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4 TOXICITY

4.1 Phorbol Esters of *J. curcas* - Biological Activities and Potential Applications

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Abstract

Toxicity of *Jatropha curcas* seeds can be caused by several components, including saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid, and phorbol esters. Phorbol esters which activate the important cellular target protein kinase C (PKC) constitute the most active components which must be removed if oil or seed cake is being used for animal or human nutrition. *Jatropha* oil and phorbol esters exhibit insecticidal and molluscicidal activities over a wide range of organisms, suggesting their potential use in agriculture as biorational pesticides and as mollusc control agents (against water snails which transmit parasites, such as schistosomes or flukes). Phorbol esters are known tumor promotors, but are not mutagenic or carcinogenic themselves. Before using them in agriculture or health control certain precautions and toxicological studies on the fate of phorbol esters in water, soil and plants are necessary to assess the potential environmental risks.

Introduction

Jatropha curcas L. (family Euphorbiaceae), a shrub of 3 to 8 m height, originates from Central America but is presently cultivated in Central and South America, West- and South Africa, India and South-East Asia. *J. curcas* is well adapted to marginal areas with poor soils and low rainfall and is resistant to diseases and herbivores. Thus it does not compete with conventional food or feed crops for land and water and can also used as a live hedge (not browsed by livestock) [1].

J. curcas produces relatively big seeds (mean weight 0.64 ± 0.10 g). The kernels contain up to 53 % oil (range 43 - 59 %), 26 % protein (range 19 - 31 %), 5 % neutral detergent fibres (range 3.5 - 6.1 %), and 4.2 % ash (range 3.4 - 5 %). Trypsin inhibitor activity in the degreased kernels (meal) varies from 18.4 - 27.5 mg trypsin inhibited/g, saponins from 1.8 - 3.4 % as diosgenin equivalent, phytate from 6.2 - 10.1 % as phytic acid equivalent, and lectin activity from 0.85 - 6.85 using a latex agglutination test and 51.3 - 204 using a hemagglutination assay [2]. The level of phorbolesters ranged from 0.87 to 3.32 mg/g kernel [2]. Tannins, amylase inhibitor, glucosinolates and cyanogens were not detected [2].

The oil can be used as petrol for diesel motors to drive tractors or pumps. Furthermore, the oil can be exploited for the production of soap or candles [1]. Since the oil is rich in unsaturated fatty acids (C16:0 = 15.3 %, C18:0 = 6.6 %, C18:1 = 40.1, C18:2 = 35.9 %, and C18:3 = 0.2 %) it might be useful for human nutrition (if the purgative curcalonic acid has been removed). But the oil also contains up to 1 - 2 % phorbol esters (Fig. 1) which make it unpalatable and toxic if the phorbol esters are not completely removed. The oilseed cake (left after extraction of oil) is presently exploited as a fertiliser but it has potential to be used as livestock feed as it is rich in crude protein (50 - 58 % depending on the residual oil) [1].

