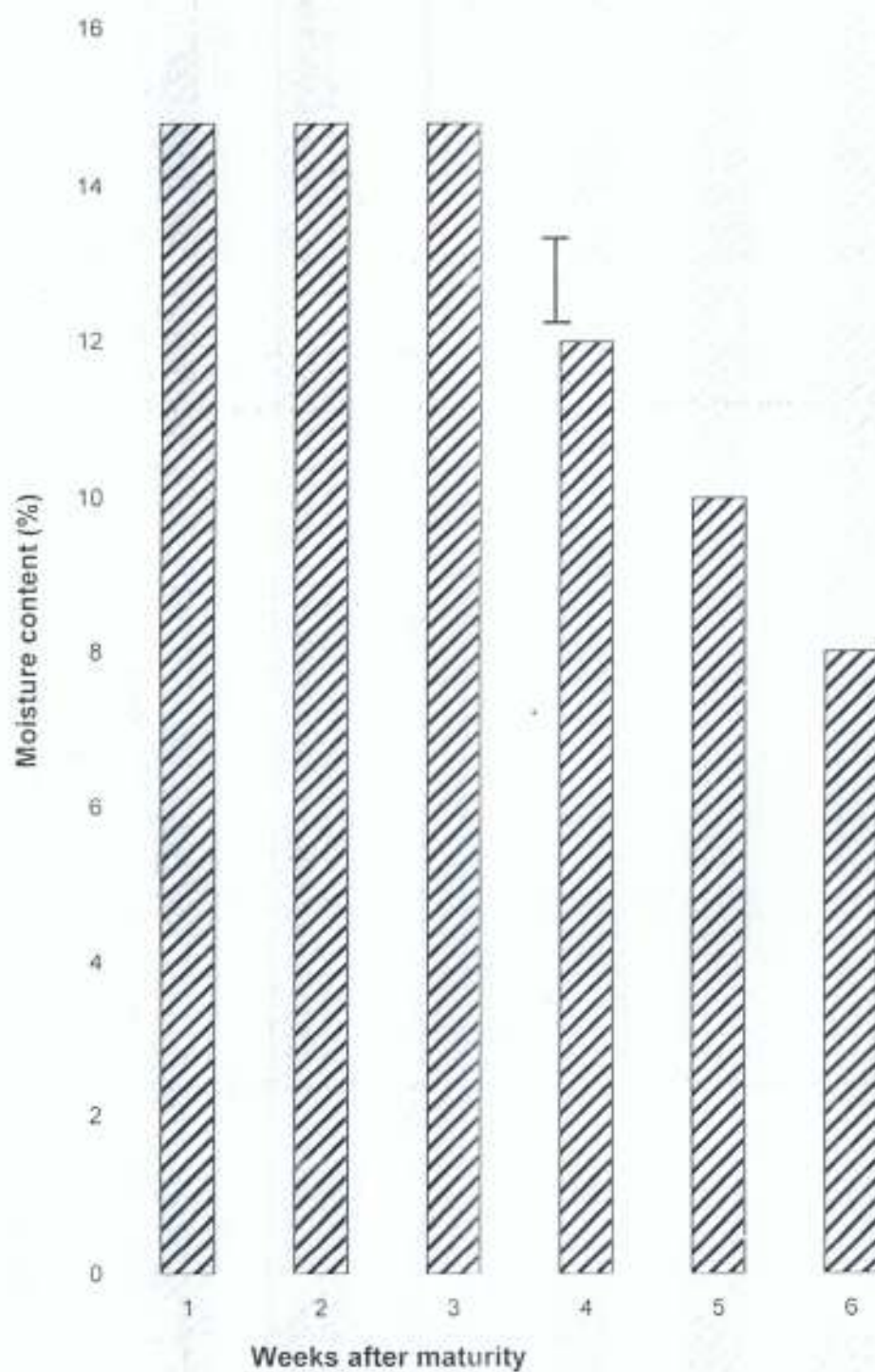


Fig. 7: Effect of black pod disease on moisture content (m/c) of dry cocoa beans

