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DETOXIFICATION AND ANTI-NUTRIENT REDUCTION OF *J. CURCAS* SEED CAKE BY FERMENTATION USING *BACILLUS COAGULANS*

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ABSTRACT

Jatropha curcas seed cake is a by-product generated from the oil extraction of *J. curcas* seed- a biodiesel producing plant's seed. Although, the seed cake contains a high level of protein, it has Phorbol ester and some anti-nutritional factors such as phytic acid, saponin, lectin and trypsin inhibitor making it not to be applied directly in the food or animal feed industries. This study was aimed at detoxifying the toxin and reducing the anti-nutritional factors in *J. curcas* seed cake by fermentation using *Bacillus* species. *Bacillus coagulans* of 1.0×10^8 cells MacFarland's standard per 100ml were used in the study. The seed cake used for the detoxification was extracted both manually and with the use of a machine. This fermentation was carried out on 10g of seed cake in 100ml of distilled water for 5 days with submerged fermentation. Temperature (27^{0}C, 30^{0}C, and 370C), pH (4.5, 6.5, and 8.5) and Time (24 hours, 48 hours, 72 hours, 96 hours and 120 hours) were also varied. After fermentation the toxin and anti-nutritional factor level was determined. Results showed that *Bacillus coagulans* was able to degrade the toxin and reduce the anti-nutritional factors in the seed cake. After fermentation phorbol ester, phytic acid, saponin, lectin and trypsin inhibitor were reduced by 45.27%, 28.95%, 18.87%, 32.29% and 31.03% respectively. The reduction may be due to the activities of esterase, phytase and protease enzymes. *Jatropha curcas* seed cake was detoxified by bacterial fermentation using a *Bacillus* strain and the rich protein fermented seed cake could be potentially used as animal feed.

KEYWORDS: *Jatropha curcas*, *Bacillus coagulans.*

1.0 INTRODUCTION

Jatropha curcas is a species of flowering plant in the Euphorbiaceae family. It is native to the American tropics, especially Mexico and Central America (Janick and Robert, 2008). It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. The specific epithet, *"curcas"*, was first used by the Portuguese doctor Garcia de Orta more than 400 years ago with uncertain origin. Common names includes Barbados Nut, Purging Nut, Physic Nut and JCL (*J. curcas* Linnaeus), whereas "Lapalapa" (Yoruba) "Binidazugu" (Hausa) and "Owulo idu" (Ibo) in Nigeria. It is a multipurpose tree because of industrial and medicinal uses. *J. curcas* is a poisonous, semi-evergreen shrub or small tree, reaching a height of 6 m (Janick and Robert, 2008). It is resistant to a high degree of aridity, allowing it to be grown in deserts. The seeds contain an average of 34.4% oil (Achten *et al.,* 2008) with a range between 27-40% (Achten *et al.,* 2007). Besides the economic potential of processing the oil to produce high-quality biodiesel fuel usable in a standard diesel engine, the seeds also contain the highly poisonous toxalbumin curcin. Esterases and lipases catalyze the hydrolysis of ester bonds and are widely distributed in animals, plants and microorganisms. In organic media, they catalyze reactions such as esterification, intesterification and transesterification (Kawamoto *et al.,* 1987). Esterases differ from lipases mainly on the basis of substrate specificity and interfacial activation (Long, 1971). Esterases are found in plants, animals and microbes, but the majority of industrially produced esterase are derived from microbial sources. This is because they can be engineered for production of esterase with desirable properties for industrial need. The

microbial sources include bacteria, fungi, yeasts and actinomycetes (Torres *et al.,* 2005). The applications of esterases are found in various fields, including inorganic synthesis process.

The seeds of *J. curcas* contain oil, which can be used as a renewable biodiesel source and applications in the manufacture of soaps and cosmetics (Makkar *et al.,* 1998). *J. curcas* seed cake is a by-product generated from the oil extraction of *J. curcas* seeds in a biodiesel processing plant. It has high protein content of approximately 50 -60% (Haas and Mittelbach, 2000) and could be used in animal feeds and also as protein hydrolysate. However, it contains the phorbol esters, which are toxic compounds, and the anti-nutritional factors such as trypsin inhibitors, phytic acids, lectins and saponins.