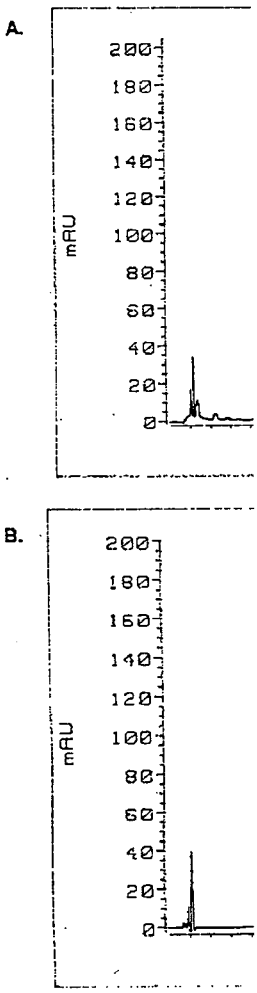

Fig. 1: Separation of phorbc
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HPLC equipment: Hewlett
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Analytical column: Reverse
Solvents: (A) 1.75 ml o-pho
Gradient: Start with 60 % A
a linear gradient to 25 % A
washed with 100 % C and
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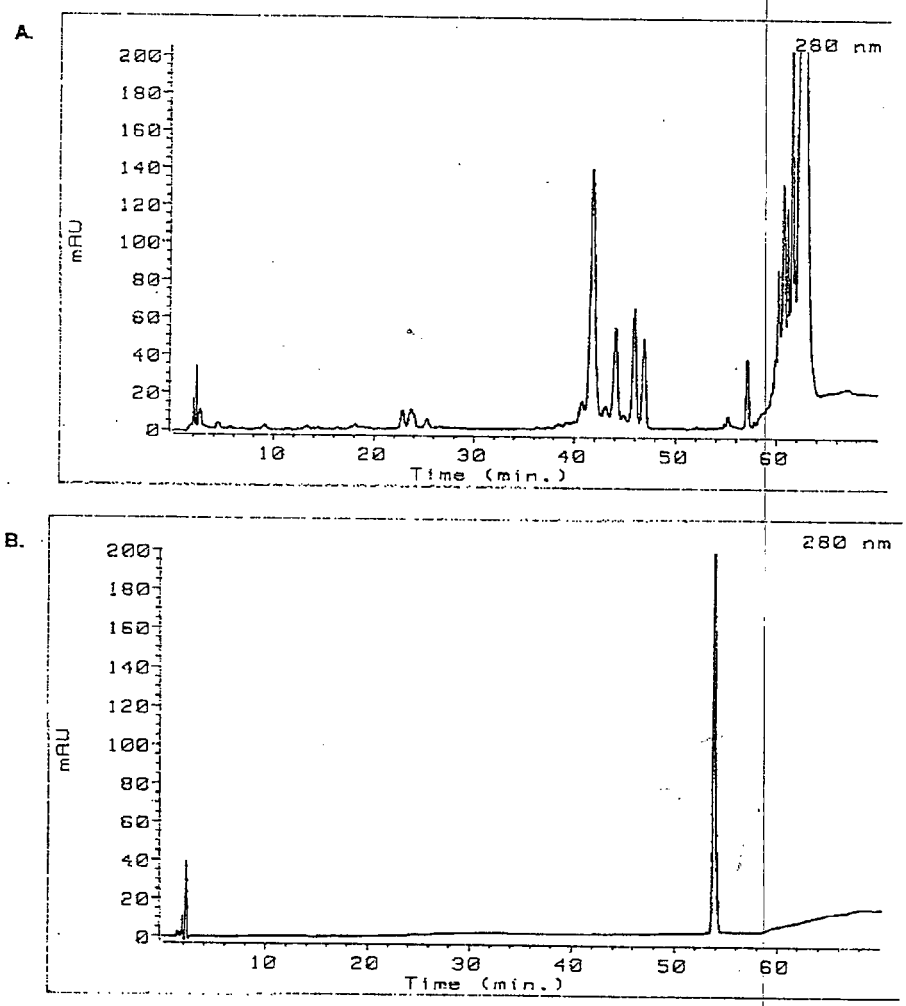

Fig. 1: Separation of phorbol esters from *J. curcas* oil by HPLC
A. HPLC profile from *Jatropha* oil; compounds which elute between 40 and 48 min are phorbol esters.
B. HPLC profile of a pure phorbol ester, phorbol-12-myristate 13-acetate (TPA), which is used as an external standard for quantification.

HPLC equipment: Hewlett Packard 1050 HPLC pump, Hewlett Packard 1040A photo diode array detector, and a Spark Holland-Basio Marathon autosampler.
Analytical column: Reversed phase C18 (LiChrospher 100, endcapped 5 µm), 250 x 4 mm I.D. (Lichrocart)
Solvents: (A) 1.75 ml o-phosphoric acid (85 %) in 1 liter distilled water, (B) acetonitrile and (C) tetrahydrofuran.
Gradient: Start with 60 % A and 40 % B; for 10 min decrease A to 50 % and increase B to 50 %; for the next 30 min a linear gradient to 25 % A and 75 % B; then increase of B to 100 % within the next 15 min. Then the column is washed with 100 % C and adjusted to the starting conditions with 60 % A and 40 % B. Separation is performed at room temperature (ca. 22°C) with a flow rate of 1.3 ml/min.

Results and Discussion

Toxicity of J. curcas

The seeds from *J. curcas* have been reported to be toxic to humans, rodents and livestock [1, 3]. Reports on the accidental intoxication of humans after ingestion of oil or seeds have appeared in Hawaii, Florida and the Philippines. The symptoms of intoxication in humans are: burning and pain in mouth and throat, vomiting, delirium, muscle shock, decrease of visual capacity and a high pulse [4]. A high mortality rate has been reported for rodents (mice, rats) and domestic animals (sheep, goats, zebu calves and chicks) when feeding *J. curcas* seeds [5 - 10]. 0.1 or 0.5 % seed meal in a diet for chicks caused growth depression, hepatonephropaties, hemorrhages and congestion [8]. When given to rats orally, oil exhibited an LD_{50} with 6 ml/kg body weight and an LD_{100} with 9 or 13 ml/kg body weight; symptoms were diarrhoea, gastro-intestinal inflammation, and hemorrhagic eyes [3].

