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DETOXIFICATION PHORBOL ESTERS OF *Jatropha curcas* SEEDS BY SOME TECHNOLOGICAL TREATMENTS

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ABSTRACT

The toxic phorbol ester content of *Jatropha curcas* seeds cultivated in Egypt in raw whole and a kernel seed was ranged between 24.45 and 43.53 mg/kg, respectively. Detoxifications of phorbol esters from *Jatropha curcas* by physical treatments were studied. Soaking in water removed 46.13 % and 36.18 % phorbol ester in whole and kernel seeds, respectively. While germination and roasting treatments did not affect the phorbol ester level. Detoxification of phorbol ester by some chemical treatments was also studied. The $NaHCO_3$ treatments / 121ºC/ 25 min, the treatment (ethanol 90 % /2 h), (ethanol 90 % + $NaHCO_3$/121 º C / 25 min) treatment, sodium hydroxide (4 % NaOH) treatment followed by washing with distilled water, the (4 % NaOH) treatment followed by washing with methanol, the (4% NaOH +10 % NaOCl) treatment and the alkali (2%NaOH or 2%Ca $(OH)_2$) treatment were decreased significantly the phorbol esters content by (38.21 to 90.81 %) and (42.58 to 95.87 %) with non-defatted whole and kernel seeds, respectively, as well as (40.57 to 93.87 %) and (43.69 to 96.44 %) with defatted whole and kernel seeds, respectively. So effects of some physical and chemical treatments were successful detoxification of phorbol esters content.

Keywords: *Jatropha curcas*, Phorbol esters, Detoxification, Physical treatments, Chemical treatments.

INTRODUCTION

Jatropha curcas (Physic Nut) a tropical plant belonging family of *Euphorbiaceae*, is a drought-resistant multipurpose small tree of significant economic importance because of its several industrial and medicinal uses (Makkar *et al*., 2008). This plant thrives on degraded and poor stony soils. The seed kernels are known to contain 55-60% oil, which can be used as fuel directly or as a substitute to diesel in the transesterified form. The oil is also used for making candles, soap, lubricants and varnishes and is used for illumination. However, seeds of *Jatropha curcas* contain highly toxic lipo-soluble but thermo-stable co-carcinogenic phorbol esters (PEs) (Goel *et al.,* 2007 and Chivandi *et al*., 2004).

Jatropha curcas (physic nut or purging nut) the new cultivated and promising crop is convcient to adapt in Egypt for increasing the local planted production (MSEA, 2008). The primary use of *J. curcas* seeds in Egypt is for oil extraction which is a good alternative to biofuel, and has proven success used either independently or by mixing the diesel to operate farm machinery, household lighting, in Soap and Candles (El-Gamassy, 2008).

Growers of *Jatropha* are increasingly demanding seeds in Egypt for the production of biofuel. In 2004–05, the area planted with *J. curcas* was about 238 feddan, increased seven times to about 1666 feddan in 2007. The rate of increase is 175%, which is really very high (MSEA, 2008).

In Egypt, about 155.6 feddan on wastewater treatment in Luxor, Ismailia, Suez and Giza. This is grown in feddan between 350 to 500 saplings, and seed production range between 1.5 to 12 tons per feddan or the yield per feddan is up to 5 tons seed given about 1.85 tons of oil in the year (El- Gamassy, 2008).

Phorbol esters (phorbol-12-myristate 13-acetate) have been identified as the major toxic principle in Jatropha (Makkar *et al*., 1997; Becker and Makkar,1998). Phorbol esters are bioactive diterpene derivatives that have a multitude of effects in cells (Goel *et al*., 2007), but else phorpol ester depend on the soil and climatic conditions. A part from these phorbol esters that are present at high levels in the kernels have been identified as the main toxic agent responsible for toxicity (Makkar *et al*., 1998).

The phorbol ester and their different derivatives are reported to be potent tumor promoters. In addition to this effect these induce a remarkable diversity of other biological effects at exceptionally low concentration. These are responsible for skin irritant effects and tumor promotion because they stimulate protein kinase C (PKC), which is involved in signal transduction and developmental processes of most of the cells and tissues, producing a variety of biological effects in a wide range of organisms (Goel *et al*., 2007).

The biological effects of these compounds in addition to tumour promotion, bring about a wide range of biochemical and cellular effects, alter cell morphology, serve as lymphocyte mitogens and induce platelet aggregation (Blumberg, 1981). The whole seed as well as dehulled seed meal are reported to be highly toxic in animal studies (Adam and Magzoub, 1975).

The phorbol esters (PEs) have to be removed or lowered to levels that do not elicit a toxic response from animals for the *Jatropha curcas* seed meal to be used as an ingredient in livestock feeds. Makkar and Becker (1997a) report that phorbol esters to be highly soluble in ethanol giving some possibility of detoxification of the meal. Therefore, various chemical and physical treatments have been evaluated to extract or inactivate phorbol esters (Goel *et al*., 2007).

