academicJournals

Vol. 16(31), pp. 1660-1669, 2 August, 2017
DOI: 10.5897/AJB2017.15889
Article Number: 5AB3F8565597
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Evaluating auto-detoxification of *Jatropha curcas* Linnaeus, 1753 kernel cake with brine shrimp *Artemia salina* (Linnaeus, 1758) lethality test

Divine Ewane[1]*, Pius M. Oben[1], Kenneth J. N. Ndamukong[1], Benedicta O. Oben[1], Kingsley A. Etchu[2] and Kennedy F. Chah[3]

[1]Department of Animal Science, Faculty of Agriculture and Veterinary Medicine, University of Buea, Cameroon.
[2]Institute of Agricultural Research for Development, Yaounde, Cameroon.
[3]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

Receieved 12 January, 2017; Accepted 6 July, 2017

***Jatropha curcas* Linnaeus, 1753 is a plant with several uses for communities in Cameroon and other African countries. However, present methods of detoxifying *J. curcas* kernel cake (JKC) to add value to its further utilization are too sophisticated for cottage operations in rural areas. This study sought to evaluate two auto-detoxification methods using the shellfish brine shrimp, *Artemia salina* (Linnaeus, 1758). Two identical *J. curcas* auto-detoxification apparatuses (JADA) were developed: one simulating diffuse daylight (DJADA) and the other solar irradiation (SJADA) conditions. JKC was pretreated by either soaking or boiling in water or lye. The pretreated samples were then placed in the JADA and either re-moistened to 66% dry matter (DM) or left un-moistened. Among the 14 pretreated samples, water soaked solar without re-moistening (WS1), lye soaked solar without re-moistening (LS1), and water soaked solar with re-moistening (WS2) had LC_{50} values above 1000 ppm after four weeks. On the other hand, un-moistened diffuse daylight spread (UDC), boiled in water and exposed to diffused daylight without remoistening (WD3), and un-moistened solar spread (USS) were the least detoxified within the same period of four weeks with LC_{50} values less than 60 ppm. Irrespective of the pretreatment, extracts from the SJADA had higher LC_{50} values when compared to that of DJADA. This finding suggests that sunlight is an important factor in auto-detoxification. The top three most efficient detoxified treatments: WS1, LS1 and WS2 are recommended for further development and testing with livestock and fish models. This study confirms that JKC is toxic, but under natural conditions is exposed to auto-detoxifying factors which are both endogenous and environmental. When these conditions are optimally manipulated, the detoxification rate is enhanced. Farmers in rural communities can use this strategy to detoxify JKC, transform it into livestock and fish dietary ingredient and consequently enhance its contribution to climate change mitigation.**

Key words: *Jatropha curcas*, auto-detoxification, solar irradiation, *Artemia salina*.

INTRODUCTION

The reality of climate change has encouraged a search for renewable energy sources. However, the cultivation of energy crops for biofuel production has resulted in an increased competition between food and fuel. Therefore,

renewable energy crops such as *J. curcas* L. which are not consumed by humans have gained interest (Devappa, 2012). As an endemic plant of tropical America, *J. curcas* is widely distributed throughout the tropics as an ornamental and medicinal plant. It produces high-quality biodiesel fuel after the appropriate processing of oil extracted from its seeds. The nutrient profile of the resultant *J. curcas* seed cake (JSC) or *J. curcas* kernel cake (JKC) surpasses the Food and Agriculture Organization of the United Nations (FAO) reference protein apart from lysine (Makkar et al., 1998). JSC is toxic to humans and animals even after its further processing. *J. curcas* can provide opportunities for additional income and therefore improve food security (Lyimo, 2010). To enhance the value of JKC, several methods have been proposed for its detoxification, such as: (1) solvent extraction methods (Chivandi et al., 2004; Martınez-Herrera et al., 2006; Makkar et al. 2008; Rachadaporn et al., 2012); (2) chemical treatment methods (Makkar and Becker, 1998; Haas and Mittelbach, 2000; Aregheore et al., 2003; Devappa and Swamylingappa, 2008; Azza and Ferial, 2010); (3) bio-detoxification methods such as fungal detoxification (Belewu and Akande, 2010; Belewu et al., 2010 Azhar et al., 2014; Sulaiman et al., 2014), bacterial detoxification (El-Zelaky et al., 2011; Phengnuam and Suntornsuk, 2013; Widiyastuti et al., 2013; Chin-Feng et al., 2014) and ensilage (Oliveira et al., 2012); physical methods such as ionizing radiation (Runumi et al., 2014), ozonation and solar irradiation (Susan et al., 2015), heat (Workagegn et al., 2013), roasting (Azza and Ferial, 2010), germination (Azza and Ferial, 2010), soaking (Azza and Ferial, 2010), and boiling (Annongu et al., 2010; Fakunle et al., 2013; Alatise et al., 2014). All these methods are characterized by different levels of success. An improved efficiency is observed after the combination of two or more approaches. The challenge today therefore is not only the JKC detoxification to levels safe enough for animal consumption, but also whether the detoxification method can stimulate local interest by ensuring its biological, chemical, technical and economic feasibility for large scale industrial as well as cottage operations in rural communities of resource-poor countries like Cameroon.

The majority of the current successful detoxification methods is sophisticated and cannot be applied at a cottage industry level in rural communities where *J. curcas,* which has already several uses, is additionally expected to contribute in energy security and poverty alleviation. This fact poses limitations on its potential contribution to sustainable agriculture strategies, since climate activists suggest that 80% of current crude oil reserves should remain in the subsoil in order to avoid the critical 2°C rise in global temperature. Easily applied methods such as boiling need further investigation, because phobol esthers (PE), the main toxic component in JKC, are heat stable. They remain in the JKC even when autoclaved at 121°C for 30 min (Aregheore et al., 2003). However, the naturally occurring phobol esters are unstable and are susceptible to oxidation, hydrolysis, transesterificaion and epimerization during isolation procedures (Runumi et al., 2014). By taking into consideration this background, an effort needs to be made to find an easy and cheaper method to detoxify *J. curcas* for rural communities in countries like Cameroon. The present study was therefore aimed to develop two *J. curcas* auto-detoxification apparatuses (JADA) and test their efficacy using brine shrimp lethality test.