In Nigeria and Burkina Faso seeds and seed oil are added to *Strophanthus* arrow poison. In Gabon seeds are grated with palm oil to kill rats [9]. The Shamba of the Usambara region of Tanzania used *Jatropha* seeds as an ordeal poison [3].

Toxicity of *J. curcas* seeds could be caused by several components, including saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid (which is a stronger purgative than ricinolic acid) and phorbol esters [2, 4, 9, 11, 12]. A variety from Mexico, which was non-toxic in feeding trials, still contained levels of saponins, lectins, protease inhibitors similar to those in toxic varieties, but was almost free from phorbol esters. Therefore, we have concluded that phorbol esters contribute predominantly to the toxicity of *Jatropha* seeds, seed cake and oil [2].

J. curcas has also been used in Traditional Medicine as a cathartic purgative, and to treat skin ailments, dropsy, gout, paralysis, and rheumatism [9]; often extracts from leaves and roots are preferred to those from seeds [9].

Biological activities of phorbol esters (PE)

Phorbolesters have been found to be responsible for skin-irritant effects and tumor promotion since they stimulate protein kinase C (PKC) [11, 12]. The natural substrate of PKC is diacylglycerate (DAG) whose structure is mimicked by phorbolesters. Since PKC is involved in signal transduction and developmental processes of most cells and tissues, a variety of biological effects should be expected over a wide range of organisms.

Insecticidal activities of oil containing phorbol esters or of concentrated phorbolester fractions have been recorded for *Manduca sexta* [13], *Helicoverpa armigera*, *Aphis gossypii*, *Pectinophora gossypiella*, *Empoasca biguttula*, *Callosobruchus chinensis*, *Sitophilus zeamays* [14], *Phthorimaea operculella* [15], *Culex* spec. (M. Wink, unpublished), *Sesamia calamistis*, *Busseola fusca* [16], *Periplaneta americana*, *Blatella germanica* and *Oncopeltus fasciatus* [17].

The effect of 0.1 % and 1 % oil on the survival of some insects is shown in Tab. 1 indicating that topical applications of phorbol ester containing oil have insecticidal properties over a wide range of insects. It is not a very strong activity, but it should be recalled that extracts and formulations had not been optimised for these trials.

Tab. 1: Insecticidal activity of phorbol ester containing oil of *Jatropha curcas* (in collaboration with laboratory Dr. Wachendorf; Bayer AG, Monheim)

Species	Mortality (% dead animals)			
	1 % oil		0.1 % oil	
	6 d	13 d	6 d	13 d
Phaedon cochliariae	33	50	n.d.	33
Plutella xylostella	33	60	n.d.	33
Spodoptera frugiperda	33	33	n.d.	n.d.
Mycus persicae	100	100	90	95
*Tetranychus urticae**	100	100	50	45

* this is a spider mite, not an insect

In larvae of the Tobacco horn moth (*Manduca sexta*), the effect of PE was studied in more detail: Larvae obtained an artificial diet to which different amounts of *Jatropha* oil or PE extracts were added. Larvae were weighted every day, so that the effect on the growth and development of the larvae could be compared with untreated controls. Fig. 2A shows that the addition of *Jatropha* oil at concentrations of 1 or 5 % to the diet inhibits the growth of the larvae which do not die immediately but stop growth and development. Methanolic extracts of *Jatropha* oil, which contain an enriched PE fraction show significant inhibitory effects (Fig. 2B) with concentrations higher than 250 ppm (= 0.025 %).

Substances were added to the artificial diet which was exchanged daily. 5 larvae were used for each treatment / concentration. In addition, phorbol ester containing oil and phorbol ester extracts exhibit a significant molluscidal activity against water snails, for example those which transmit parasites, such as *Biomphalaria glabrata*, and *Oncomelania hupensis* [18] thus confirming earlier reports [19]. Phorbol esters are probably responsible for this activity, since pure phorbol esters unambiguously showed this effect. When added to the water, PE (4ß-phorbol-13-decanoate) killed 100 % of the snails at concentrations of 0.001 % [18] and also the schistosomiasis causing parasite *Schistosoma mansoni* [20]. Molluscicidal activity against *Lymnaea auricularia* which transmits river fluke in the Philippines has also been recorded [21].

Potential applications of phorbol esters (PE)

Phorbol esters act as tumor promotors in mice which have been treated with a carcinogen beforehand but not in untreated animals. Therefore, PE are called co-carcinogens, although they are no carcinogens themselves (also the hormones present in the pill are co-carcinogens according to this definition).