It is not possible to destroy phorbol esters by heat treatment because they are heat stable and can withstand roasting temperature as high as 160 ºC for 30 min. however, it is possible to reduce its concentration in the meal by chemical treatments (Makkar and Becker, 1997a, b). After removing the toxic and heat-stable factors through solvent extraction, using 92 % methanol, the extracted meal was found to be non-toxic to rats (Makkar and Becker, 1997 a). The heat treatment in combination with the chemical treatment of sodium hydroxide and sodium hypochlorite has also been reported to decrease the phorbol ester level in Jatropha seed meal to 75% (Hass and Mittelbach 2000).

Utilization of *Jatropha curcas* flour after detoxification of phorbol ester in mixture of semolina for enriched Spagetti in order to increase the protein

content (Flores- Miranda *et al*., 2008); fortify wheat flour for preparation of bread (Guemes *et al*., 2008). While, Lopwz *et al.* (2008) studied the evaluation of the effects of *Jatropha curcas* flour on the quality of cookies.

In spite of the food- related uses of the *Jatropha curcas*, information on their toxic components (phorbol esters) in *Jatropha curcas* seeds are scantly in the literature. The objective of the present study was to evaluate the effect of some various processing treatments included some physical and chemical treatments on detoxification of phorbol esters of Egyptian *Jatropha curcas* seeds can be used in food industry. This study could provide some basic information, which would help determine in an application, for *Jatropha curcas* flours in food products.

MATERIALS AND METHODS

Materials:

Jatropha species (*Jatropha curcas L.)* were purchased from Luxor city, Luxor governorate, Egypt that harvested at April, 2009. The sample was cleaned manually to remove all foreign materials such as dust, dirt, small branches and immature seeds. The cleaned and graded seeds were de--hulled to gain access to a cream-coloured endosperm, which is the sample material. The portions of sample materials were blended to powder (0.5 mm) form with a high-speed blender (Braun KMM 30 mill, type 3045, CombiMax, Germany), then stored in an air tight polyethylene bags and kept in a refrigerator prior to analysis.

Chemicals and reagents

All used chemicals and reagents (sodium hydrogen carbonate, sodium hydroxide, sodium hypochlorite, methanol, ethanol, diethyl ether, dichloromethane and tetrahydrofuran) were purchased from Sigma chemical Co. (St. Louis, Mo, USA). The used water was distilled using water distillation apparatus.

Standard Phorbol esters (phorbol-12-myristate 13-acetate) purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Methods:

Physical treatments:

1) **Soaking:** Seeds were soaked in distilled water at ratio of 1:10 (w/v) at room temperature (25 ± 2°C) for 12 h, then dried in a hot air oven at 40 ºC to a constant weight. The samples were milled in a Braun (KMM 30) mill to pass through a 0.5 mm sieve and stored in polyethylene bags until required for further analysis.
2) **Germination:** The seeds were germinated at room temperatures (25 ± 2 °C) for 5 days by keeping them in trays lined with wet filter paper. The germinated seeds were dried in a hot air oven at 40 ºC to a constant weight. The samples were milled in a Braun (KMM 30) mill to pass through a 0.5 mm sieve and stored in plastic bags until required for further analysis.
3) **Roasting:** The seeds were generally roasted on trays at 160 ºC/30 min according to the method of (Yanez *et al*., 1986)**.** The samples were milled

in a Braun (KMM 30) mill to pass through a 0.5 mm sieve and stored in plastic bags until required for further analysis.

Chemical treatments:

The whole seeds and kernel were divided into eight equal experiments (500 g of each):

- The first experiment not treated as control.
- The second experiment was treated with 0.07% $NaHCO_3$ solution in the ratio of 1:5 (w/v) and immediately autoclaved at 121ºC for 25 min. The samples were dried in hot air oven at 40 ºC.
- Experiment third was extracted with 90 % ethanol for 2 h. at room temperature (25 ± 2°C) with constant stirring. The sample to solvent ratio was 1:10 (w/v). The solvent was removed by filtration and the residue was dried in hot air oven at 40 ºC.
- The four experiment of sample, after treatment similar to experiment with portion (3) was air-dried, mixed with 0.07% $NaHCO_3$ solution in the ratio of 1:5 (w/v) and subjected to autoclaving at121ºC for 25 min and the residual was dried in hot air oven at 40 ºC.
- In experiment five, the seeds were weight into 1000 ml beakers, followed by the addition 4 % NaOH solution to form a paste. The paste was heat treated (autoclaving at 121ºC for 25 min), then dried by hot air oven at 40 ºC. The dried paste was grounded using a simple laboratory mill to give the sample. Consequently, the grounded sample was washed with distilled water three times, prior to milling.
- The sixth experiment treated sample by NaOH similar to that mentioned above in treatment (5). The NaOH treated sample was washed four times with water instead of 92 % methanol that was used as in treatment (5).
- In experiment seven, the seeds was weighed into a 1000 ml capacity beaker and mixed with 4 % sodium hydroxide (NaOH) to form a paste. This was followed by the intermittent addition of 30 ml sodium hypochlorite (NaOCl) to the paste using a variable microliter pipette. In between the addition of NaOCl, the paste was thoroughly mixed to bring the moisture content to approximately 66 %. Consequently the beaker was covered with aluminum foil, placed inside autoclavable disposable bags and autoclaved at 121 °C for 25 min. the beaker content was allowed to cool, dried in hot air oven at 40 ºC. The dry paste was ground using a simple laboratory mill to give the sample.
- The eight experiment, the seeds Jatropha was treated with aqueous solution of either 2 % NaOH or 2 % Ca $(OH)_2$. Alkali was added in a ratio of 1:1 (w/v) mixed well till it becomes a thick paste, covered with aluminium foil and kept for 30 min. at room temperature. The material was autoclaved at 121°C for 25 min. The samples were dispersed in water in a ratio 1:5 (w/v) and kept for 1h and filtered in cheesecloth to remove excess alkali and soluble tannins. The residue was dried at 40 ºC and powdered to pass through 60-mesh sieve for further analysis.