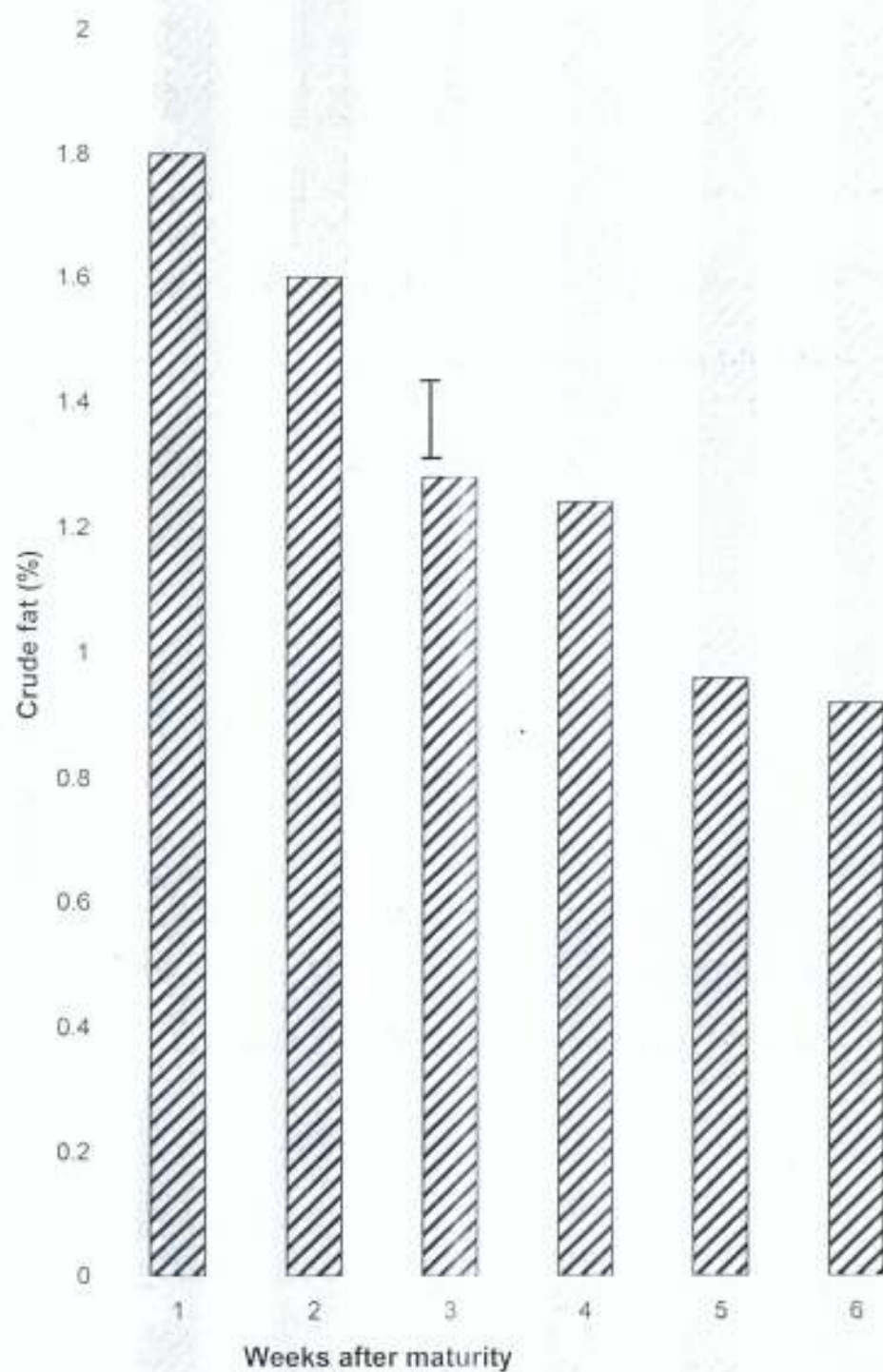


Fig. 8: Effect of black pod disease on crude fat content (cfc) of dry cocoa beans