Since PE or PE containing fractions might be used in agriculture as biorational pesticides [22] or to irradiate schistosomiasis transmitting snails, it is necessary to assess the risks of PE for the people who have to work with these compounds or for humans who come into contact with treated water or agricultural products. In the first instance precautions must be taken when handling the oil or PE containing fractions to avoid skin contact and ingestion because of skin irritance.

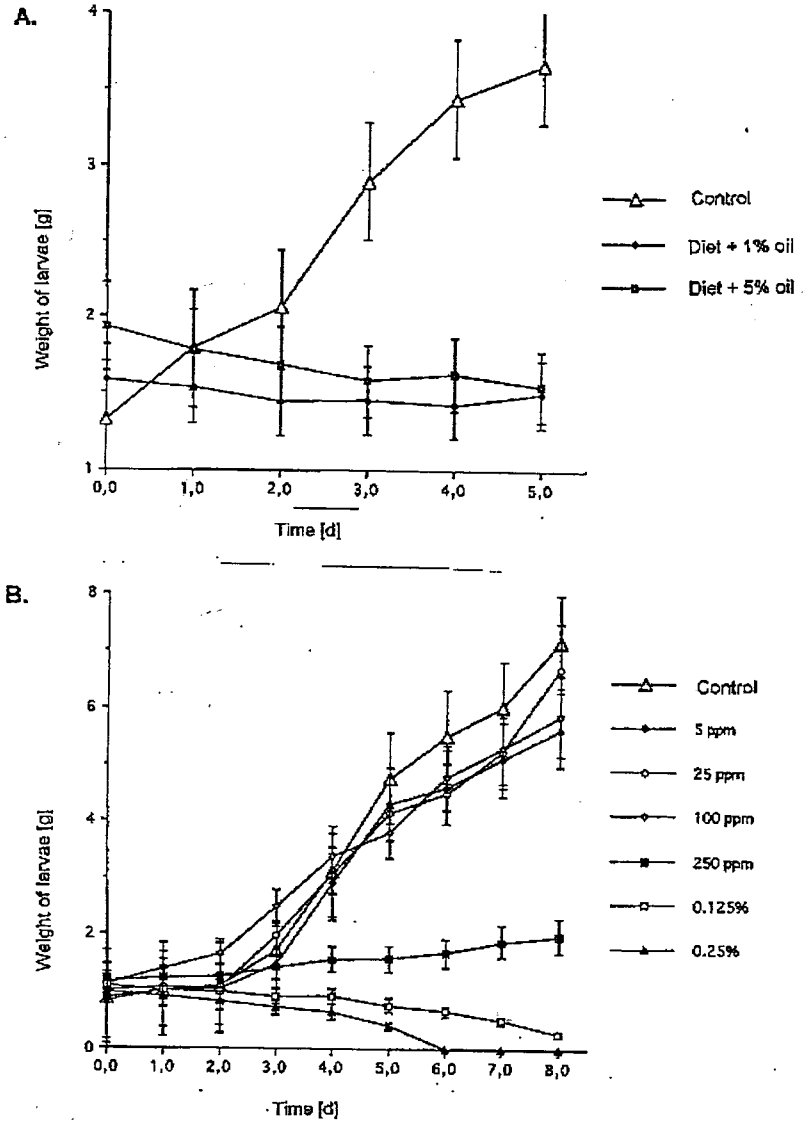

Fig. 2: Effect of *Jatropha* oil and PE fraction on the growth and development of *Manduca sexta* larvae
A. Effect of 1 or 5 % oil in the diet
B. Effect of different concentrations of the PE containing methanolic extracts from *J. curcas* oil

Secondly, any mutagenic or carcinogenic potential must be determined in detail. As a first measure into this direction, we have analysed whether *Jatropha* oil or isolated phorbolesters exhibit mutagenic effects in vertebrate cells. Human liver cells were selected as an assay system in order to obtain relevant data for human toxicology. It was assayed if single strand breakage occurs in the DNA of liver cells after treatment with *Jatropha* oil, phorbol ester extracts or a

positive mutagenic control $Na_2Cr_2O_7$. In a first set of experiments standard dilutions were chosen. Since no evidence for mutagenicity was found, higher concentrations were studied in a second set of experiments. Important is the last column which reflects the mutagenic potential: values between -10 and +10 are in the range of variation of this method. Values from 10 to 20 indicate that a very low mutagenic potential cannot be ruled out with certainty. Mutagenic compounds usually display values which are much higher than 20 even in very low concentrations (see our positive controls). In our experiments, only the PE fractions tested at high concentrations reached values around 20. Since the dosage which will be applied in agriculture or mollusc control will be several orders of magnitude lower than those used in these assays, the mutagenic risk should be very low if it exists at all. Besides DNA strand breakage, also the viability of the liver cells was analysed. As can be seen from Tab. 2 oil and PE result in a decrease of cell viability of maximally 27 % when the PE fraction was given in a 1 : 2 dilution. Further toxicological studies are needed to evaluate the fate of PE in plants, water, soil and the ecosystem.