MATERIALS AND METHODS

Design and construction of *J. curcas* auto- detoxification apparatuses (JADA)

A natural convection greenhouse solar drier with chimney (Ekechukwu, 1999) was modified to a *J. curcas* auto detoxification apparatus (JADA) by compartmentalizing the access door air inlet in such a way that various levels of air access could be achieved. This modification allowed the adjustment of the apparatus from full detoxification (when air access is completely closed) to full drying mode (the access is fully open). Between these two extreme modes, partial drying and detoxification was also possible. The JADA was constructed to have a triangular drying chamber with a height of 2.7 m, fitted on a rectangular base measuring 2.3 m wide and 5 m long. This chamber had an attached 2.3 m high vertical cylindrical chimney at one end, while at the other end a door existed for air inlet and access to the chamber. Thus, the total height of the chimney from the base of the apparatus was 5 m and had a north-south orientation. In Buea, located at 4.1667° N, and 9.2333° E, the chimney was located in the south and the door in the north. The floor was insulated with 30 cm thick compacted wood shaving covered with black plastic to keep it in place and absorb light. On the top of the floor were two rock piles gathered to retain any trapped heat during the day. The rock piles were covered externally by black corrugated sheets which trapped heat from the sun.

Two identical *J. curcas* auto-detoxification apparatuses were constructed at the Faculty of Agriculture and Veterinary Medicine Farm in the University of Buea. In the first, the north was covered with transparent plastic, and the rest of the apparatus covered with black plastic to simulate diffuse daylight conditions and was named diffuse daylight *J. curcas* auto-detoxification apparatus (DJADA). In the second, the northern end with the door was covered with black plastic sheets, while the rest of the apparatus was covered with transparent plastics to maximize the effects of solar irradiation and was called solar *J. curcas* auto-detoxification apparatus (SJADA).

Preparation and pre-treatment of *J. curcas* kernel cake (JKC)

Jatropha curcas seeds were harvested from farms, live fences and plantations within six regions of Cameroon

*Corresponding author. E-mail: dewane381@gmail.com. Tel: +237 677 761 854.

(South West, North West, Littoral, Adamawa, North and Extreme North). The seeds were transported to the University of Buea where they were manually de-shelled to produce kernels. The kernels were de-oiled using a hydraulic press and *J. curcas* kernel cake (JKC) was produced. The JKC was ground into powder using a plate mill, and the powder was homogenized by hand mixing. The JKC samples were either physically or chemically pre-treated before being placed in the two types of JADA for detoxification.

Physical pretreatment was performed by soaking or boiling the JKC samples in water. In the physical pretreatment by soaking, homogenized JKC was soaked overnight in water at the ratio of 1:2 w/v by adding fixed weight of JKC to fixed volume of water and stirring the mixture to ensure complete dispersion in water. This procedure resulted in a pretreatment additional moisture content of 66.67%. This material was thereafter divided into 4 treatments (WS1, WS2, WD1 and WD2) with 4 replicates per treatment. In the boiling method, homogenised JKC was poured into boiling water in an aluminium pot at the ratio of 1:2 w/v and stirred continuously while still on fire for 30 min to produce dough. The resultant dough was sub-divided into two treatments (WS3 and WD3) each with 4 replicates.

Chemical pretreatment was achieved by soaking or boiling the JKC samples in lye. The lye used was produced locally by modifying the procedure described by Kent et al. (2014). About 50 kg of wood ash from a local palm oil processing plant was leached for three days in a 120 L plastic tank fitted with a tap. In the chemical pretreatment by soaking, homogenized JKC was over soaked overnight in lye at the ratio of 1:2 w/v by adding fixed weight of JKC to fixed volume of lye and stirring the mixture to ensure complete dispersion in lye. This procedure offered a pretreatment additional moisture content of 66.67%. This material was thereafter divided into 4 treatments (LS1, LS2, LD1 and LD2) with 4 replicates per treatment. In the boiling method, homogenised JKC was poured into boiling lye at the ratio of 1:2 w/v and stirred continuously while still on fire for 30 min to produce dough. The resultant dough was sub-divided into two treatments (LS3 and LD3) each having 4 replicates.

Auto-detoxification of the JKC

The 12 pretreated JKC samples (WS1, WS2, WS3, WD1, WD2, WD3, LS1, LS2, LS3, LD1, LD2 and LD3) plus two untreated or unmoistened samples (USS and UDC) were subjected to auto-detoxification using the JADA. The two JADA modifications were operated at full detoxification mode. The samples were placed in steel plates that were arranged on two tables within the JADA; each treatment had four replicates. The WS1, WS2, WS3, LS1, LS2, LS3 and USS were placed in the SJADA while WD1, WD2, WD3, LD1 LD2, LD3 and UDC were placed in DJADA (Table 1). During the detoxification process, WS2 and WD2 and LS2 and LD2 were remoistened daily with water and lye, respectively, to obtain a moisture level of 66% every morning. However, there was no remoistening on the day proceeding the day of sample collection for evaluation (Table 1).

Assessment of the level of detoxification of JKC by performing the brine shrimp lethality test

Preparation of crude methanol auto-detoxified J. curcas kernel cake extracts (CMJKCE)

Forty grams of each treatment sample (10 g per replicate) were collected weekly to assess the level of auto-detoxification. The four replicates per treatment were pooled and further dried within the JADA for another week. Each sample was homogenised, powdered and 20 g of it were mixed with 200 ml of methanol for 72 h with regular agitation. Filtration using Whatman No. 541 filter papers followed and the CMJKCE were obtained by complete evaporation of the solvent using a rotary evaporator.