Phorbol esters are the most potent tumor promoters known. They exhibit a remarkable ability to amplify the effect of a carcinogen but are themselves not carcinogenic (Wender *et al.,* 1998). The seeds from *J. curcas* had been reported to be orally toxic to humans, rodents and ruminants of which phorbol esters had been identified as the main toxic agent (Becker and Makkar, 1998). Pure phorbol esters can kill when administered in microgram quantities (Heller, 1996). Ingestion of phorbol esters (LD_{50} for mice: 27mg/kg body mass) can cause lung and kidney damage, resulting in fatality (Li *et al.,* 2010).

Detoxification of toxin is necessary for *J. curcas* seed meal utilization, after which the detoxified seed cake may be used as animal feed and its protein hydrolysate (fermented liquid) as plant growth promoter. Biological detoxification of *J. curcas* seed cake has not been widely studied. However, toxins in cotton seed were successfully detoxified by microbial fermentation (Zhang *et al.,* 2006).

Despite its intrinsic advantages, *J. curcas* seed like soybean seed has the problem of antinutritional factors. In addition to thermos-labile lectins and trypsin inhibitors, *J. curcas* contains toxic lipo-soluble but thermo-stable phorbol esters (Heller, 1996: Makkar and Becker, 1997). Phorbol esters have to be removed or lowered to levels that do not elicit a toxic response from animals in order for the *J. curcas* seed meal to be used as an ingredient in livestock feeds. Makkah and Becker (1997) reported that phorbol esters were highly soluble in ethanol, giving some possibility of detoxification of the meal.

J. curcas seed cake is well adapted to grow in marginal areas with low (480mm) rainfall and poor soils. In such areas, it grows without competing for space with food crops (Gaydou *et al.,* 1982: Heller, 1996). *J.* curcas seed meal (10-20g Kg^{-1} residual oil) has a crude protein content ranging from 580-640g Kg^{-1} of which 90% is true protein (Makkar *et al.,* 1997: Makkar and Becker, 1997). The plant's ability to thrive in marginal areas and its high crude protein makes it an attractive complement and or substitute to soybean meal as a protein source in livestock feeds. The use of *J. curcas* will reduce the competition between man and livestock for soybean that is currently prevailing since soybean is used in both livestock and human feeds. Phorbol esters are the major impediment to the wide commercial use of Jatropha meal as feedstock. During extraction of oil from Jatropha seed, 70-75% of Phorbol esters associate with the oil and 25-30% remain strongly bound to the matrix of seed meal (Wink *et al.,* 1997). The Phorbol esters have been found to be responsible for skin-irritant effects and tumor promotion (Wink *et al.,* 1997). *J. curcas* seed cake is mainly used as manure and can be made more useful when detoxified and hence its use in animal feeds. The aim of this study was to detoxify and reduce the anti-nutritional factors in *J. curcas* seed cake by fermentation using *Bacillus species.*

2.0 MATERIALS AND METHODS

2.2 Collection of sample

Bacterial strains, *Bacillus coagulans* and *Paenibacillus polymyxa and Paenibacillus macerans* were used in this study. *P. macerans* and *P. polymyxa*- were obtained from dump site (soil) *and B. coagulans* from kilishi (food) using a minimal medium that contains *Jatropha curcas* seed cake as the only source of carbon.

2.3 Isolation of *Bacillus* species

These strains were isolated from soil samples using the surface spread method on a minimal medium containing the *J. curcas* seed cake. The minimal medium was be prepared according to the modified method of Lateef *et al.,* (2010) as follows; $NaNO_3$ 2g/L, NaCl 2g/L, KH_2PO_4 2g/L, $MgSO_4$ 0.05g/L, $FeSO_4.7H_2O$ 0.1g/L, $CaCO_3$ 0.1g/L, *Jatropha curcas* seed cake 2g/L, Agar Powder 15g/L.

The medium was sterilized in the autoclave at 121^oC for 15 minutes, and supplemented with 0.05g/l of sterile nystatin to inhibit the growth of fungi. The *J. curcas* seed cake that was used served as the sole source of carbon, nitrogen, sulfur and energy. The plates were incubated at 37^oC for up to 3 days. Distinct colonies, observed using morphological features, were selected, isolated, and purified on minimal medium and nutrient agar medium.