Sample preparation:

The whole and kernel seeds were grounded, using a mechanical grinder (Braun KMM 30 mill). The whole seeds and kernel seeds were

divided into two portions. The first portion not defatted and the second portion defatted in soxhlet apparatus, using diethyl ether (boiling point of 40-60 ºC), for 16 h (fat <1%). The defatted seeds were air dried at room temperature (25 ± 2°C) and stored in a separate plastic container at 4 ºC until required for further analysis.

Extraction and estimation of phorbol esters by HPLC:

The phorbol ester concentration was estimated in the untreated and treated samples. Five seeds were weighed, ground with a small amount of sand using a pestle and mortar and then 20 ml dichloromethane was added. The mixture was again ground for about 5 min with the mortar. The material was allowed to settle and the liquid phase was filtered. The residues on the filter paper and in the pestle were pooled using about 20 ml dichloromethane and then ground for about 5 min using the mortar. The liquid phase was again collected. The extraction procedure was reported three more times and the filtrate from all five extractions were pooled. The filtrate was dried under vacuum at 40 ºC. The dried residue was dissolved in 5 ml tetrahydrofuran, passed through a 0.2 µm glass filter and injected (20 µl) into the HPLC (Makkar *et al*., 1997). A waters HPLC system consisting of a 600 controller pump, a temperature control Module, a tunable absorbance detector water 486. Chromatographic separation was achieved on phenomonex (Luna 5 µ C18 (2) column (250 x 4.6 mm). The separation was performed at room temperature (23 ºC) and the flow rate was 1.3 ml min^{-1}. The phorbolesters peaks were identified and integrated at 280 nm. The results are expressed as equivalent to a standard, phorbol-12-myristate 13- acetate, which appeared between 34 and 36 min (Fig. 1).

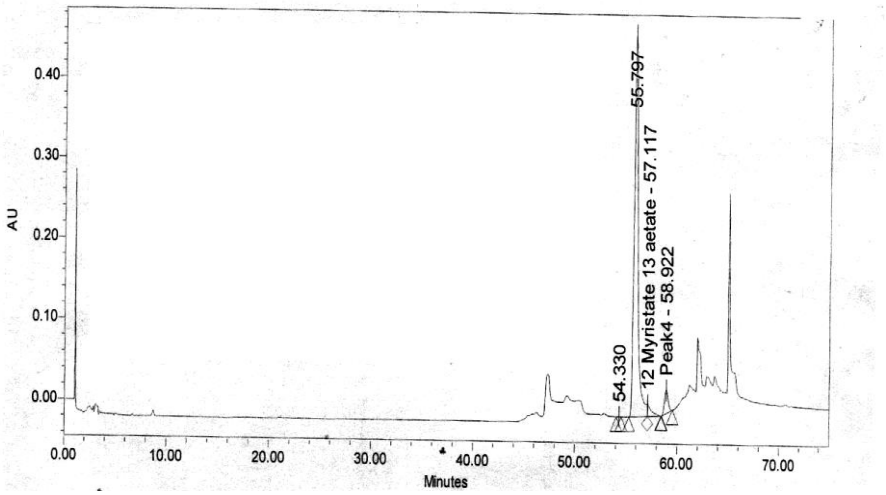

Fig. 1: HPLC Chromatogram of phorbol esters standard (phorbol-12-myristate 13- acetate)

Statistical analysis:

Obtained data were statistically analyzed by analysis of variance and least significant difference (L.S.D.) at 0.05 levels according to the method described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Phorbol esters content in raw non-defatted and defatted *Jatropha curcas* seeds:

Phorbol esters content in whole and kernel of *Jatropha curcas* seeds are shown in Table 1. Data indicate that whole and kernel seeds contained 27.32 and 47.21 mg/kg phorbol esters, respectively in raw (non-defatted) seed. With meal (defatted seed) phorbol esters ranged between 24.45 and 43.53 mg/kg. These results confirmed by analysis of variance highly significant differences were ($P < 0.05$) was observed in the contents of phorbol esters in kernel seeds than those whole seeds.