Brine shrimp bioassay

The brine shrimp lethality test (BSLT) as described by Meyer et al. (1982) was used to test toxicity of CMJKCE after some modifications. The toxicity is reported as LC_{50} values in mg/ml (ppm). Brine shrimps [*Artemia salina* (Linnaeus, 1758)] were hatched from eggs in rectangular dishes divided by a perforated barrier, under constant aeration using natural seawater. The perforated barrier divided the dish into two chambers. The bigger chamber receiving the eggs was in the dark while the smaller chamber receiving the hatched nauplii in anticipation was under constant light. Forty eight hours were allowed for the eggs to hatch and the photropic nauplii to mature. Ten nauplii were collected using a pipette and placed in marked vials, each containing 4 ml of natural seawater. Two hundred miligrams of each examined CMJKCE were dissolved in 2 ml of pure dimethyl sulfoxide (DMSO) to get a solution of 100,000 ppm (100,000 mg/l). A unit volume of this solution was diluted in one mililitre of natural seawater to give a concentration of 50,000 ppm. Ten-fold serial dilutions were further performed in sea water to produce concentrations of 5000, 500, 50 and 5 ppm. One milliliter of each of these dilutions was delivered into pre-marked vials containing 4 ml of natural sea water and 10 nauplii. As a result, the final concentrations of CMJKCE were 10,000, 1000, 100, 10 and 1 ppm. Three replicates of each concentration were prepared for CMJKCE samples. The negative controls were dilutions of DMSO in seawater without CMJKCE. Un-detoxified whole *J. curcas* kernel cake served as the positive control. The vials were kept incubated under light for 24 h after which the surviving nauplii were counted. The nauplii were counted against a lighted background using a 3× magnifying hand lens. They were considered dead if they lay immobile at the bottom of the vial. The mortality percentage was then calculated. The mortality data was corrected using Abbott's (1925) formula (Apu et al., 2013), since a mortality percentage more than 10% was recorded in the controls. Subsequently there were corrections for 0 and 100% as proposed by Ghosh (1984). The formulae used in calculating and correcting brine shrimp mortality are presented in Table 2: The surviving nauplii were killed by addition of 5 ml of 5% v/v phenol to each vial. The vials were crosschecked to ensure that all the nauplii were dead before discarding.

Determination of 50% lethal concentration (LC_{50})

The lethal concentration of CMJSCE resulting in 50% mortality of brine shrimp (LC_{50}) was determined from the 24 h counts by a plot of percentage of the shrimps killed against the logarithm of the CMJKCE concentration. The best-fit line was obtained from the curve data by means of regression analysis (MS Excel version 7) and the LC_{50} was derived from the slope of the best-fit line obtained.

Statistical analysis

Levene's test for equality of variances and t-test for equality of means were performed on LC_{50} using the IBM SPSS Statistics version 22 (IBM Corp. Released 2013). The mean (±SEM) were quarreled.

Selection of the most promising auto-detoxified JKC (ADJKC) treatments for further development

Selection of the most promising ADJKC treatments was achieved

Table 1. Summary of JKC auto-detoxification treatment groups.

Form of treatment of JKC	Lighting exposure (JADA)	
	Solar spread (SJADA)	**Diffuse daylight spread (DJADA)**
Un-moistened	USS (un-moistened solar spread)	UDC (un-moistened diffuse daylight spread)
Soak in water. No remoistening	WS1 (water soaked, solar spread without remoistening)	WD1(water soaked, exposed to diffuse daylight without remoistening)
Soak in lye. No remoistening	LS1 (lye soaked, solar spread without remoistening)	LD1 (lye soaked exposed to diffuse daylight without remoistening)
Soak in water. Remoistening to 66% DM	WS2 (water soaked, solar spread and remoistened to 66 % DM)	WD2 (water soaked exposed to diffuse daylight and remoistened to 66 % DM)
Soak in lye. Remoistening to 66% DM	LS2 (lye soaked, solar spread and remoistened to 66 % DM)	LD2 (lye soaked exposed to diffuse daylight and remoistened to 66 % DM)
Boil in water. No remoistening	WS3 (boil in water and solar spread, without remoistening)	WD3 (boil in water, exposed to diffuse daylight without remoistening)
Boil in lye. No remoistening	LS3 (boil in lye, solar spread without remoistening)	LD3 (boil in lye exposed to diffuse daylight without remoistening)

Table 2. Formulae used in calculating and correcting brine shrimp mortality.

Method	Formulae
Percentage mortality	% of mortality = (number of dead nauplii / total number) × 100
Abbott (1925) formula	Corrected % mortality = {(Mobs - Mcontrol) / (100 - Mcontrol)} × 100. Where, Mobs = observed % mortality; Mcontrol = control % mortality
Ghosh (1984) 0 and 100% correction	For 0% mortality: 100 × (0.25 × n). For 100% mortality: 100 × (n - 0.25/n); Where, n= no. of test animal in each group

by ranking the LC_{50} from the largest to the smallest values with the largest values indicating the least toxicity. A t- test was used to compare the LC_{50} of ADJKC from the SJADA and DJADA to determine the significance of solar radiation in auto-detoxification of JKC at an experimental level.

RESULTS

The level of auto detoxification of the different experimental groups has been ranked using the mean of the LC_{50} values for all the treatments from weeks 1 to 4 (Table 3). From the results presented in Table 3, WS1, LS1 and WS2 treatments all had LC_{50} values above 1000 ppm after four weeks of exposure in the JADA, indicating that they were the most detoxified. On the other hand UDC, WD3 and USS were the least detoxified within the same period of 4 weeks with LC_{50} of less than 60 ppm.

The extent of lethality was found to be proportional to the concentration of the CMJKCE. High mortality rates were recorded at 1000 and 10,000 ppm, while lower values were recorded at 1 and 10 ppm. Mortality rates of brine shrimp exposed to whole *J. curcas* kernel cake just after oil extraction are presented in Figure 1. Thereafter, corrected brine shrimp mortality rates in the 4^{th} week for UDC, WS1, LS1 and WS2 are presented in Figures 2 to 5, respectively.

The means (and standard error of the means) of the LC_{50} of the extracts of solar and diffused daylight detoxified JKC extracts over the four week detoxification period are presented in Table 4. Irrespective of the pretreatment, SJADA treated extracts had higher LC_{50} values compared to the DJADA ones, with the WS1, LS1 and WS3 having significantly higher values ($P<0.05$) compared to WD1, LD1 and WD3 groups, respectively (Table 4).

DISCUSSION

The JADAs were easy to construct onsite because of their simple design and low cost which is characteristic of natural convection greenhouse solar driers with chimney. These characteristics attributed to natural convection greenhouse driers (Weiss and Buchinger, http://www.aee-intec.at/0uploads/dateien553.pdf) make development of the JADA a suitable tool for *J. curcas* detoxification in remote rural communities. Other solar dryer designs may not be appropriate for re-design into a

Table 3. LC_{50} of crude methanol auto detoxified *J. curcas* kernel cake extracts (CMJKCE) ranked from highest to least detoxified (mean of 4 weeks).