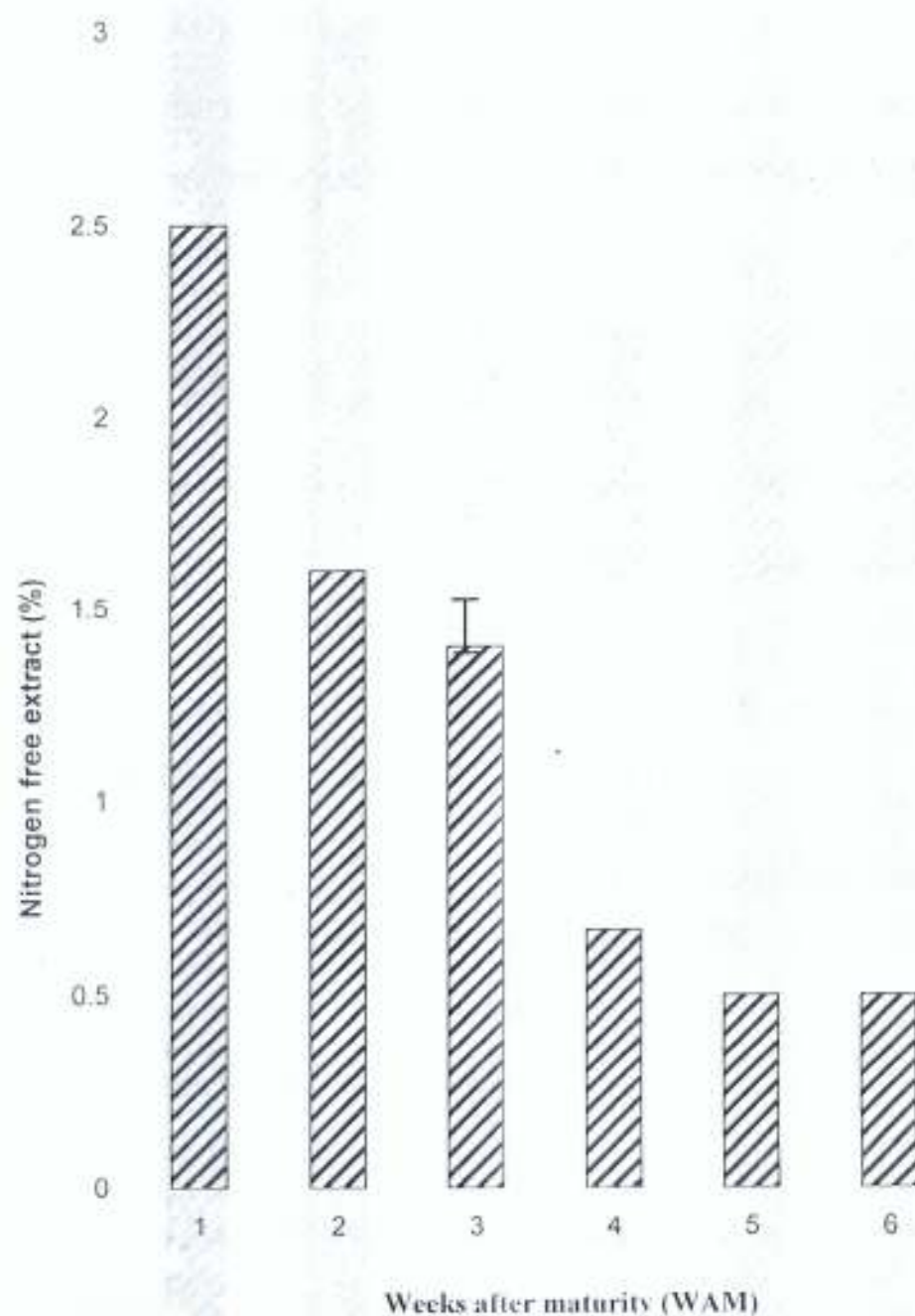


Fig. 9: Effect of black pod disease on nitrogen free extract (NFE) of dry cocoa beans

4.11 Effect of time of harvesting on the Ash Content of the beans

The ash content when tested for the dry beans was found to be decreasing with increase in time of harvesting. It was similar to what was obtained when the moisture, crude fat and nitrogen free extracts were determined on the beans and was found to be in six significantly different groups whose members were not significantly different from one another (within) but were different from members of the other groups as shown in fig 10 as each time of harvesting constitute a group of its own.

Weeks five and six after maturity recorded the least ash content of 3.40mg and 2.20mg respectively and were significantly different from one another. However the highest ash content 9.90 and 8.90mg were obtained in one and two weeks after maturity respectively.

4.12 Effect of time of harvesting on the Dry Matter Contents (Crude Fibre) of the dried beans

In the dry matter contents of the dried beans, the result 6, 6.4, 7, 7.2, 7.6 and 8.8mg obtained were found to be increasing with age (delay in harvesting) and similar to those obtained for the *mycelial* growth when artificially inoculated as the crude fibre content is increasing with age.

At one, two, three and four weeks after maturity dry matter recorded were 6.0, 6.4, 7.0 and 7.20g respectively while the highest of 8.70g was recorded for 6 weeks after maturity. (fig 11).

4.13 Effect of time of harvesting on (%) processability of pods and fermentability of the beans produced

The effect of time of harvesting on the pods produced showed that all the pods were processable i.e. the beans can be easily extracted from the pods but all the beans can not be fermented because some of the beans were already dried shrivelled and weevilled.