PEs will be a side product of *Jatropha* oil production which might be exploited in tropical agriculture as a biorational pesticide or as a mollusc control agent. Since protein kinase C is such an important target, it is likely that PE could also be of use as therapeutic agents or as biochemical probes.

Tab. 2: Mutagenicity test for *Jatropha* oil and phorbol esters (in collaboration with P. Schmezer, DKFZ, Heidelberg)

Sample	concentration	vitality %	% DNA	Relative activity*
First experiment				
Control (C)	---	100	62	0
Mutagen	0.5 µmol	78	23	39
Oil	1:10	73	69	(-7)
	1:100	88	67	(-5)
	1:1000	88	69	(-7)
PE	1:10	75	47	15
	1:100	83	62	0
	1:1000	98	64	(-2)
Second experiment				
Control	---	100	82	0
Mutagen	0.5 µmol	77	53	29
Oil	1:2	73	74	8
	1:5	77	78	4
	1:10	97	71	11
PE	1:5	77	62	20
	1:10	92	61	21

*difference between control (C) and treatment

Acknowledgements

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4.2 Rumen Digesti
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Abstract

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4.2 Rumen Digestion of Raw, Roasted and Boiled Seeds of *J. curcas* from Chiapas, Mexico

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Abstract

Two rumen cannulated *Bos indicus* bulls were used to study the *in situ* digestion of DM, OM, CP and NDF of untreated (US) and heat treated: roasted (RS) and boiled (BS) seeds of *Jatropha curcas* harvested in Chiapas, Mexico. Temperature reduced the CP solubility, potential degradation and degradation rate of seeds of *Jatropha curcas*. In particular the RS may be considered as a appropriate source of escape protein in ruminant rations, however the antinutritional factors must be tested in animal growth trials. Further work is required to evaluate the *in situ* digestion of the cakes of *Jatropha curcas*.

Introduction

Jatropha curcas, a *Euphorbiaceae* native from Central America and Mexico has great potential to be used as a protein source for the feeding of ruminant livestock, however no information is available regarding the *in situ* rumen digestion of the protein contained in the seed [2]. The nutritional value of protein sources for ruminants depends on the rumen digestion of the protein (rate and extent) and on the time the protein remains in the reticulo-rumen [8, 3] also the new animal rationing models require accurate determination of the dynamic aspects of rumen nitrogen degradation and the *in situ* technique has been used [6, 10]. In the tropical areas of the world, the nutritional value (intake, chemical composition, digestibility) and the availability (dry matter production) of pastures varies with season (dry / wet) [7], therefore protein supplements are necessary to improve animal performance mainly during the (> 6 month-long) dry season (when quality and availability of the pasture decreases), however, protein supplements in Mexico have been gradually increasing their price during the last few years, and cattle producers cannot afford to buy imported protein sources (soybean, fishmeal) from overseas. The on-farm production of protein supplements locally available may increase the profitability of ruminant production in Mexico. The aim of the work reported here, was to evaluate the *in situ* rumen digestion of raw and heat treated *J. curcas* as conventionally employed by the human population before consumption in Mexico.

Materials and Methods

Mature seeds of *J. curcas* harvested in Villaflores, Chiapas (Awo"(w)ig climate) [4] were used. Two heat treatments were applied to the seeds. Whole seeds were roasted (RS) at 100°C for 10 min simulating the traditional process used before consumption in Veracruz, Mexico. After roasting the seeds were grounded. Whole seeds were grounded and boiled (BS) at 80°C for 120 min and left to stand at room temperature during another 120 min. A further treatment (US) consisted in the whole