Treatment	Week 1	Week 2	Week 3	Week 4	Mean LD_{50}
WS1	776.24712	986.12330	1,050.32265	1,483.32158	1,074.00366
LS1	873.32616	913.38623	981.89826	1,087.76440	964.09376
WS2	92.08173	419.98449	1,057.21209	1,836.53834	851.45416
WS3	58.42075	120.43669	280.94207	395.32296	213.78062
WD2	63.90805	80.78246	183.36201	383.08736	177.78497
LS3	30.07266	74.42734	121.45608	304.24754	132.55091
WD1	38.74078	50.79456	123.68662	204.03416	104.31403
LD1	38.22643	51.12171	131.46350	154.46779	93.81986
LS2	9.32810	17.22766	127.26505	201.52612	88.83673
LD2	0.88057	1.91720	92.08173	63.89076	39.69256
USS	15.79273	16.66887	49.32035	58.28871	35.01766
LD3	3.02178	5.56577	26.00650	98.35801	33.23802
WD3	0.37125	1.08390	5.12075	21.06534	6.91031
UDC	0.09094	0.35058	2.90193	5.21849	2.14049
Whole *J. curcas* kernel cake	0.00035				0.00009

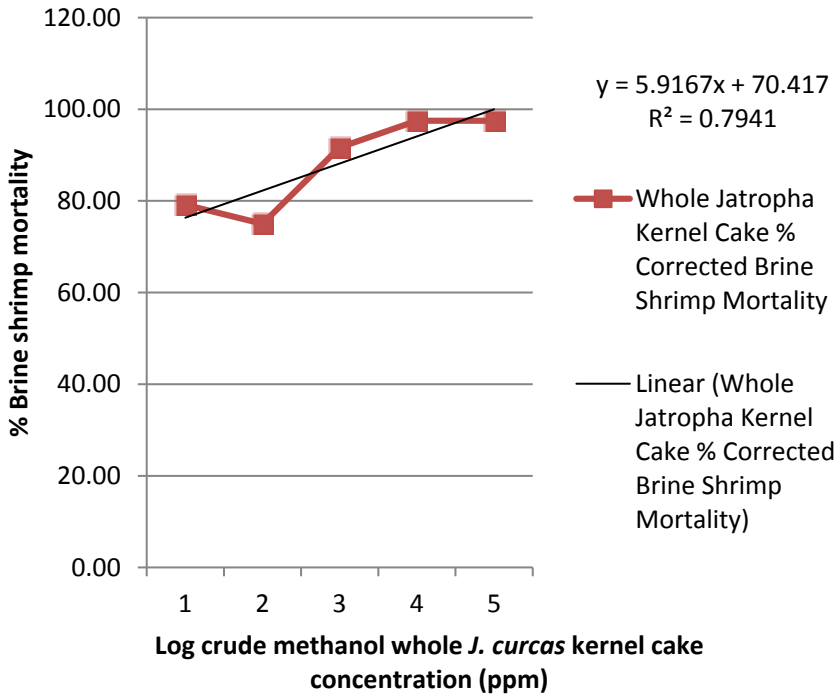

Figure 1. Mortality rates (%) of brine shrimp exposed to whole *J. curcas* kernel cake just after oil extraction.

JADA. This is because in the JADA, there is need for the crop to have optimised access to environmental conditions such as light, temperature, moisture, wind, oxygen and microbes that contribute to its auto-detoxification. Besides, *J. curcas* is a tropical and sub-tropical crop, grown in areas with ubiquitous solar radiation.

The proportional response of brine shrimp to the increased concentration of the CMJKCE confirms that auto-detoxification was effective in reducing JKC deleterious effects. The present study also supports the earlier findings of Chikpah and Demuyakor (2012), who found that approximately 60% reduction in crude phorbol ester levels can be achieved within 21 days of

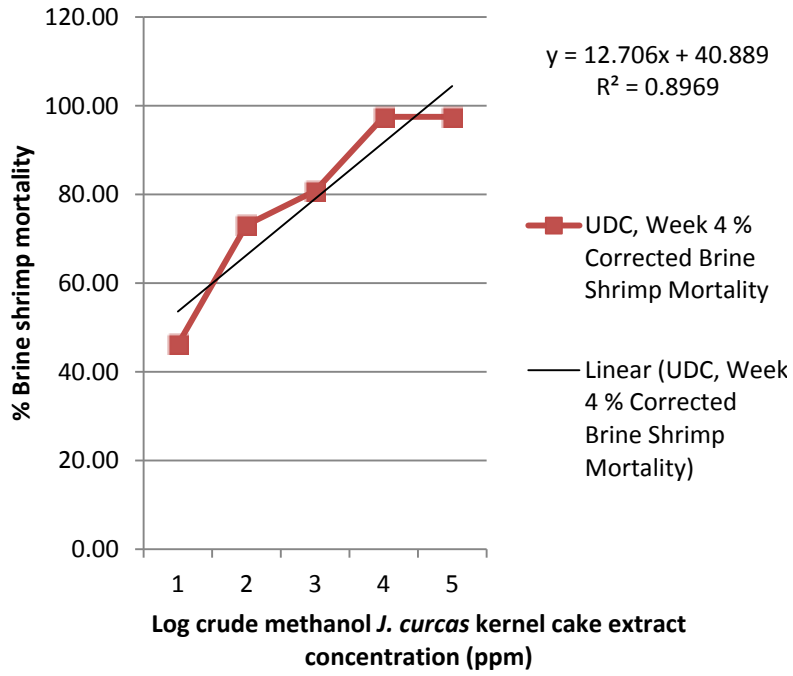

Figure 2. Corrected brine shrimp mortality rates (%) for UDC (Un-moistened diffused daylight spread) JKC treatment after 4 weeks of auto detoxification.