Beans from one, two and three weeks after maturity were all processable and fermentable while the beans produced from four, five and six weeks after maturity were only processable (easily extracted) not fermentable and weevilled. Fig. 12. Chemical qualities started declining with increased pod infection as five and six weeks after maturity were the most affected while those of one, two and three weeks after maturity has more or large amount of nutrients in form of ether extract, moisture content, nitrogen free extract, crude fat and lower or smaller crude fibre content, those of four, five and six weeks after maturity has a relatively high crude fibre and low or smaller ether extract, nitrogen free extract ash and crude fat content due to heavy infection by the black pod disease of cocoa and dryness of the pods and beans.

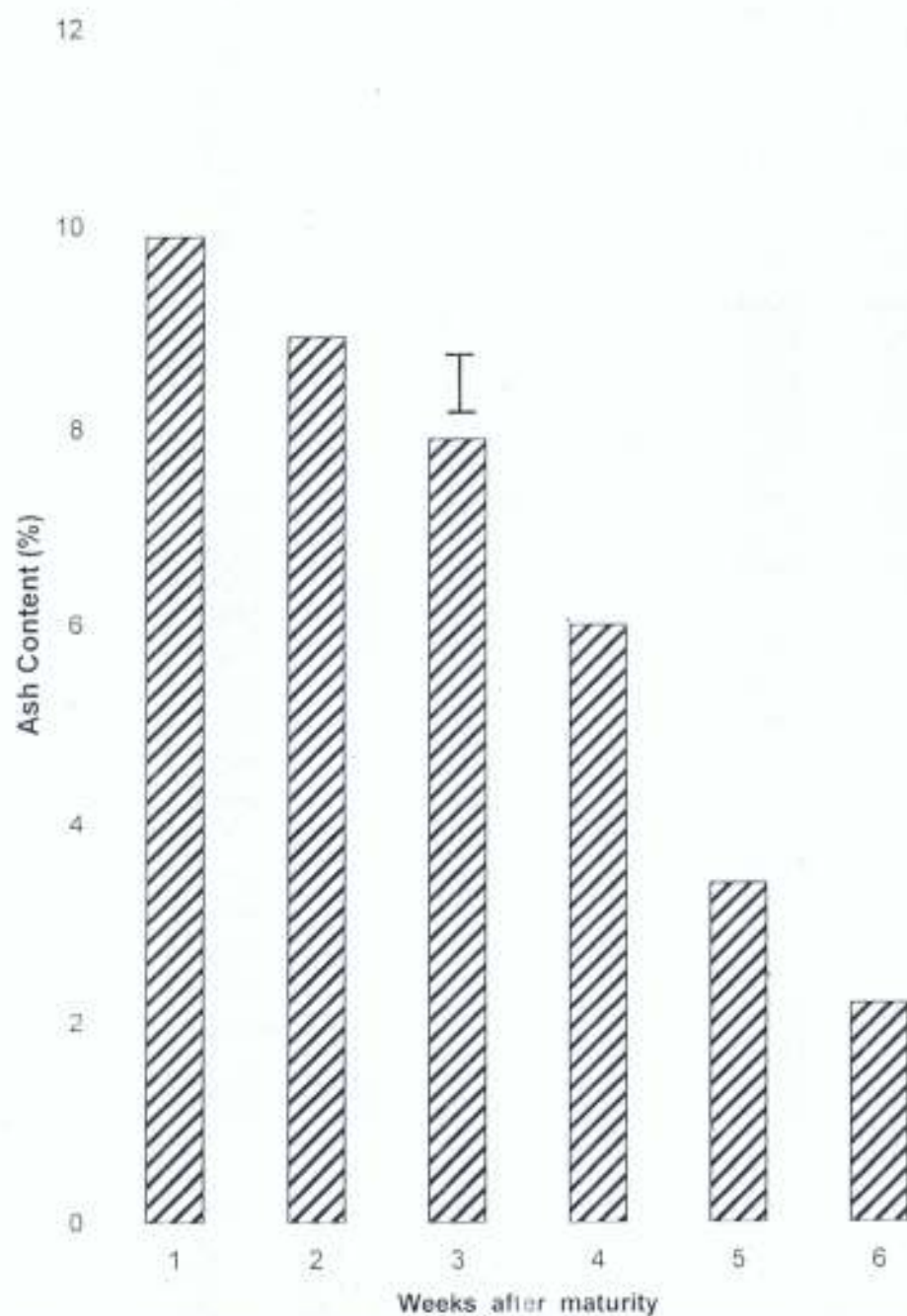


Fig. 10: Effect of black pod disease on ash content (A/C) of dry cocoa beans

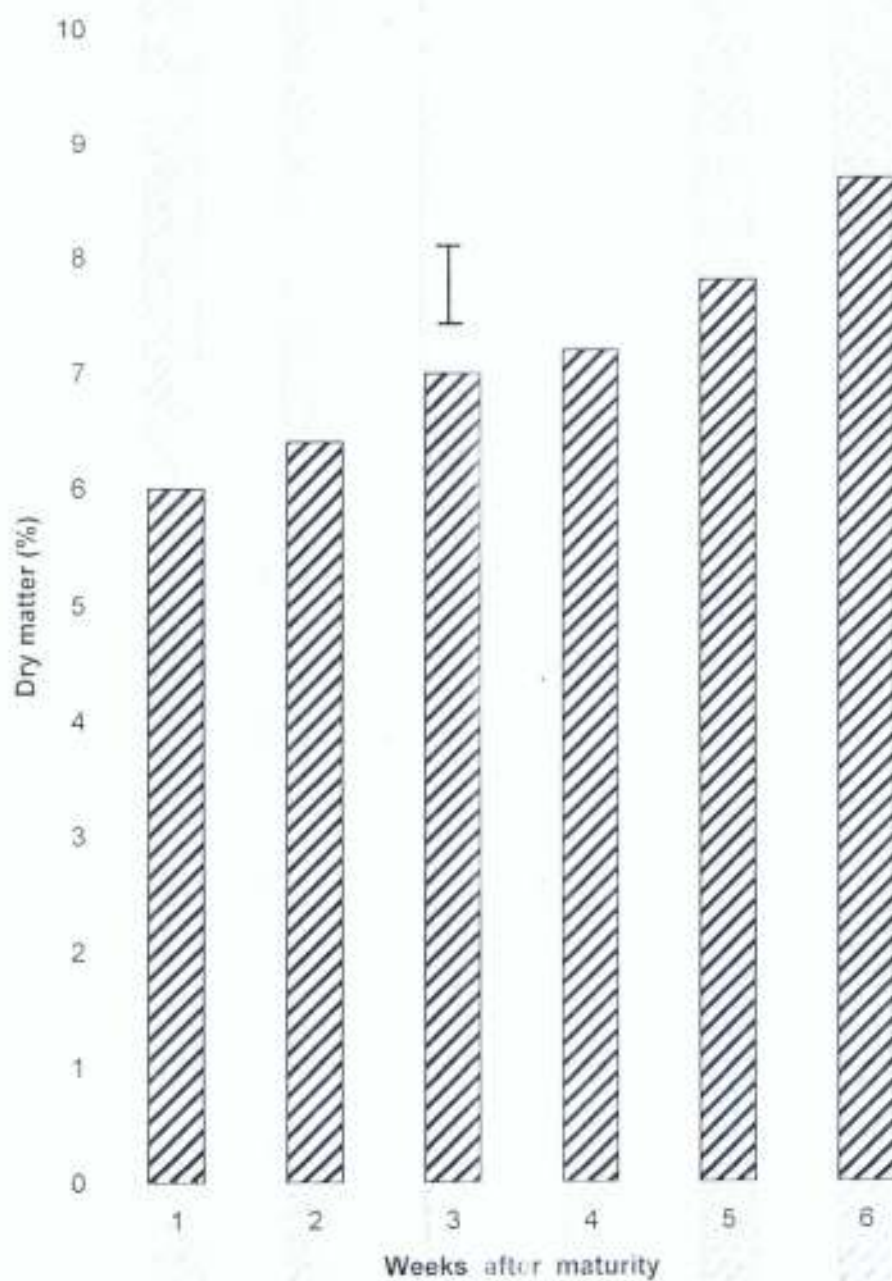


Fig. 11: Effect of black pod disease on dry matter content (crude fibre) of dry cocoa beans