Table 1: Phorbol esters content in non-defatted and defatted raw *Jatropha curcas* seeds

Samples	Phorbol esters content (mg/kg dry weight basis)	
	Whole seeds	Kernel seeds
Raw (Non-defatted)	27.32^{b}±1.91	47.21^{a}±2.21
Defatted (Meal)	24.45^{b}± 0.02	43.53^{a} ± 0.03

-All values are means of triplicate determinations ± standard deviation (SD). - Means within row with different letters are significantly different ($P < 0.05$)

Similar results reported by Chivandi *et al*. (2004) who showed that phorbol esters (PEs) were present in high concentrations in raw *Jatropha curcas* kernels indicating that each of the processing methods managed to remove varying concentrations of the toxic phorbol esters.

Effect of different physical treatments on phorbol esters content:

The results given in (Tables 2 & 3 and Fig. 2 & 3) showed the effect of soaking, germination and roasting treatments on detoxification of phorbol esters of *Jatropha curcas* seeds. Data proved that phorbol esters content of non-defatted whole Jatropha and kernel seeds were 15.41 and 31.14 mg/kg, respectively (Table 2 and Fig.2). Data showed that phorbol esters content decreased by 43.59 % and 43.04 % in whole and kernel seeds, respectively after soaking.

Phorbol esters content of defatted Jatropha and kernel seeds were 13.17 and 27.78 mg/kg, respectively after soaking (Table 3 and Fig.3). These levels indicate that phorbol esters content decreased by 46.13 % and 36.18 % in whole and kernel seeds, respectively after soaking compared with untreated samples.

The effect of germination treatment on the phorbol esters level was 45.89 mg/kg of non-defatted kernel seeds compared with control (Table 2 and Fig.2), it could be noticed that the phorbol esters level is did not significantly affect by the previous treatment in kernel seeds. However, the phorbol esters level was 42.25 mg/kg of defatted kernel seeds, so. It could be noticed that the phorbol esters level is not affected significantly by the previous treatment in kernel seeds (Table 3 and Fig. 3).

Table 2: Effect of physical treatments of non-defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

Treatments	Non-defatted whole seeds		Non-defatted kernel Seeds	
	Content (mg/ kg)	Detoxification %	Content (mg/kg)	Detoxification %
Control (untreated)	27.32^{a}±1.91	-	47.21^{a}±2.1	-
Soaking	15.41^{c}± 3.03	43.59	31.14^{d}±2.0	43.04
Germination	-	-	45.89^{c}±2.0	2.80
Roasting	26.97^{b}±2.47	1.28	46.15^{b}±2.0	2.25
LSD at 5 %	0.04	-	0.04	-

-All values are means of triplicate determinations ± standard deviation (SD). - Means within columns with different letters are significantly different (P<0.05).

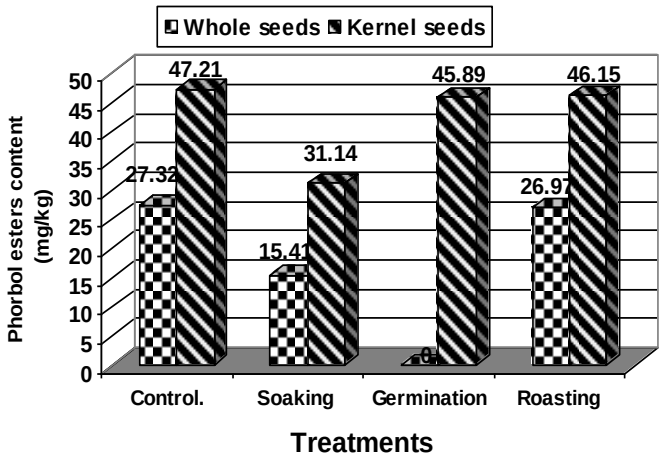

Fig 2: Effect of physical treatments of non-defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

The present investigation revealed that roasting did not significantly affect on phorbol esters levels. Phorbol esters content, it's not significantly affect on non-defatted whole Jatropha and kernel seeds which phorbol esters content were 26.97 and 46.15 mg/kg, respectively (Table 2 and Fig.2). However, phorbol esters content of defatted whole Jatropha and kernel seeds was 23.98 and 42.68 mg/kg, respectively (Table 3 and Fig. 3).

Table 3: Effect of physical treatments of defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

Treatments	Defatted whole seeds		Defatted kernel seeds	
	Content (mg/ kg)	Detoxification %	Content (mg/kg)	Detoxification %
Control (untreated)	24.45^{a}± 0.02	-	43.53^{a} ± 0.03	-
Soaking	13.17^{b}±0.02	46.13	27.78^{b} ±1.9	36.18
Germination	-	-	42.25^{a} ± 2.21	2.94
Roasting	23.98^{a} ±1.91	1.92	42.68^{a} ±0.02	1.95
LSD at 5 %	1.63	-	1.88	-

-All values are means of triplicate determinations ± standard deviation (SD).
- Means within columns with different letters are significantly different (P< 0.05).

These results confirmed by analysis of various. Data proved that significant differences were observed with soaking and germination and insignificant differences with roasting in non-defatted whole seeds and kernel seeds. However, in defatted whole seeds and defatted kernel seeds, insignificant differences were observed between soaking, germination and roasting.