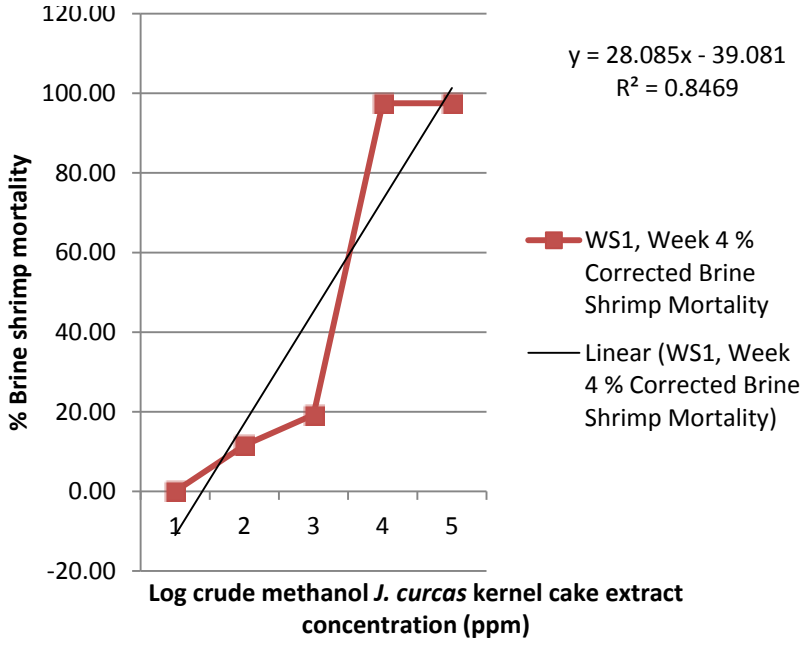

Figure 3. Corrected brine shrimp mortality rates (%) for WS1 (water soaked, solar spread, without remoistening) JKC treatment after 4 weeks of auto detoxification.

spontaneous fermentation of *J. curcas* kernel meal.

According to Meyer et al. (1982), several extracts derived from natural products which had LC_{50} ≤ 1000 µg/ml using BSLT were known to contain physiological active principles while those with LC_{50} values > 1000 ppm are considered inactive. Thus, exposure of the JKC in the JADA for four weeks rendered them inactive and therefore the detoxified cake extract was less toxic to

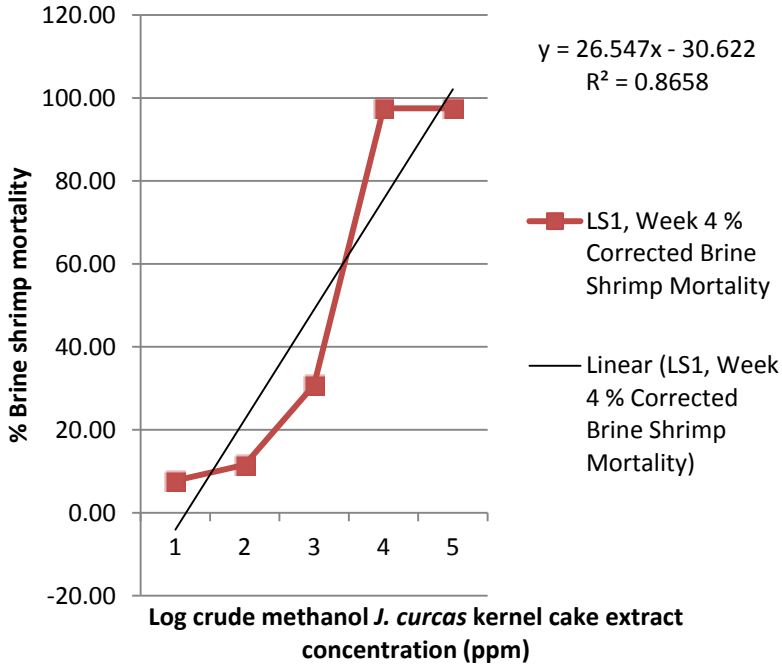

Figure 4. Corrected brine shrimp mortality rates (%) for LS1 (Lye soaked, solar spread, without remoistening) JKC treatment after 4 weeks of auto detoxification.

brine shrimps. However, working with phorbol ester enriched fraction (PEEF) of *J. curcas* seeds, Devappa et 72%, which was achieved at 47 mg L^{-1}, further increase in concentration was not effective in increasing mortality rates of *A. salina*. 100% mortality was only achieved at a high concentration of 6000 mg L^{-1}. This observation prompted the testing of CMJKCE at a concentration of 10,000 ppm in the present study.

The *J. curcas* plant contains many biologically active phytochemicals including proteins, peptides and diterpenes which exhibit a wide range of biological activities (Devappa et al., 2010, 2011a). However, the seeds contain anti-nutritional and toxic factors such as phytate, trypsin inhibitor, lectin, curcin and phorbol esters (PEs) (Makkar et al., 1997). In the majority of the cases, al. (2011b) observed that after a mortality "plateau" of toxicity of seeds and seed derivatives is attributed to the presence of PEs (Devappa et al., 2011b). In a previous study, Kinghorn et al. (1977) had demonstrated that there are differences in the toxicity of specific purified phorbol esters. While purified PEs such as phorbol 12-tetradconoate 13-acetate, phorbol 12, 13-didecanoate, and phorbol 12, 13-dibenzoate induced toxicity with an effective mortality dose (ED_{50}) of 3.8, 6.8 and 11.8 mg L^{-1} respectively, the phorbol and 4a-phorbol 12, 13-didecanoate were relatively nontoxic (ED_{50} > 1000 mg L^{-1}). Referring to the above study, Devappa et al. (2011b) concluded that bioactivity of PEs depends on their chemical structure/configuration and purity.

The BSLT only indicates the level of toxicity of an extract or a substance and does not identify the toxic component. However, it can be used anywhere in the world as a general toxicity test because the eggs are readily available. It is a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties (McLaughlin et al., 1991). This assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and the cytotoxicity testing of dental materials (Harwing and Scott, 1971; McLaughlin et al., 1991; Martinez et al., 1998; Barahona and Sanchez-Fortun, 1999; Pelka et al., 2000). Comparing the BSLT with the respective snail bioassay, Devappa et al. (2011b) concluded that the BSLT is less sensitive towards *J. curcas* PEs with respect to toxicity screening. Nonetheless, the use of *A. salina* and *Daphnia magna* Straus, 1820 is preferred for assaying a large number of PE samples because the test can be performed in 96-well plates (Ruebhart et al., 2008). At the same time, BSLT is the only feasible choice in countries like Cameroon, where the common bladder snail [*Physa fontinalis* (Linnaeus, 1758)], un-adapted to tropical environments, is not readily available and the response of local snails to PEs has not yet been studied. In addition, the preliminary testing of ADJKC for toxicity should not only be focused on PEs but should detect additional toxins developed during the process. Makkar et al. (2012) recognized that no information is available on the nature of the phorbol ester degraded products and