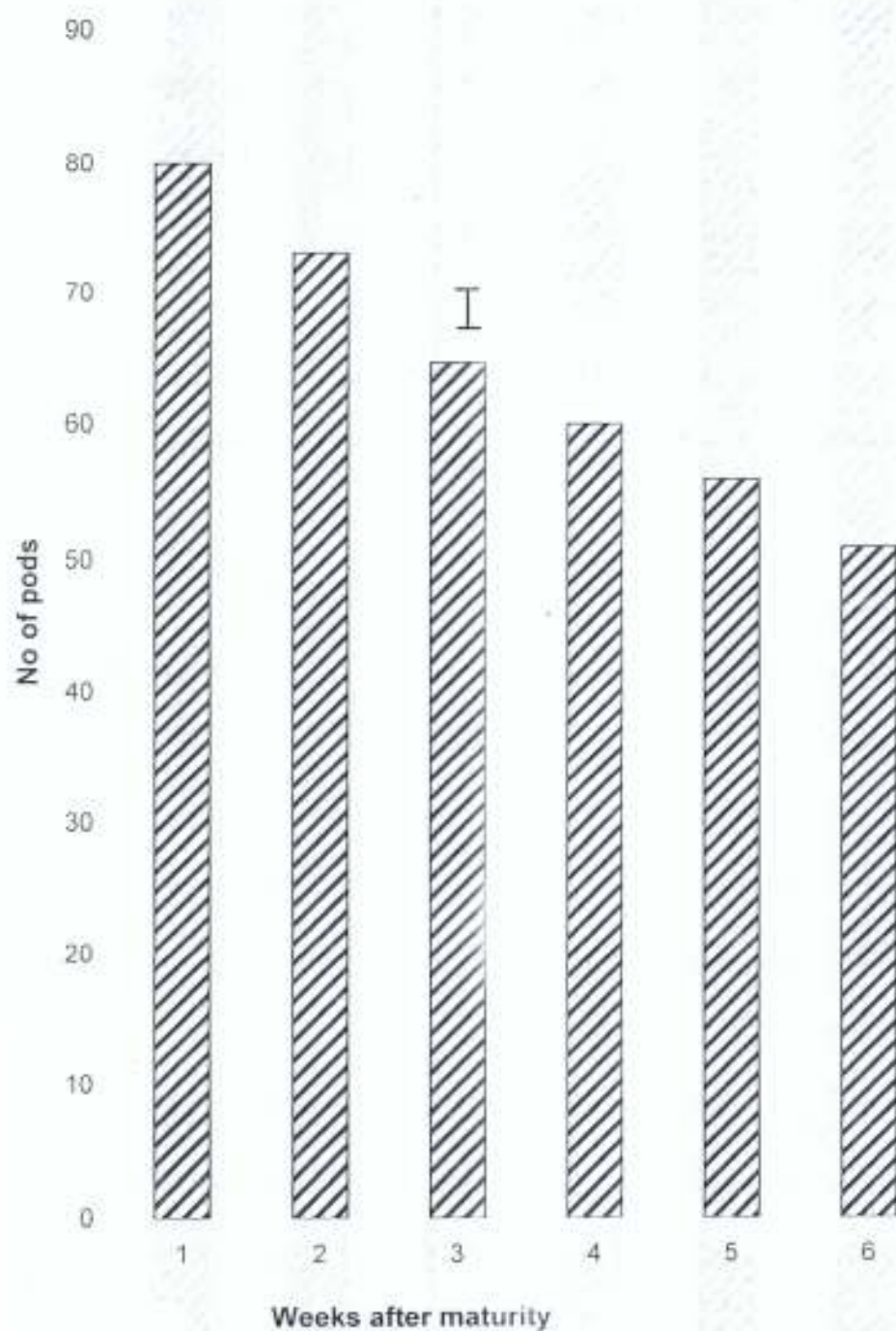


Fig. 12: Effect of black pod disease on the ability to process and ferment cocoa pods and beans

5.0 DISCUSSION

Of the six harvesting periods considered, one and two weeks after inoculation showed considerable control of the black pod disease of cocoa caused by *Phytophthora palmivora*. The degree of control compares favourably with results obtained in Ghana (Anon, 1979) where Ridomil and Perenox were used. This is also in consonance with the work of (Adekagun, 1997) where it was reported that Bordeaux mixture at low concentrations controlled cocoa black pod.

Cocoa pods which mature in twenty weeks and suffers no infection up to that stage (Adebayo, 1975) agrees with this report since pods at twenty weeks i.e point of maturity shows no visible sign of infection by *Phytophthora palmivora* (Adenikinju, 1972 and Asare Nyako, 1973) as the beans were still succulent, processable and fermentable.

The variability in pod size, weight and circumference were also in agreement with (Sorias 1971). The remarkable increment in both the number of infected pods and mycelial growth of the fungus were also in agreement with Blencove 1971; Turner 1965; Asare Nyako 1975; Anon 1995a and Anon 1995b that the longer the pods (matured) are left on the field the more they are infected and the beans one loose, weevilled and dried in the pods.

Pods harvested six weeks after maturity were nearly dried – up and the beans were loose in the pods, this agrees with (Adenikinju 1974 and Adenikinju 1989). The amount of mucilage around the beans generally increased with maturity and were less tightly packed than what obtained

in pods harvested at one week after maturity which agrees with the work of Esan (1989).