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raw seed grounded. Samples of RS, BS and US were incubated in the rumen of cattle to assess rate and extent of digestion of *J. curcas*. Two entire male *Bos indicus* cattle with an average liveweight of 450 kg were fitted with flexible rumen cannulas (Bar Diamond Inc., Parma, Idaho, USA) with 10 cm internal diameter. Cattle were allowed to recover for at least two months after surgery. Cattle were fed freshly chopped Taiwan (*Pennisetum purpureum*) grass plus *Leucaena leucocephala ad libitum*. The nylon bag technique [9, 10] was employed to assess rate and extent of digestion of DM, OM, CP and NDF of *J. curcas* [1, 5]. Five grams of sample were weighed into nylon bags (Bar Diamond Inc. Parma, Idaho, USA) with dimensions 10 x 20 cm and 53 micron pore size. Bags were incubated by duplicate in reverse order in large lingerie bags provided with a zipper. After withdrawal from the rumen the bags were washed in a washing machine five times until the draining water was clear, after allowing the excess water to drain, the bags were introduced in a forced air-oven at 60°C during 72 h. The disappearance of the relevant component in the bag was calculated by subtracting the residual material in the bag from the material originally incubated in the rumen. Disappearance data was fitted into the exponential model $p = a + b\ (1 - \exp^{-ct})$ to estimate a: rapidly soluble fraction, b: insoluble but potentially digestible fraction, c: rate of digestion of b and t: time [9].

Results and Discussion

Tab. 1 gives the kinetics of rumen digestion of DM, OM, CP and NDF. The values for the soluble fraction of DM and OM were 39.26, 21.76 and 18.39 % and 38.65, 19.81 and 18.09 % for US, BS and RS respectively. These results suggest that heat treatment reduces the solubility of DM and OM of seeds of *J. curcas;* solubility being higher for RS. Potential degradation (a + b) of DM and OM was 66.77, 44.51 and 57.04 % and 65.75, 43.01 and 57.5 % for US, BS and RS, respectively. These results suggest that in BS the meat kernel was lost when the water was decanted and the proportion of testa in the sample was greater reducing the a + b fraction. Rates of digestion (c) of DM and OM were 0.034, 0.138 and 0.048 and 0.044, 0.127 and 0.046 for US, BS and RS, respectively. It can clearly be shown that BS had a considerably greater rate of digestion than US and RS. Figs. 1 and 2 show that DM and OM quickly disappeared within the first 12 h of incubation and then a plateau was reached which remained until 96 h, while US and RS showed different initial soluble fractions (a) but the plateaus tended to be similar (converge) at 96 h. The data so far shown seem to suggest that the digestion of the protein of *J. curcas* in the rumen of cattle is lower than conventional protein sources as soybean meal [9].

Tab. 1 shows the solubility of the protein of *J. curcas*, the values were 56.33, 39.42 and 9.53 % for US, BS and RS respectively, the data for BS and RS suggest that temperature reduces protein solubility, the effect being greatest for the RS treatment. For potential degradation of protein (a + b fraction) the values were 91.27, 80.54 and 86.57 % for US, BS and RS, respectively. This shows that at 96 h the values were similar and that the temperature reduced the solubility of the protein but not the potentially digestible fraction. For the rate of digestion (c) the values were 0.1039, 0.1056 and 0.0519 for US, BS and RS, respectively. It can be seen that the rates of protein digestion are similar for US and BS and considerably lower for RS. Fig. 3 shows that solubility of US and BS was higher than for RS and that the nitrogen disappearance for RS was lower, although at 96 h the values are similar. This suggest that RS is an alternative treatment to modify the nitrogen availability in the ruminal ecosystem and could be used as a treatment to obtain a by-pass protein depending on rumen outflow rate.

Tab. 1: Degrad

DM	
a (%)	
b (%)	
a+b (%)	
c (%)	
t (h)	
rsd	
OM	
a (%)	
b (%)	
a+b (%)	
c (%)	
t (h)	
rsd	
CP	
a (%)	
b (%)	
a+b (%)	
c (%)	
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NDF	
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b (%)	
a+b (%)	
c (%)	
t (h)	
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Tab. 1: Degradation characteristics of DM, OM, CP and NDF for *J. curcas*

	Untreated Seeds (US)	Boiled Seeds (BS)	Roasted Seeds (RS)
DM			
a (%)	39.26	21.76	18.39
b (%)	26.69	22.68	39.25
a+b (%)	65.95	44.44	57.64
c (%)	0.0344	0.1381	0.0481
t (h)	2.9	0.9	4.1
rsd	1.59	1.74	2.92
OM			
a (%)	38.65	19.98	18.09
b (%)	22.39	22.73	38.38
a+b (%)	61.04	42.71	56.47
c (%)	0.0441	0.1268	0.0464
t (h)	3.9	0.6	4.3
rsd	3.12	1.95	2.89
CP			
a (%)	56.33	39.82	9.53
b (%)	34.94	40.72	77.04
a+b (%)	91.27	80.54	86.57
c (%)	0.1039	0.1056	0.0519
t (h)	2.9	-1.3	4.8
rsd	3.23	1.36	5.95
NDF			
a (%)	32.51	8.90	24.83
b (%)	30.90	19.70	23.42
a+b (%)	63.41	28.60	48.25
c (%)	0.0109	0.0297	0.0694
t (h)	17.7	-12.7	11.8
rsd	3.40	3.66	4.96