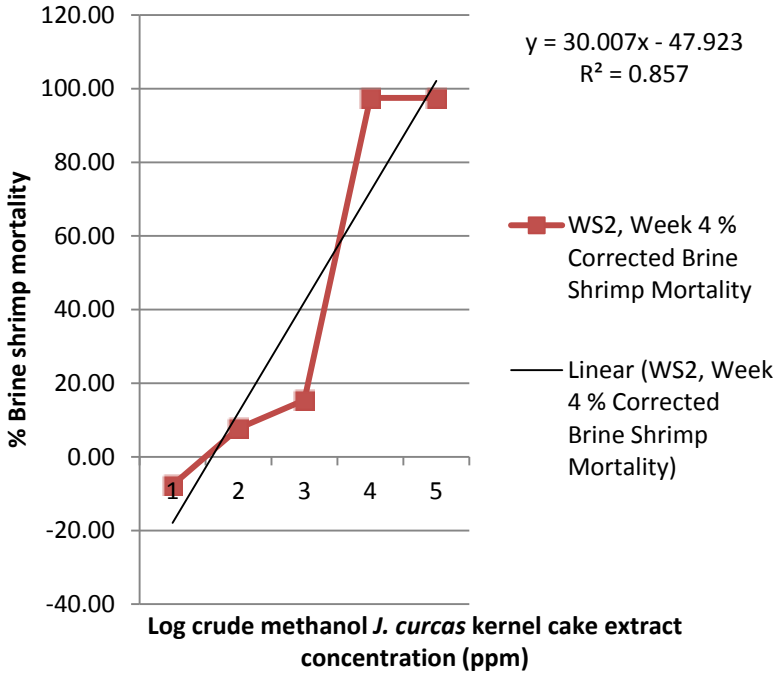

Figure 5. Corrected brine shrimp mortality rates (%) for WS2 (water soaked, solar spread and remoistened to 66% DM) JKC treatment after 4 weeks of auto detoxification.

Table 4. Comparism of mean (± sem) LD_{50} for solar JADA and diffused daylight JADA.

Pre-treatment	Mean (± sem) LC_{50} SJADA	Mean (± sem) LC_{50} DJADA	P-value
WS1/WD1	1,074.004 ± 148.56	104.314 ± 38.170	0.001
LS1/LD1	964.094 ± 46.923	93.820 ± 28.881	0.000
WS2/WD2	851.454 ± 384.658	177.785 ± 73.347	0.136
WS3/WD3	213.781 ± 76.550	6.910 ± 4.833	0.036
LS3/LD3	132.551 ± 60.196	33.238 ± 22.310	0.173
LS2/LD2	88.837 ± 46.211	39.693 ± 22.846	0.377
USS/UDC	35.018 ± 11.001	2.140 ± 1.206	0.025

Sem = Standard error of the mean.

their possible toxicity. The naturally occurring PEs are unstable and are susceptible to oxidation, hydrolysis, trans-esterification and epimerization during isolation procedures (Runumi et al., 2014). Auto detoxification could exploit the opportunities presented by these PEs properties to render JKC innocuous.

Irrespective of the pretreatment, SJADA extracts had higher LC_{50} values compared to the DJADA ones. This observation indicates that exposure of JKC to sun-light has a significant effect on the auto-detoxification process. Auto-detoxification is a self-detoxification process induced by endogenous and environmental factors including enzymes, microbes, sunlight, temperature, humidity and wind. It is the natural way to transform the toxic *J. curcas* seeds into an innocuous material. These processes take considerable time under natural conditions but their duration can be shortened by human manipulation. Materials are pre-treated through physical, chemical or both ways, in order to induce the necessary changes that contribute in their detoxification. One of the known changes is a process called autoxidation. Autoxidation is any oxidation that occurs in open air or in presence of oxygen (and sometimes UV radiation) and forms peroxides and hydroperoxides. In a wider sense, any oxidation process which produces peroxy radicals, irrespective of their origin, is also considered as an autoxidation process (Michael, 1981). The main toxin of *J. curcas* seeds is represented by the phorbol esters that are highly susceptible to autoxidation. Schmidt and Hecker (1975) investigated the various products arising from two PEs - 2-O-tetradecanoylphorbol-13-acetate (TPA) and 4a-phorbol-12, 13-diacetate – derived by

autoxidation under different conditions of storage. It was found that in addition to the traces of polar products that were not isolated, the aldehyde, 0-epoxide, hydroperoxide, hydroxylated A5-6 isomer, ketone and 6, 7-seco hemiacetal are formed from TPA. If TPA is stored as a powder at 25°C in diffuse daylight for 3 months, it is converted preferentially into the hydroperoxide, with smaller amounts of the other products. If spread on a large surface as a thin film (on glass plates, beads, or silica gel thin layers) and kept in diffuse daylight at 25°C, TPA is oxidized very rapidly. In this case, the 6, 7-seco hemiacetal is formed preferentially, in addition to smaller amounts of the other products. This rapid conversion is dependent on light and the reaction is slowed down in the dark. Moreover, among the various autoxidation products that could be obtained from TPA, a sample of 4a-phorbol-12, 13-diacetate yielded (~30%) the corresponding 20-aldehyde as the only conversion product after storage for 1 year at room temperature in the dark. Storage in the dark is mandatory to avoid the known "lumi reaction" of 4a-phorbol, which is independent of the presence of oxygen (Schmidt and Hecker, 1975). It can be concluded from the above study that all phobol esters do not follow the same path of autoxidation in the presence of light. This may explain the differences in auto-detoxification observed between the SJADA and DJADA. The challenge for auto-detoxification of *J. curcas* seed materials is to maintain an adequate post-detoxification value for their utilization as feed ingredients for livestock and fish.

The top three most detoxified treatments: WS1, LS1 and WS2 are recommended for further development and testing as livestock and fish dietary ingredients by using the respective farm animal models. WS3 could also be considered for further development. It could be possible that pre-treatment heating influenced some factors that subsequently affected the level of auto-detoxification of WS3. Aregheore et al. (2003), found that autoclaving at 121°C for 30 min did not change the PE content in *J. curcas* kernel meal. However, the concentration of lectins was significantly ($P<0.05$) reduced by the heat. Makkar et al. (2012) recognize that the main category of toxic compounds, the phorbol esters, is to a large extent heat stable. Therefore, other strategies must be applied for their removal. Alatise et al. (2014), Fakunle et al. (2013) and Annongu et al. (2010) concluded that *Clarias gariepinus* (Burchell, 1822) could tolerate up to 30% of boiled *J. curcas* kernel meal in the diet in place of soybean meal. One of the challenges concerning the future development of WS3 treatment is that it uses heat as a pre-treatment prior to auto-detoxification. This may increase its carbon footprint particularly in countries like Cameroon where 98.7% of rural households rely on wood as their only fuel source (Ego, 2009). Moreover, Cameroon's wood biomass demand grows nearly at the same pace with total energy demand; approximately 2.4% per year (AEEP, 2013).