Lastly, at six weeks the beans become more easily processable as they were already dried and loose in the pods as a result of heavy infection by the fungus *Phytophthora palmivora* Akinwale-Ojo, 1995, 1996; Debenham, 1997; Bamigboye, 1997 and Oluyide, 1997 reported similar results about dryness and dehydration and that the beans are not fermentable.

The laboratory analysis (page 34-37, 40 and 41) showed that the longer the harvesting intervals, the higher the crude fibre content and the lower the crude Protein, Glucose, Sucrose. This harvesting interval also confirmed the increasing rate of the volatile acids while the non-volatile acids decreases with age (Adenikinju, 1989; Dehenham 1997 and Esan 1989). (Appendix VI).

It should be noted that while the *mycelial* growth and the Crude Fibre content were increasing with delay in harvesting the Nitrogen Free Extract (NFE) crude protein and crude fat were decreasing which is in agreement with (Akinwale-Ojo, 1996). The flavour and aroma of the beans were not tested for as the flavour and aroma is a result of the two way processes of fermenting on the farm and roasting in the factory (Iward, 2000). Cocoa flavour will change within a few months of production. (Anon, 1999)

It should be noted that the harvesting dates can be influenced on the one hand by the choice of days before harvesting and on the other hand by the level of maturity and the period for which the mature harvested pods

are left on the field before processing as it also affects the fungi development. The method of harvesting also plays an important role in pods infection. Advantages of timely harvesting over chemical methods are specificity i.e. specific with interval in days or weeks and has no direct or indirect negative impact on the environment, it is non toxic to both the producer and consumer as well as to the cocoa tree itself, it has no chemical use, it is a financially and time attractive option as there is no additional cost thereby saving the time for application of chemicals. It is compatible with other methods even chemical ones, users need not be literate as it requires no technical know how. Disadvantages associated with cultural method of control is that it becomes fully apparent after a long period, it requires the full cooperation and coordination of all the farmers in a given farming area as a single deviation may result in a total loss (CTA, 1977; Anon 2000).

5.1 CONCLUSION AND RECOMMENDATION

The use of any economical method of control of the black pod disease of cocoa that requires low cash, management, inputs and time in place of using pesticides, (as using or implementing a full chemical control programme increase pod production cost by 30-50% therefore the use of harvesting intervals, a cultural, low costing and effective means) should be seen as an alternative black pod disease control method to the use of chemicals.

Apart from being too costly, the breaking down of the resistance in resistant programmes makes the use of resistance varieties to be an

effort in futility and this still leaves harvesting interval as the only alternative option and to achieve this the following must be adhered to strictly:

Harvesting should commence two weeks after maturity i.e. twenty-two weeks after pollination as cocoa pods mature in twenty weeks after the day of pollination.

Tree canopies should be well and thoroughly reduced by way of pruning, removal of chuppons and overhead shades before flower cutting and pollination commence.

Leftover of last year harvest should be well and thoroughly harvested to prevent disease inoculum from spreading downwards from the unharvested and infected pods hanging on the trees.

Husks of harvested and processed pods should either be burnt or buried away from the plantation.

Harvesting intervals i.e. day of harvesting should be adhered to strictly (specificity).

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APPENDIX I

ANOVA: MYCELIAL GROWTH

Source	DF	SS	MS	F Cal	T Tab
Total	29	146.55			
Treatment	5	19.57	3.91	15.64	2.71
Blk	4	1.94	0.49	1.96	2.81
SSE	20	5.04	0.25		

LSD = 0.66

ANOVA: WEIGHT OF FRESH PODS AND BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	282.34			
Treatment	5	254.04	50.81	242.00	2.71
Blk	4	24.13	6.03	28.71	2.87
SSE	20	4.17	0.21		

LSD = 0.61

ANOVA: WEIGHT OF FRESH BEANS ALONE

Source	DF	SS	MS	F Cal	T Tab
Total	29	363.67			
Treatment	5	304.57	60.91	41.72	2.71
Blk	4	3.00	7.50	5.14	2.87
SSE	20	29.10	1.46		

LSD = 1.59

ANOVA: WEIGHT OF DRY POD AND BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	122.04			
Treatment	5	100.50	20.10	36.55	2.71
Blk	4	10.53	2.63	4.78	2.87
SSE	20	11.01	0.55		

LSD = 0.98

ANOVA: WEIGHT OF DRY BEANS ALONE

Source	DF	SS	MS	F Cal	T Tab
Total	29	784.22			
Treatment	5	699.65	139.93	177.14	2.71
Blk	4	69.57	17.39	22.30	2.87
SSE	20	15.60	0.78		