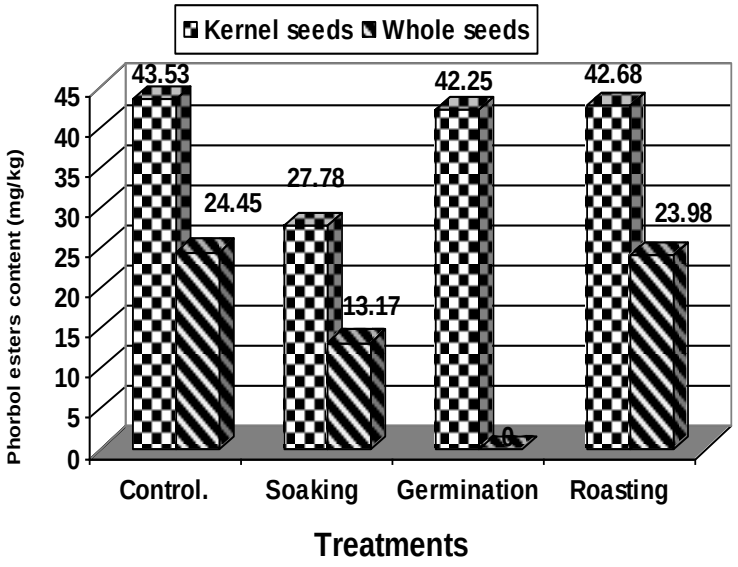

Fig. 3: Effect of physical treatments of defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

These results are in agreement with those reported by Makkar and Becker (1997a, b). They found that it is not possible to destroy phorbol esters by heat treatment because they are heat stable and can withstand roasting temperature as high as 160 °C for 30 min. Also, Aregheore *et al*. (2003) reported that the heat treatment alone has not been able to decrease the concentration of phorbol esters.

Effect of different chemical treatments on phorbol esters content:

Data in Tables (4 and 5) and Figs (4 and 5) indicate the effect of various chemical treatments on detoxification of phorbol esters of *Jatropha carcus* seeds.

Table 4: Effect of chemical treatments of non-defatted *Jatropha curcas* on the detoxification of phorbol esters (on dry weight basis)

Treatments	Non-defatted whole seeds		Non-defatted kernel seeds	
	Content (mg/ kg)	Detoxification %	Content (mg/kg)	Detoxification %
Control (untreated)	27.32^{a}± 1.91	-	47.21^{a}±2.21	-
0.07 %$NaHCO_3$/ 121ºC / 25 min.	7.35^{d} ±0.02	73.09	10.97^{d}±1.01	76.76
90 % ethanol /2 h	2.77^{f}±0.01	89.86	2.98^{f}±0.01	93.69
90 % ethanol /2 h followed by 0.07% $NaHCO_3$/121ºC / 25 min.	2.51^{g}±0.01	90.81	1.95^{g}±0.01	95.87
4 % NaOH followed by washing with distilled water	8.44^{c}±0.02	69.11	13.22^{c}±1.99	71.99
4 % NaOH followed by washing with 92 % methanol	16.88^{b}± 1.01	38.21	27.11^{b}±1.0	42.58
4 % NaOH + 10 %NaOCl	16.88^{b} ±0.02	70.06	13.21^{c} ±1.0	72.02
2 % NaOH +2 % Ca $(OH)_2$.	4.71^{e} ±0.01	82.76	7.45^{e}±0.02	84.22
L.S.D. at 5 %	2.87		2.90	-

-All values are means of triplicate determinations ± standard deviation (SD).
- Means within columns with different letters are significantly different (P<0.05).

Table 5:Effect of chemical treatments of defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

Treatments	Defatted whole seeds		Defatted kernel seeds	
	Content (mg/ kg)	Detoxification %	Content (mg/kg)	Detoxification %
Control (untreated)	24.45^{a}± 0.99	-	43.53^{a} ±1.0	-
0.07 %$NaHCO_3$/ 121ºC / 25 min.	6.12^{c}±0.99	74.97	9.75^{c}±1.0	77.60
90 % ethanol /2 h	2.24^{e}±0.99	90.84	2.59^{e}±0.99	94.05
90 % ethanol /2 h followed by 0.07% $NaHCO_3$/121ºC / 25 min.	1.50^{e} ± 0.0	93.87	1.55^{e} ± 0.91	96.44
4 % NaOH followed by washing with distilled water	6.99^{c}±0.58	71.41	11.35^{c}±0.99	73.93
4 % NaOH followed by washing with 92 % methanol	14.53^{b}±0.99	40.57	24.51^{b}±1.0	43.69
4 % NaOH + 10 %NaOCl	7.02^{c} ±0.99	71.29	11.28^{c}±1.0	74.09
2 % NaOH +2 % Ca $(OH)_2$.	3.96^{d} ± 0.02	83.80	5.81^{d}±0.02	86.65
L.S.D. at 5 %	1.54	-	1.62	-

-All values are means of triplicate determinations ± standard deviation (SD).
- Means within columns with different letters are significantly different (P <0.05).

Effect of $NaHCO_3$ treatment followed by autoclaved at 121ºC for 25 min:

The results given in (Table 4 and Fig. 4), indicate that, phorbol esters content were10.97 and 7.35 mg/kg in non-defatted kernel and whole Jatropha seed, respectively. This treatment detoxificated the phorbol esters content by76.76 % and 73.09 % of kernel and whole seeds, respectively. This treatment was slightly affected significantly on the phorbol esters content.