Conclusion

This study has confirmed that JKC is quite toxic. However, under natural conditions, it is exposed to auto-detoxifying factors which are both endogenous and environmental. When these conditions are optimally manipulated as in the SJADA, the detoxification rate is enhanced. Solar radiation plays an important role in auto detoxification process of JKC. Farmers in rural communities can use this strategy to detoxify JKC, transform it into animal feed and consequently enhance its contribution to climate change mitigation. The role of microbial succession in auto-detoxification needs to be further investigated. Although the BSLT is rather inadequate in identifying the toxic ingredients in ADJKC, it is useful to determine the level of detoxification. The top three most detoxified treatments: WS1, LS1 and WS2 are recommended for further development and testing as livestock and fish dietary ingredient.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abbot WS (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.

AEEP (2013). Country Power Market Brief: Cameroon. Available at https://www.ruralelec.org/sites/default/files/aeep_cameroon_power_sector_market_brief_en.pdf

Alatise SP, Adedokun MA, Adelodun OB, Ajiboye GE (2014). Effects of Boiled *Jatropha* Kernel Meal as a Substitue for Soyabeans Meal in Diet of African Mud Catfish (*Clarias gariepinus*) Fingerlings. J. Fish. Aquat Sci. 9:446-454.

Annongu AA, Joseph JK, Apata DF, Adeyina AO, Yousuf MB, Ogunjimi, KB (2010). Detoxification of *Jatropha curcas* Seeds for Use in Nutrition of Monogastric Livestock as Alternative Feedstuff. Pak. J. Nutr. 9(9):902-904.

Apu AS, Bhuyan SH, Khatun F, Liza MS, Matin M, Hossain FMd (2013). Assessment of Cytotoxic Activity of Two Medicinal Plants Using Brine Shrimp (*Artemia Salina*) as an Experimental Tool. IJPSR, 4(3):1125-1130.

Aregheore EM, Becker K, Makkar HPS (2003). Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats S. Pac. J. Nat. Sci. 21:50-56.

Azhar N, Abdullah N, Saad WZ, Ahmad S, Oskoueian E, Abas F, Gherbawy Y (2014). Detoxification of toxic phorbol esters from Malaysian Jatropha curcas Linn. kernel by Trichoderma spp. and endophytic fungi. Intl. J. Mol. Sci. 15(2):2274-2288.

Azza AA, Ferial MA (2010). Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors. Afr. J. Food Sci. 4(3):93-103.

Barahona MV, Sanchez-Fortun S (1999). Toxicity of Carbamates to the Brine Shrimp *Artemia salina* and the Effect of Atropine, BW284c51, iso-OMPA and 2-PAM on Carbaryl Toxicity. Environmental Pollution, 104:469-476.

Belewu MA, Akande BA (2010). Biological upgrading of the nutritional quality of *Jatropha curcas* kernel cake: effect on performance characteristics of goat. Intl. Res. J. Biotechnol. 1(2):019-022.

Belewu MA, Belewu KY, Ogunsola FO (2010). Nutritive value of dietary fungi treated *Jatropha curcas* kernel cake: Voluntary intake, growth