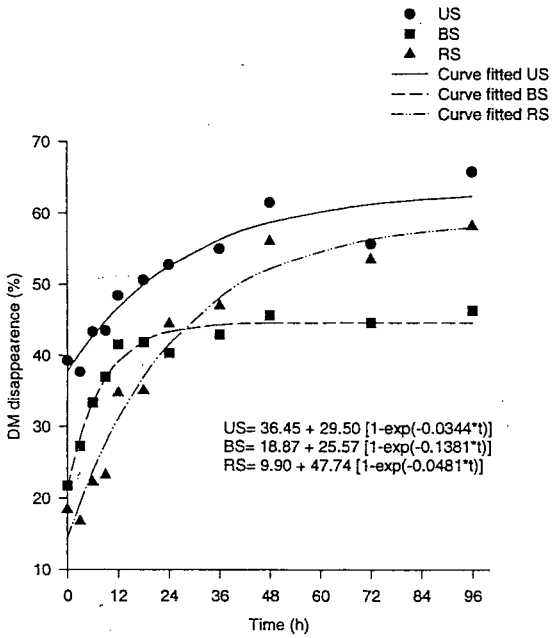

Fig. 1: DM disappearence of untreated (US), boiled (BS) and roasted (RS) seeds of *J. curcas* in situ

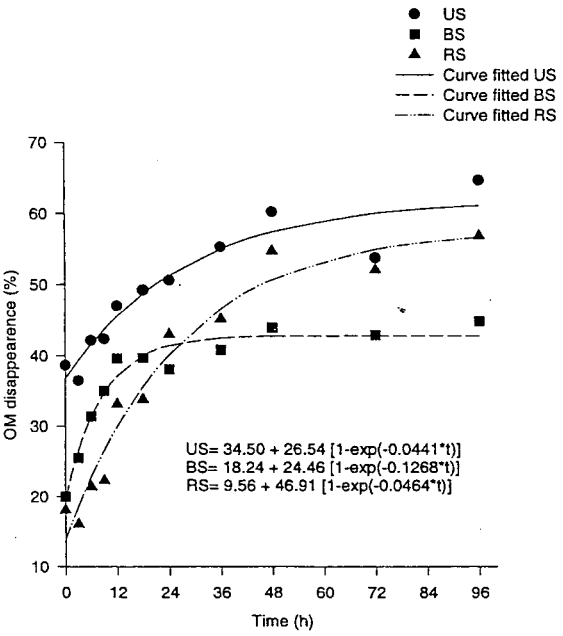

Fig. 2: OM disappearence of untreated (US), boiled (BS) and roasted (RS) seeds of *J. curcas* in situ

Fig. 3: CP disappea

Fig. 4: NDF disapp

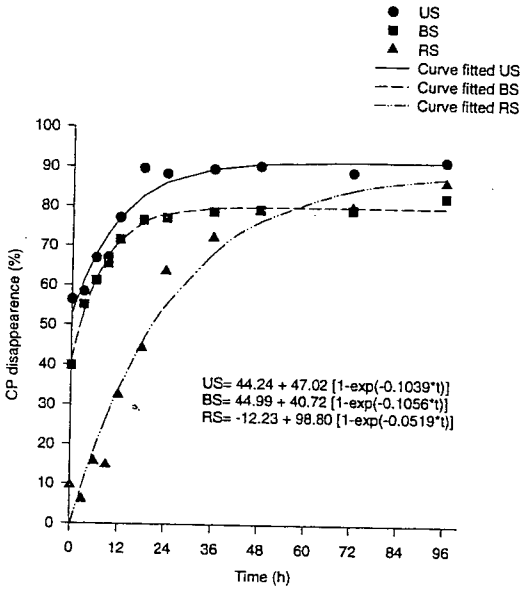

Fig. 3: CP disappearence of untreated (US), boiled (BS) and roasted (RS) seeds of *J. curcas in situ*