2.4 Morphological Identification

Gram staining, endospore staining and other characterization were done using the conventional biochemical technique according to the flow chart of Bergey's manual and also with the Microgen kit for *Bacillus* identification which included the Microgen Bacillus-ID identification system consists of 2 microwell strips (labelled BAC 1 and BAC 2) and the Microgen Identification System Software (MID-60) for the identification of *Bacillus* species. The strains, after being characterized, were stored on Nutrient agar slants at 4^oC till further use.

2.5 Raw Material and Proximate Composition

J. curcas seed cake was collected from the National Research Institute for Chemical Technology (NARICT) Zaria. It was analysed for moisture, crude protein, crude fat, ash and crude fibre using the AOAC (Association of Official Analytical Communities) methods (AOAC, 1995).

2.6 Analysis for Toxin and Anti –Nutritional Factors

Phorbol ester

Phorbol esters were determined according to the method of Saetae and Suntornsuk (2010). The dry seed cake was extracted with 95% ethanol. The phorbol ester concentration was determined by High performance liquid chromatography (HPLC), using phorbol 12–myristate 13–acetate (Sigma, Steinheim, Germany) as a standard.

Phytic acid

Phytic acids were determined according to the method of Reddy *et al* (1982). Four (4.0g) of sample was soaked in 100ml of 2% hydrochloric acid for 5 hours and filtered. Twenty-five (25ml) of the filtrate was decanted into a conical flask and 5ml of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of iron (III) chloride until a brownish yellow colour persists for 5 minutes. 1ml of 0.025M $FeCl_2$ will give 6.601mg phytate.

Lectin

Lectins were determined according to the modified method of Gordon and Marqaurdt (1974). The dry seed cake was extracted with normal saline. A serial dilution of sample with normal saline (1:1) in a micro titration plate was prepared. Sheep red blood cells were added into the diluted sample. The lectin concentration was displayed as of a haemagglutination unit which is the minimum amount of the sample required to show the agglutination.

Trypsin inhibitor

Trypsin inhibitor was determined by spectrophotometric method and N-α-benzol-L-arginine-*p*-nitroanilide (BAPNA) was used as a standard (Onwuka, 2005). One gram (1g) of the sample was dispensed in 50ml of 0.5M NaOH solution, stirred for 30 minutes and centrifuged at 3,600rpm and then filtered. Exactly 2ml of standard trypsin was added to 4ml of aliquot in a test tube and was allowed to stand for 5 minutes. The aliquot was then measured in a spectrophotometer at 410nm. The blank analysis was also carried out simultaneously. One trypsin inhibitor unit is equal to an increase of 0.01 absorbance unit from the blank.

Saponin

Saponins were determined according to Hudson and El-Difrawi (1979). Ten grams (10g) of the sample was added to 100ml of 20% aqueous ethanol in water and agitated with a magnetic stirrer for 12 hours at 55^0C. The solution was filtered using Whatmann No 1 filter paper and the residue was re-extracted with 300ml of 20% aqueous ethanol. The extracts were combined and reduced to about 40ml under vacuum using a rotary evaporator. The extract and 20ml diethyl ether were transferred into a 250ml separatory funnel and shaken vigorously. The aqueous layer was discarded. The process of purification was continued until a colourless aqueous extract was obtained. The pH of the remaining aqueous solution was adjusted to 4.5 by adding 4g of sodium chloride and the solution then shaken successively with butanol. The butanolic extract was washed twice with 10ml of 5% (w/v) sodium chloride and evaporated to dryness in a fume cupboard, to give the saponin, which was weighed and expressed as a percentage.

2.7 Submerged fermentation using different *Bacillus* strains

Ten grams (10g) of seed cake and 100ml of water were added into 250ml Erlenmeyer flask. The flask was sterilized and then inoculated aseptically with each bacterial strain at 1.0×10^8 cells MacFarland's standard per 100ml. The control contained the 10g of the seed cake and 100ml of water. The cultures and control were incubated at 30^0C on a rotary shaker operated at 150rpm. The flasks were sampled at days 0 and 5 to determine the levels of toxin- phorbol ester and anti-nutritional factors- Phytic acid, Lectin, Trypsin inhibitor and Saponin. Samples were withdrawn at different time intervals for residual toxin and anti-nutritional factors analyses. The percentage (%) reduction of toxin- Phorbol ester and anti-nutritional factors were calculated from differences of samples in day 0 and 5.