and digestibility coefficient of goat. Agric. Biol. J. N. Am. 1(2):135-138.
Chikpah SK, Demuyakor B (2012). Effects of Spontaneous Solid-State Fermentation On The Proximate and Antinutrients Composition of *Jatropha curcas* Kernel Meals Obtained from Four Different Agro-Climatic Areas of Ghana: Detoxification Approach. IJSTR, 1:11.
Chin-Feng C, Jen-Hsien, W, Kao-Yung L, Li-Yun L, Shang-Shyng Y (2014). Phorbol Esters Degradation and Enzyme Production by *Bacillus* using *J. curcas* Seed Cake as Substrate. Intl. J. Environ. Pollut. Remediation 2:1.
Chivandi E, Mtimuni JP, Read JS, Makuza SM (2004). Effect of processing method on phorbol esters concentration, total phenolics, trypsin inhibitor activity and the proximate composition of the Zimbabwean *Jatropha curcas* provenance: a potential livestock feed. Pak. J. Biol. Sci. 7(6):1001-1005.
Devappa RK, Makkar HPS, Becker K (2010). Nutritional, biochemical and pharmaceutical potential of proteins and peptides from *Jatropha* : Review. J. Agric. Food Chem., 58:6543–6555.
Devappa RK, Makkar HPS, Becker K (2011a). *Jatropha* diterpenes-a Review. J. Am. Oil Chem. Soc. 88:301-322.
Devappa RK, Rajesh SK, Kumar V, Makkar HPS, Becker K (2011b). Activities of *Jatropha curcas* phorbol esters in various bioassays. Ecotoxicol. Environ. 78:57-62.
Devappa RK, Swamylingappa B (2008). Biochemical and nutritional evaluation of *Jatropha* protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors. J. Sci. Food Agric. 88(5):911-919.
Devappa RK (2012). Isolation, characterization and potential agro-pharmaceutical applications of phorbol esters from *Jatropha curcas* oil. Dissertation Submitted in fulfillment of the requirements for the degree “Doktor der Agrarwissenschaften (Dr. Sc. Agr. / Ph.D. in Agricultural Sciences) University of Hohenheim Stuttgart, Germany.
Ego L (2009). *Jatropha* versus Palm Oil: which biofuel will best protect the environment in Cameroon? (http://www.gvepinternational.org/en/business/news/J.curcas-versus-palm-oil-which-biofuel-will-best-protect-environment-cameroon).
Ekechukwu OVNB (1999). Review of Solar energy drying systems II: an overview of solar energy drying technology. Energy conversion and Management, 40:615-655.
El- Zelaky OA, Khalifa EI, Mohamed AH, Bahera KM, Hussein AM, (2011). Productive and Reproductive Performance of Rahmani Male Lambs Fed Rations Containing *Jatropha* Cake. Egyptian J. Sheep Goat Sci. 6(2):15-24.
Fakunle JO, Alatise SP, Effiong BN, Tiamiyu K (2013). Effects of Replacing Soyabean Meal with Graded Levels of Boiled Jatropha Kernel Meal in Diets of *Clarias gariepinus* Fingerlings. Bull. Environ. Pharmacol. Life Sci. 2 (9):112-117.
Ghosh MN (1984). Fundamentals of Experimental Pharmacology. Scientific Book Agency, Second Edition, P 25.
Haas W, Mittelbach M (2000). Industrial Crops and Products, 12:111-118.
Harwing J, Scott P (1971). Brine shrimp (*Artemia nauplii* L.) larvae as a screening system for fungal toxins. Appl. Microbiol. 21:1011-1016.
IBM Corp. Released (2013). IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.
Kent G, Krystle C, Rubenstein B, Ash F, 39 others (2014). How to make Lye. (http://www.wikihow.com/Make-Lye). Accessed 1/02/2015.
Kinghorn AD, Harjes KK, Doorenbos, NJ (1977). Screening procedure for phorbol esters using brine shrimp (*Artemia salina*) larvae. J. Pharm. Sci. 66:1362-1363.
Lyimo R (2010). *Jatropha curcas* cultivation and its impact on land use and land cover change in Mon-duli and Mpanda Districtis, Tanzania [MSc thesis]. Morogoro, Tanzania: Sokoine University.
Makkar HPS, Becker K, Sporer F, Wink M (1997). Studies on nutritive potential and toxic constituents of different provenances of *J.curcas curcas* J. Agric. Food Chem. 45:3152-3157.
Makkar HPS, Aderibigbe AO, Becker K (1998). Comparative evaluation of non-toxic and toxic varieties of *J.curcas curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chem. 62:207-215.
Makkar HPS, Becker K (1998). Toxic Plants and other Natural Toxicants (Proceedings of the International Symposium on Poisonous Plants), 5th Ed., San Angelo, Texas, 554-558.
Makkar HPS, Francis G, Becker K (2008). Protein concentrates from *Jatropha curcas* screw pressed seed cake and toxic and antinutritional factors in protein concentrate J. Sci. Food Agric. 88:1542-1548.
Makkar HPS, Kumar V, Becker K (2012). Use of detoxified *Jatropha* kernel meal and protein isolate in diets of farm animals. In FAO (2012). Biofuel co-products as livestock feed - Opportunities and challenges, edited by Harinder PSM, Rome. 45.
Martinez-Herrera J, Siddhuraju PF, Davila-Ortiz G, Becker K (2006). Chemical composition, toxic/antimetabolic constituents, and effect of different treatments on their levels, in four provnances of *Jatropha curcas* L. from Mexico. Food Chem. 96:80-89.
Martinez M, Del ramo J, Torreblanca A, Diaz-Mayans J (1998). Effect of cadmium exposure on zinc levels in the brine shrimp *Artemia partenogenitica*. Aquaculture 172:315-325.
McLaughlin JL, Chang CJ, Smith DL (1991). Bench-top bioassays for the discovery of bioactive natural products: an update. In: Rhaman, A. U. (Ed.), Stud. Nat. Prod. Chem. Elsevier 383-409.
Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, McLaughlin JL (1982). Brine shrimp: a Convenient general bioassay for active plant constituents. Planta Med. 54:31.
Michael GS (1981). Free radical mechanisms in autoxidation processes. J. Chem. Educ. 58(2):125.
Oliveira AS, Schwambach TI, Sinhorin AP, Oliveira MRC, Alessi KC, Filho FAO, Pina DS (2012). Capacity of ensilage of *Jatropha curcas* L. cake to degrade forbol esters R. Bras. Zootec. 41:6.
Pelka M, Danzl C, Distler W, Petschelt A (2000). A new screening test of dental materials. Journal of Dentology, 28:341-345.
Phengnuam T, Suntornsuk W (2013). Detoxification and anti-nutrients reduction of *Jatropha curcas* seed cake by Bacillus fermentation. J. Biosci. Bioeng. 115(2):168-172.
Rachadaporn S, Balaji V, Shubhen K (2012). A sustainable source of biofuels: *Jatropha curcas*. http://www.labint-online.com/featured-articles/a-sustainable-sourceof-biofuels-jatropha-curcas/index.html.
Ruebhart DR, CockIE, Shaw GR (2008). Brine shrimp bioassay: importance of correct taxonomic identification of Artemia (Anostraca) species. Environ. Toxicol. 23:555-560.
Runumi G, Utpal KN, Ajay KT (2014). Reduction of phorbol ester content in *Jatropha curcas* cake using high energy gamma radiation. J. Radiat. Res. Appl. Sci. 7:305-309.
Schmidt R, Hecker E (1975). Autoxidation of Phorbol Esters under normal storage conditions. Cancer Res. 35:1375-1377.
Sulaiman AF, Ahmed EIMA, Afeez, AA, Rihanat BM, Abiodun MS, Abdulfattah OA, Oluyomi SA (2014). *Aspergillus*-fermented *Jatropha curcas* seed cake: proximate composition and effects on biochemical indices in Wistar rats. Biological Lett. 51(1):37-46.
Susan M, Brent S, Susan H, Paramjeet P, Alla A, Boubacar D, Mel Y (2015). Removal of Phorbol Ester from *Jatropha* Seedcake Using Ozonation and Solar Irradiation. Ozone: Science & Engineering: The J. Intl. Ozone Association 37:1.
Weiss W, Buchinger J (2012). Solar Drying Training Course Within The Project: Establishment of Production Sales and Consulting Infrastructure for Solar Thermal Plants in Zimbabwe Supported by the Austrian Development Cooperation, Arbeitsgemeischaft ERNEUERBARE ENERGIE, Institute for Sustainable Technologies, Austria (http://www.aee-intec.at/0uploads/dateien553.pdf. (Accessed 5/17/2012).
Widiyastuti T, Prayitno CH, Iriyanti N (2013). Digestibility and Blood Metabolite Profiles of Chicken Fed Fermented Jatropha Seed Meal. Anim. Prod. 15(2):98-105.
Workagegn KB, Ababbo ED, Tossa BT (2013). The Effect of Dietary Inclusion of *Jatropha curcas* Kernel Meal on Growth Performance, Feed Utilization Efficiency and Survival Rate of Juvenile Nile tilapia. J. Aquac. Res. Dev. 4:193.