LSD = 1.17

ANOVA: EITHER EXTRACT (E/E) OF THE DRY BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	345.17			
Treatment	5	336.67	67.33	224.43	2.71
Blk	4	2.59	0.65	21.66	2.87
SSE	20	5.11	0.30		

LSD = 0.72

ANOVA: MOSTURE CONTENT (M/C) OF THE DRY BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	305.20			
Treatment	5	212.80	42.56	3.58	2.71
Blk	4	68.53	17.13	1.44	2.87
SSE	20	23.87	1.19		

LSD = 1.44

ANOVA: CRUDE FAT CONTENT (CFC) OF THE DRY BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	3.65			
Treatment	5	2.96	0.59	29.50	2.71
Blk	4	0.24	0.07	3.50	2.87
SSE	20	0.31	0.02		

LSD = 0.19

ANOVA: NITROGEN FREE EXTRACT (NFE) OF THE DRY BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	14.37			
Treatment	5	13.77	2.75	137.50	2.71
Blk	4	0.09	0.02	1.00	2.87
SSE	20	0.45	0.02		

LSD = 0.19

ANOVA: ASH CONTENT (A/C) OF THE DRY COCOA BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	266.84			
Treatment	5	237.74	47.55	190.20	2.71
Blk	4	24.13	6.03	24.12	2.87
SSE	20	4.97	0.25		

$$\text{LSD} = 0.66$$

ANOVA: DRY MATTER (CRUDE FIBRE) OF THE DRY BEANS

Source	DF	SS	MS	F Cal	T Tab
Total	29	37.21			
Treatment	5	24.87	4.97	12.43	2.71
Blk	4	4.38	1.10	2.75	2.87
SSE	20	7.96	0.40		

$$\text{LSD} = 0.83$$

**ANOVA: PROCESSABILITY AND FERMENTABILITY OF
THE POD**

Source	DF	SS	MS	F Cal	T Tab
Total	29	4584.70			
Treatment	5	2931.50	586.30	43.01	2.71
Blk	4	1300.53	328.13	23.85	2.87
SSE	20	352.67	13.63		

LSD = 4.87

APPENDIX II

COCOA PRODUCTION (ONDO STATE) 1990-2000

YEARS	PRODUCTION (mt) ONDO	NIGERIA (mt)
1990	55,004.50	137,511.25
1991	68,129.50	170,000.00
1992	41,973.00	130,000.00
1993	65,292.00	130,000.00
1994	58,255.25	138,000.00
1995	44,894.24	155,000.00
1996	44,770.00	158,000.00
1997	41,181.00	152,000.00
1998	27,895.00	165,000.00
1999	31,154.50	170,000.00
2000	24,047.00	156,000.00

STATISTICS ON POD INFECTION BY DISEASES.

PATHOGEN	1970	1971	1972	1973	1974	Total	Average
<i>P. palmivora</i>	71	75	56	63	54	319	63.8
<i>B. theobroma</i>	20	18	24	16	23	101	20.2
<i>Fusarium spp</i>	6	3	12	12	14	47	9.4
Others	3	4	8	9	9	33	6.6
Total	100	100	100	100	100	500	100

Culled from 5th Int. Cocoa Res. Conf. Report 1977.

APPENDIX IV

PERCENTAGE DAMAGE CAUSED BY PHYTOPHTHORA PALMVORA AND COCOA SWOLLEN SHOOT VIRUS OF COCOA.

	P. PALMVORA	C.S.S.V
1975	31.30	19.00
1965	53.20	33.50
1975	71.40	37.70
1985	73.70	34.20
1995	83.40	59.90

APPENDIX V

MONTH	RAINFALL	TEMPERATURE	RELATIVE HUMIDITY
April	1150mm	35°C	57%
May	1375mm	19°C	71%
June	1750mm	18°C	88%
July	1600mm	17°C	83%
August	1550mm	31°C	81%
September	1400mm	30°C	72%
October	1210mm	34°C	63%
November	1150mm	35°C	57%
TOTAL	11185mm	219°C	572%
AVERAGE	1398.13mm	27.37°C	71.50%

APPENDIX VI**ACCEPTABLE NUTRIENT LEVEL IN COCOA BEAN**

CONTENTS	PERCENTAGE (%)
Moisture content	10.70
Ash Content	08.53
Crude Protein Content	19.28
Ether Extract Content	09.78
Crude Fibre Content	06.28
NFE Content	45.43
TOTAL	100.00

Called from 5th Int. Cocoa Res. Conf. Report for 1977.