2.8 Optimization of detoxification conditions

Using the modified method of Kamath *et al*. (2010), Various process parameters influencing detoxification include fermentation time (24 hours, 48 hours, 72 hours, 96 hours, 120 hours), pH (4.5, 6.5, 8.5) and temperature (27^0C, 30^0C, 37^0C). The optimization medium contained the 10g of the seed cake, 100ml of water and the bacterial strains at 1.0×10^8 cells MacFarland's standard per 100ml. At constant temperature other parameters like pH and Time were varied.

3.0 RESULTS AND DISCUSSION

Table 1: Microscopic characteristics of the Isolate.

Isolates	Gram reaction	Morphology	Arrangement	Endospore(location)
A	Positive(+)	Rod	Singly	Present (sub terminal)

Table 2: Biochemical test characteristics of the isolates.

ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONPG	ARG	CIT	V P	NIT	Tentative identity of the isolat
+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	+	-	-	+	+	*B coagulans*

Key: + Positive - negative,
ARA- Arabinose, CEL- Cellobiose, INO- Inositol, MAN- Mannitol, MNS- Mannose, RAF- Raffinose, RHA- Rhamnose, SAL- Salicin, SOR- D-Sorbitol, SUC- Sucrose, TRE- Trehalose, XYL- Xylose, ADO- Adonitol, GAL- Galactose, MDM- Methyl-D-Mannoside, MDG- Methyl-D-Glucosides, INU- Inulin, MLZ- Melezitose, IND- Indole, ONPG- ONPG Hydrolysis, ARG- Arginine Dihydrolase, CIT- Citrate utilization, V P- Voges Proskauer, NIT- Nitrate.

Table 3: Proximate Composition of Dry *J. Curcas* Seed Cake.

Parameters	Seed cake (%)
Moisture	5.45
Ash content	8.20
Crude Protein	23.75
Fat and oil	9.80
Digestible carbohydrate	46.45
Crude fibre	11.38

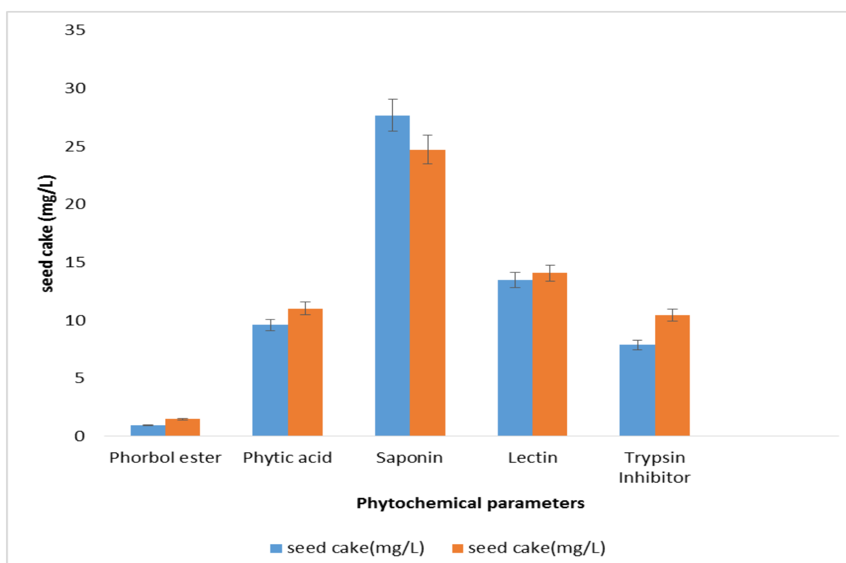

Figure 1: Phytochemical Content of Dry *J. Curcas* Seed Cake Processed Using Different Oil Extraction Methods.

Table 4: Percentage Reduction of Fermented *J. curcas* Seed Cake.