On the other hand, the results showed that, the phorbol esters levels were 6.12 and 9.75 mg/kg in defatted whole and kernel seeds, respectively (Table 5 and Fig. 5). This treatment detoxificated the phorbol esters content by 77.60 % and 74.97 % of defatted kernel and whole Jatropha seed, respectively. These results are agreement with those reported by Herrera et al., (2006) who reported that $NaHCO_3$ treatment decreased the phorbol esters content by 75 .3%.

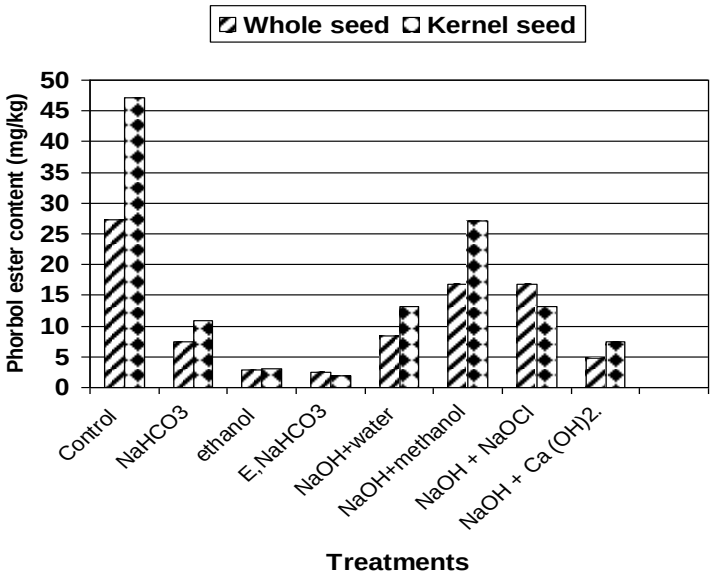

Fig. 4: Effect of chemical treatments of non-defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

Effect of ethanol for 2 h treatment:

The effects of (ethanol 90 % for 2 h) treatment on detoxification the phorbol esters content in non-defatted kernel and whole seeds are presented in (Table 4 and Fig. 4). The results showed that the phorbol esters levels were 2.98 and 2.77 mg/kg of non-defatted kernel and whole seeds, respectively. So this treatment was successful in detoxification the phorbol esters content by93.69 % and 89.86 % of non-defatted kernel and whole seeds, respectively.

On the other hand, the results given in Table 5 and Fig. 5 showed that the phorbol esters content were 2.24 and 2.59 mg/kg of defatted whole and kernel seeds, respectively. Our treatment (ethanol 90 % for 2 h) was successful in detoxification the phorbol esters content by 94.05 % and 90.84 % of defatted kernel and whole seeds, respectively. These results confirmed by analysis of variance. Highly significant differences were observed with this treatment compared with the untreated samples, may be because of Phorbol ester was highly soluble in ethanol which giving some possibility of detoxification of the meal as reported by Makkar and Becker, 1997b. The

authors observed that used, aqueous ethanol treatment seems to be promising as it removed phorbol esters by about 93 %

Phorbol esters are more soluble in alcohols because phorbol esters are associated with nonpolar lipids in the kernel tissue. These molecules cannot be effectively extracted using only polar solvents. The phorbol esters are moderately polar, as well as ethanol and methanol have major affinity for them. The use of ethanol has the advantage of it being a relatively non-toxic compared to methanol and the presence of any residues in the flour (even though not probable because of volatility) might not negatively affect animals consuming the treated flour (Herrera *et al.,* 2006).

Effect of ethanol for 2 h treatment followed by $NaHCO_3$ and autoclaved at 121ºC for 25 min:

The results given in Table 4 and Fig. 4 showed that, phorbol esters content of non-defatted kernel and whole Jatropha seeds were1.95 and 2.51 mg/kg, respectively compared with control.

In general from these results, it could be noticed that, the amount of detoxification of phorbol esters content were 95.87 and 90.81 % of non-defatted kernel and whole Jatropha seed, respectively. **So this** treatment was successful detoxification of phorbol esters in the Jatropha kernel seeds. These results are confirmed by statistical analysis. Data showed highly significant differences between the treatment samples and the untreated samples (control).

Regarding to the effects of (ethanol 90 % + $NaHCO_3$/121 º C / 25 min) treatment on detoxification of phorbol esters in defatted seeds (Table 5 and Fig. 5), the results showed that, phorbol esters content were1.50 and 1.55 mg/kg, defatted kernel and whole Jatropha seed, respectively compared with control.. These results indicate that the detoxification of phorbol esters content were 96.44 and 93.87 % of defatted kernel and whole Jatropha seeds respectively Our treatment was successful detoxification of phorbol esters in the Jatropha kernel seeds.

The phorbol esters are moderately polar, as well as ethanol and methanol have major affinity for them. The use of ethanol has the advantage of it being a relatively non-toxic compared to methanol and the presence of any residues in the flour (even though not probable because of volatility) might not negatively affect animals consuming the treated flour (Herrera *et al*., 2006).

Effect of sodium hydroxide treatment followed by washing with distilled water:

The results given in (Table 4 and Fig. 4), proved that, phorbol esters content of non-defatted kernel and whole Jatropha seed were 13.22 and 8.44 mg/kg, respectively. This treatment detoxificated the phorbol esters content by71.99 % and 69.11% of non- defatted, respectively.

Regarding to defatted seeds, (Table 5 and Fig.5) data showed that the content of defatted whole Jatropha and kernel seeds were 6.99 and 11.35 mg/kg, respectively compared with control. This treatment detoxificated the phorbol esters content by73.93 % and 71.41% of defatted kernel and whole

Jatropha seed, respectively. Statistical analysis proved these results, which highly significant differences were observed with non-defatted and defatted samples compared with the untreated samples.

These results are in agreement with those reported by Hass and Mittelbach (2000), who reported that the heat treatment in combination with the chemical treatment of sodium hydroxide had decreased the phorbol ester level in Jatropha to 75%. Also, Rakshit and Bhagya, (2007) reported that the Alkali treatment (NaOH-treated) was the effective method in reducing the phorbol esters and possibility of destroying phorbol esters up to 90% by treating defatted meal with chemicals.

Effect of sodium hydroxide treatment followed by washing with methanol:

Data in (Table 4 and Fig. 4) showed that the effect of sodium hydroxide (4 % solution) treatment followed by washing with methanol on detoxification of phorbol esters in non-defatted kernel and whole seeds were 27.11 and 16.88 mg/kg, respectively. This treatment detoxificated the phorbol esters content by 42.58 % and 38.21 % of non-defatted kernel and whole seed, respectively.

The effect of this treatment on detoxification of phorbol esters content in defatted seeds (Table 5 and Fig.5) results showed that, phorbol esters levels were 14.53 and 24.51mg/kg of defatted kernel and whole Jatropha seed, respectively. This treatment detoxificated the phorbol esters content by 43.69 % and 40.57 % of defatted kernel and whole Jatropha seed, respectively. Analysis of variance revealed highly significant differences between treatment samples and untreated samples.

Effect of sodium hydroxide and sodium hypochlorite treatment:

The effects of 4% NaOH and 10 % NaOCl treatment on detoxification the phorbol esters content from defatted seeds are presented in (Table 4 and Fig. 4). The results showed that the phorbol esters levels were 13.21 and 16.88 mg/kg of kernel and whole seed, respectively. This treatment detoxificated the phorbol esters content by 72.02 % and 70.06 % of non-defatted kernel and whole Jatropha seeds, respectively. With defatted seeds the results (Table 5 and Fig. 5) showed that phorbol esters content of whole and kernel seeds were 7.02 and 11.28 mg/kg, respectively. This treatment detoxificated the phorbol esters content by 74.09 % and 71.29 % of defatted kernel and whole Jatropha seed, respectively. Statistical analysis indicated highly significant differences effects of this treatment on phorbol esters contents.

Makkar and Becker (1997a, b) reported that heat alone cannot inactivate phorbol esters. However, with the additional chemical treatments of sodium hydroxide and sodium hypochlorite has been reported to decrease the phorbol esters level in Jatropha seeds to 75 % (Hass and Mittelbach**,** 2000).

Effect of sodium hydroxide and calcium hydroxide treatment:

The results given in (Table 4 and Fig. 4) showed the effect of alkali 2%NaOH or 2%Ca $(OH)_2$ treatment on detoxification of phorbol esters in non-defatted seeds. The results showed that, phorbol esters content of kernel and whole seed were 7.45 and 4.71 mg/kg, respectively. This treatment detoxification the phorbol esters content by 84.22 % and 82.76 of non-defatted kernel seeds and whole Jatropha seed, respectively.

This treatment with defatted seeds (Table 5 and Fig.5) showed that, phorbol esters content of whole and kernel Jatropha seeds were 3.96 and 5.81 mg/kg, respectively. This treatment detoxification the phorbol esters content by 86.65 % and 83.80 of defatted kernel seeds and whole seed, respectively. Highly significant differences were observed with this treatment on decreasing the phorbol esters contents comparing with the untreated samples.