Anti-nutrient	Conc. (mg/l)		% Reduction
	0 Day	5 Days	
Pborbol ester A	1.48	0.35	76.35
Phorbol ester B	1.48	0.01	99.32
Phytic acid	11.02	4.82	56.26
Saponin	24.70	13.94	43.56
Lectin	14.06	5.80	58.75
Trypsin inhibitor	10.44	3.66	64.94
Keys: Phorbol ester A- Before Optimization, Phorbol ester B- After Optimization			

Table 5: Effect of Isolate on Reduction of Phorbol Ester during Fermentation of *J. curcas* Seed Cake.

Time (hr)	Conc. (mg/L)	
	Control	*B. coagulans*
0	1.48	1.48
24	1.38	0.93
48	1.30	0.90
72	1.25	0.88
96	1.19	0.84
120	1.14	0.81
% reduction	22.97%	45.27%

Table 6: Effect of Isolate on Reduction of Phytic Acid during Fermentation of *J. curcas* Seed Cake.

	Conc. (mg/L)	
Time(hr)	Control	*B. coagulans*
0	11.02	11.02
24	10.05	8.89
48	9.85	8.62
72	9.62	8.04
96	9.43	7.94
120	9.21	7.83
% reduction	16.42%	28.95%

Table 7: Effect of Isolate on Reduction of Saponin During Fermentation of *J. curcas* Seed Cake.

Time(hr)	Conc. (mg/L)	
	Control	*B. coagulans*
0	24.70	24.70
24	23.50	21.60
48	22.98	21.42
72	22.57	21.03
96	22.23	20.55
120	21.87	19.84
% reduction	11.46%	18.87%

Table 8: Effect of Isolate on Reduction of Lectin During Fermentation of *J. curcas* Seed Cake.

Time(hr)	Conc. (mg/L)	
	Control	*B. coagulans*
0	14.06	14.06
24	13.01	11.39
48	12.68	11.01
72	12.32	10.94
96	12.01	9.78
120	11.78	9.52
% reduction	16.07%	32.29%

Table 9: Effect of Isolate on Reduction of Trypsin Inhibitor During Fermentation of *J. curcas* Seed Cake.

Time(hr)	Conc. (mg/L)	
	Control	*B. coagulans*
0	10.44	10.44
24	9.74	8.78
8	9.43	8.36
72	9.23	8.01
96	8.99	7.97
120	8.79	7.20
% reduction	15.8%	31.03%

Table 10: Effect of Temperature on Reduction of Phorbol Ester by Isolate During Fermentation of *J. curcas* Seed Cake.

Temp (^{O}C)	*B. coagulans* mean±SE
27	0.81 ± 0.05^{a}
30	0.65 ± 0.03^{b}
37	0.49 ± 0.07^{c}
P-value	**0.000***

Mean values followed by different letter within the same column are statistically significant Duncan Multiple Range Test (p<0.05).

Table 11: Effect of Fermentation Period on Reduction of Phorbol Ester on isolate During Fermentation of *J. curcas* Seed Cake.

Time (Hours)	*B. coagulans* mean±SE
24	0.71 ± 0.13^{a}
48	0.67 ± 0.14^{a}
72	0.64 ± 0.15^{a}
96	0.62 ± 0.15^{a}
120	0.59 ± 0.14^{a}
P-value	**0.486**

Mean values followed by same letter within the column are not statistically significantly (p>0.05).

Table 12: Effect of pH on Reduction of Phorbol Ester by Isolate During Fermentation of *J. curcas* Seed Cake.

pH	*B. coagulans* mean±SE
4.5	0.65 ± 0.14^{a}
6.5	0.65 ± 0.14^{a}
8.5	0.64 ± 0.14^{a}
P-value	**0.942**

Mean values followed by same letter within the column are not statistically significantly (p>0.05).

The proximate composition in Table 4.1 revealed that *J. curcas* seed cake has a moisture content of 5.45% which indicates that the seed cake used for this study had little or no moisture content. Crude protein (23.75%), crude fibre (11.38%), fat and oil (9.8%), ash content (8.2%) and digestible carbohydrate (46.45%). This study is in agreement with the study of Makkar *et al.,* (1997) and Makkar and Becker (1997), who reported *J. curcas* seed cake to have a crude protein content ranging from 580-640g Kg^{-1} of which 90% is true protein. It is also in agreement with the study of Runmi *et al.,* (2014), who reported to have obtained moisture content (5.87%), Ash content (6.23%), Fat content (12.4%), Protein content (41.14%) and residue oil (12%).

Phorbol ester, phytic acid, lectin and trypsin inhibitor were found to be higher in the *J. curcas* seed cake in which the extraction was carried out manually to be 1.48, 11.02, 14.0 and 10.44mg/l respectively when compared

to the seed cake in which the biodiesel was extracted with a machine whose values were 0.94, 9.58, 13.48 and 7.87mg/l respectively. This could be due to the fact that with the use of machine there would be complete extraction and so most of the anti-nutrients and toxin would have been crushed out alongside with the biodiesel thereby making the concentration lower than in the manually extracted biodiesel.

Post fermentation as shown on Table 4.2, the concentration of phorbol ester, Phytic acid, Saponin, Lectin and Trypsin inhibitor were reduced by 76.35, 56.26, 43.56, 58.75, and 64.94% which is in agreement with the work of Phengnuam and Suntornsuk (2013), whose Phorbol ester, phytate and trypsin inhibitor reduced after fermentation by 62, 42 and 75%, respectively.

Jatropha seeds are mechanically pressed in order to obtain oil, and generating a large amount of oil cake (41–57 percent on dry matter basis) as a by-product (Makkar *et al*., 1997). Jatropha oil cake is rich in nitrogen, phosphorus and potassium (Kumar and Sharma, 2008), thus it is thought to be an excellent candidate for use as a fertilizer for plant nutrient source. However, toxic Jatropha phorbol esters restrict its utilization as fertilizer. Fermented *J. curcas* seed cake revealed that the Phorbol ester in the seed cake reduced as the fermentation time increases. Fermented *J. curcas* seed cake solution containing the *Bacillus coagulans* ferments *J. curcas* seed cake more with a percentage of 45.27% when compared to the control which contained *J. curcas* seed cake solution only (22.97%). This may be because *Bacillus coagulans* is capable of synthesizing comparably high esterase concentrations to break down the phorbol ester. This is in agreement with the work of Chin-Feng *et al.* (2014) who was able to detoxify the seed cake by 76.5, 77.1 and 78.4% using *B. smithii, B. sonorensis* and *B. licheniformis* respectively.

Degradation of Jatropha phorbol esters has been usually conducted by various chemical and physical methods. It has been reported that high heat and pressure treatments (260^0C, 3 mbar) with moisture degraded Jatropha phorbol esters in oil completely (Makkar *et al*., 2009). Combination of alkali and autoclave treatments (121°C, 30 min) decreased Jatropha phorbol esters up to 89 percent in seed meal (Rakshit *et al*., 2008). An autoclave treatment of Jatropha seed meal followed by four repeated washes with methanol degraded 95% of phorbol esters (Aregheore *et al*., 2003). Although these chemical and physical treatments decreased Jatropha phorbol esters efficiently, detoxification method of Jatropha oil cake by incubation with a bacteria for five days is milder and more cost-effective than other methods.

Fermented *J. curcas* seed cake revealed that the phytic acid content of the fermented seed cake also reduces with time. Phytate which is the common storage form of phosphorus in plant seeds and cereal grains (*Reddy et al*., 1982) is considered to be an anti-nutritional factor for humans and animals because of its high chelating ability with cations and complex formation with the basic amino acid group of proteins, thus decreasing the dietary bioavailability of these nutrients (Wodzinski and Ullah, 1996: Martinez *et al.,* 1996).

The fermented *J. curcas* seed cake solution containing the *B. coagulans* ferments *J. curcas* seed cake more by reducing the Phytic acid level up to 28.95% when compared to the control (16.42%). This may be because *B. coagulans* can readily synthesize phytases for catalysis of phytate. This is in agreement with the work of Saetae and Suntornsuk, (2011) whose phytic acid reduced after detoxification. It is also in agreement with the work of Phengnuam and Suntomsuk, (2013) whose phytate after detoxification reduced to about 42%. Phytate is the major storage form of phosphorus in seeds and is found in diets of many animals and humans. Phosphorous is one of the major feed ingredients and is supplied to animals in required amounts through raw material and added phosphates. 50−80% of phosphorous is bound in phytates, which cannot be broken down by endogenous enzymes in poultry (Wodzinski and Ullah, 1996).