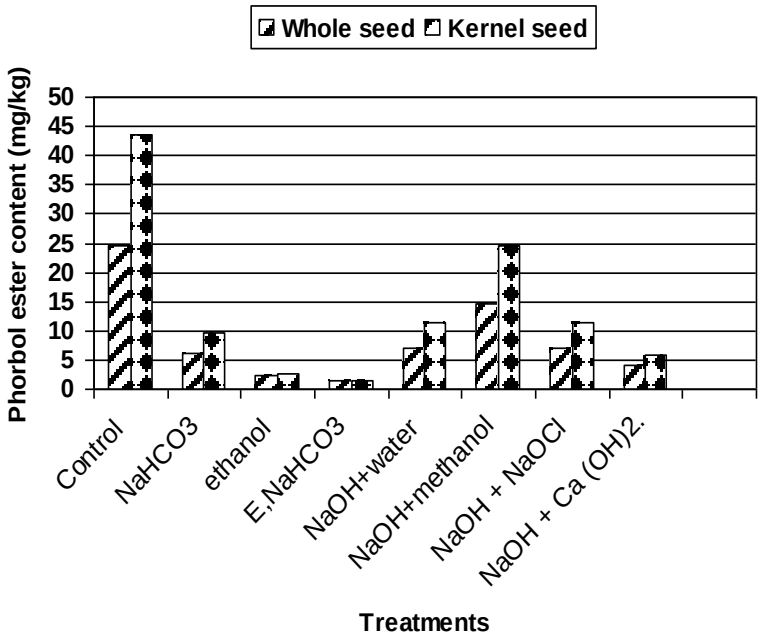

Fig . 5: Effect of chemical treatments of defatted *Jatropha curcas* seeds on the detoxification of phorbol esters (on dry weight basis)

These results are agreement with those reported by Rakshit and Bhagya (2007) who found that the Ca $(OH)_2$ and NaOH at 2% level were better in removing the phorbol ester from Jatropha seeds. Alkali and heat treatments to deactivate the phorbol ester content was reduced up to 89% in Jatropha seeds. On the other hand, Aregheore *et al.* (2003) have reported that chemical treatment in addition to heat is necessary to bring down the concentration of phorbol ester content. It can be calculated that about 72 % of the total phorbol Esters get extracted with the oil using petroleum ether (bp

40-60 ºC) and the remaining is still present in the degreased meal which is almost free of oil (Makkar and Becker, 1997b)**.** In addition, the toxic substance phorbol ester present in the oil in high concentration kills the vector snail of schistosomiasis – the second most serious human disease after malaria in the tropics (Becker, 2009). Absence of oil in the seed meal and presence of considerable amount of phorbol esters in the seed meal suggest that phorbol esters are tightly bound to the matrix of the seed meal (Makkar *et al*., 2008).

Future research areas

Further investigations are currently underway for complete detoxification of *J. curcas* and to check the efficacy of detoxification in animal model which seems to be a promising crop of future both in terms of nutritional as well as industrial applications.

1-The acceptance of raw seeds by humans and animals and levels of toxic phorbol esters in raw and roasted seeds as sold on the market.

2-Development of economically viable techniques for detoxification of chemical treatments of Jatropha meal.

Conclusion

The effects of some physical and chemical treatments were successful detoxification of phorbol esters content. These treatments included soaking in distilled water, ethanol 90 % + $NaHCO_3$, alkali treatment (2 % NaOH or 2 % $Ca(OH)^2$ and $NaHCO_3$ treatment.

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إزلة مادة الفوربول إستر السامة من بذور نبات الجاتروفا بواسطة بعض المعاملات التكنولوجية

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وجد أن محتوى بذور نبات الجاتروفا المزروعة فى مصر من مادة الفوربول إستر السامة فى البذور الكاملة والنواه الخام يتراوح بين ٢٤,٤٥و ٤٣,٥٣ ملليجم/ كجم على التوالى. تم دراسة إزالة مادة الفوربول إستر السامة من بذور نبات الجاتروفا بواسطة بعض المعاملات الطبيعية. حيث وجد أن النقع فى الماء أدى إلى إزلة ٤٦,١٣ % و ٣٦,١٨ % من مادة الفوربول إستر السامة فى البذور الكاملة والنواه على التوالى. بينما وجد أن معاملات الإنبات و التحميص ليس لها تأثير علىمستوى الفوربول إستر. كما تم دراسة ازلة مادة الفوربول إستر السامة بواسطة بعض المعاملات الكيميائية. حيث لوحظ أن المعاملة بواسطة(بيكربونات الصوديوم على ١٢١ م°/ لمدة ٢٥دقيقة) والمعاملة بواسطة (الإيثانول ٩٠ % لمدة ٢ ساعة) و(الإيثانول ٩٠% + بيكربونات الصوديوم /١٢١ م°/ لمدة ٢٥دقيقة) والمعاملة بواسطة (هيدروكسيد الصوديوم ٤%) يليها الغسيل بالماء المقطر والمعاملة بواسطة (هيدروكسيد الصوديوم ٤%) يليها الغسيل بواسطة الميثانول والمعاملة بواسطة(هيدروكسيد الصوديوم ٢% + هيبوكلوريت الصوديوم ١٠%) والمعاملة بواسطة القلويات (هيدروكسيد الصوديوم ٢% + هيدروكسيد الكالسيوم ٢%). أدت إلى حدوث إنخفاض معنوىفى محتوى الفوربول إستر (٢١, ٣٨ إلى ٩٠,٨١%) و (٤٢,٥٨ إلى ٩٥,٨٧%) مع بذور الجاتروفا الكاملة والنواه غيرمنزوعة الدهن على التوالو (٤٠,٥٧ إلى ٩٣,٨٧ %) و (٤٣,٦٩ إلى ٩٦,٤٤ %) مع بذور الجاتروفا الكاملة والنواه منزوعة الدهن على التوالى. وجد أن بعض المعاملات الطبيعية و الكيميائية كانت لها تأثيرات ناجحة فى إزالة مادة الفوربول إستر السامة من بذور نبات الجاتروفا.

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