There was a reduction of the saponin content more in *B. coagulans* when compared to the control. The saponin contents were reduced by 18.87 and 11.46% for *B. coagulans* and the control respectively. This is in partial agreement with the work of Saetae and Suntornsuk, (2011) whose saponin content reduced by 80% after fermentation. Saponins may serve both as anti-feedants and plant anti-microbial agents, but some plant saponins may enhance nutrient absorbtion and aid in animal digestion. However, saponins are often bitter to taste, and so can reduce plant palatability (Foerster, 2006).

There was a reduction in the lectin concentration of the *J. curcas* seed cake which was more in *B. coagulans* when compared to the control. The lectin contents were reduced by 32.29 and 16.07% for *B. coagulans* and the control respectively. This reduction could be as a result of *B. coagulans* being able to utilize lectin more as a source of nutrient than the others. This is in disagreement with the work of Saetae and Suntornsuk, (2011) whose results show that the lectin contents were not detected in the detoxified *J. curcas* seed cake, whereas they were observed in high levels in *J. curcas* seed cake.

There was a reduction in the trypsin inhibitor concentration of the *J. curcas* seed cake which was more in *B. coagulans* than the control. The trypsin inhibitor contents were reduced by 31.03 and 15.80% for *B. coagulans* and the control respectively. This reduction could be as a result of *B. coagulans* being able to utilize trypsin more as a source of nutrient than the others. This is in agreement with the work of Phengnuam and Suntomsuk, (2013) whose trypsin inhibitor levels were reduced by 75% after detoxification. It is also in

agreement with the work of Saetae and Suntornsuk, (2011) whose trypsin inhibitor level found in the detoxified seed cake were much lower than those found in *J. curcas* seed cake.

The mean values obtained from Tables 4.8 showed statistical difference in each of the organism. *B. coagulans* had mean values of 0.81, 0.65 and 0.49 at 27, 30 and 37^{0}C respectively. The lowest mean value of 0.49 observed for *B. coagulans* indicates that the organism had the best detoxification and anti-nutrient potential at a temperature of 37^{0}C. There were no statistical difference in the mean optimization of phorbol ester reduction with respect to time and pH.

B. coagulans displayed a low detoxification and reduction in the anti-nutrients potential. This is in contrast to the work of Mohamed and Ashraf (2014) whose results after fermenting *J. curcas* seed cake with a *Bacillus* (*B. pumilus*) indicated that fermented *J. curcas* seed cake can replace up to 50% dietary fish meal. Varying temperature, pH and time of fermentation showed that *B. coagulans* at 37°C, pH 8.5 and within the duration of 5 days will detoxify *J. curcas* seed cake in a submerged fermentation medium provided pH and temperature remains constant. This reduction is related to the activities of esterase, phytase and protease enzymes. *J. curcas* seed cake could be detoxified by bacterial fermentation and the high protein fermented seed cake could be potentially applied to animal feed.

4.0 CONCLUSION

J. curcas seed cake, a by-product of *J. curcas* with both high toxin and anti-nutritional factor levels, was detoxified during the course of this study by a Bacterial species, *B. coagulans*. Temperature is a factor which influences the rate of reduction of the phorbol ester in *J. curcas* seed cake during fermentation. The reduction of phorbol esters, phytate and trypsin inhibitor may be due to the presence of esterase, phytase and protease activities, respectively. *J. curcas* seed cake could be detoxified by bacterial fermentation using *Bacillus* strains and the rich-protein fermented seed cake could be potential for use in animal feed.

4.1 Recommendations

1. More work should be done on the complete detoxification of the phorbol ester in the *J. curcas* seed cake using *B. coagulans* by submerged and solid state fermentation so that if complete removal or detoxification can be achieved, it can be used as feed supplements for animals and even humans.
2. Identification of the gene coding for the detoxification of the ester will enhance the understanding of the mechanism involved.
3. The use of genetically modified bacteria can also be employed in the detoxification process (fermentation). The use of mutant strains and also a consortium of *Bacillus* species can also be investigated.

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