ls of *J. curcas* in situ

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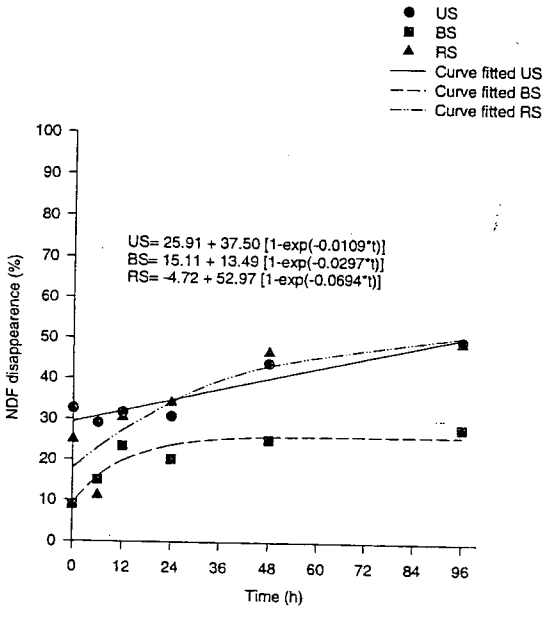

Fig. 4: NDF disappearence of untreated (US), boiled (BS) and roasted (RS) seeds of *J. curcas in situ*

eds of *J. curcas* in situ

Tab. 1 shows the results for NDF degradation, the soluble fraction and potential degradation were 32.51, 8.90 and 24.83 % and 30.90, 19.70 and 23.42 % for US, BS and RS, respectively. This suggest that the BS values are lower (than for US and RS) because there is a greater proportion of testa in the samples and the NDF disappearance was higher for US than for RS. For the rate of digestion (c) the values were 0.0109, 0.0297 and 0.0694 for US, BS and RS, respectively, suggesting that the proportion of testa in the incubated material vary and can influence the disappearance parameters. Fig. 4 shows that the disappearance of NDF in US is linear after the soluble fraction is used up, that the testa proportion between US and RS were similar and in the BS was highly undegradable. It is important to stress that the soluble fraction of NDF in BS and RS is lower than in US, probably indicating that some components in NDF may be converted into less soluble components by effect of heat.

Conclusions

Heat treatment reduced solubility, potential degradation and degradation rate of CP of *J. curcas*. The results obtained in this trial suggest that RS may be an appropriate treatment to render less digestible the protein of *J. curcas* in the rumen. Further work is needed to evaluate the effect of heat treatment on the concentration of antinutritional factors in seeds of *J. curcas*. Trials involving the rate of growth of ruminant animals are necessary to assess the potential of *J. curcas* in animal production systems. The concentration of oil in the seeds in this experiment could be a potential source of energy for the ruminant. Additional work is needed to evaluate the *in situ* digestion of the cakes of *Jatropha*.

Acknowledgements

We are grateful to Mrs. Cynthia Rosado, Rosario Quijano and Beatriz Gutierrez for chemical analysis of *J. curcas*. J.A.R.L. acknowledge CONACYT-Mexico for granting a Ph.D. scholarship and for financial support through the project "Utilization de la pasta de *J. curcas* en la alimentacion de rumiantes (# 4057PB-9607)".

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4.3 Toxicity o

M. Trabi[1], G.M. Gi

[1]Institute of Biotechn
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Abstract

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Introduction

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4.3 Toxicity of *Jatropha curcas* Seeds

M. Trabi[1], G.M. Gübitz[1], W. Steiner[1], N. Foidl[2]

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Abstract

The seeds of *Jatropha curcas* L. contain up to 60 % oil with a fatty acid pattern similar to that of edible oils. The amino acid composition, the percentage of essential amino acids and the mineral content of the press cake can be compared to those of other seeds and press cakes used as fodder. Due to several different toxic principles including a lectin (curcin), phorbol esters, saponins, protease inhibitors and phytates neither the seeds nor the press cake nor the oil of *Jatropha curcas* can be used for human or animal nutrition.

Feeding experiments with fish were carried out to determine the toxicity of different fractions as well as the influence of heat and alkali treatment on the press cake. The heat treated seeds and seed meal showed less toxicity than the untreated material, whereas the toxicity of the alcoholic oil extract did not change after treatment with hot alkali.

Introduction

The name *Jatropha* is likely to derive from the Greek word "jatros" meaning doctor or healing. Especially in Africa and Asia almost all parts of *Jatropha curcas* L. including seeds, leaves, bark and roots are used in traditional medicine as well as for veterinary purposes. The latex for example is used to stop wounds from bleeding, whereas a decoction of the leaves is said to soothe the pain caused by rheumatism. The seeds as well as the oil are mainly used as purgative, but also to treat scabies, gout or dropsy [1].

Some of these medicinal aspects might be due to the different toxic components in the seeds of *Jatropha curcas*.

In 1913 Felke [2] isolated a lectin called curcin from the seeds of *Jatropha curcas*. Like many other lectins this toxic glycoprotein hinders the ribosomal protein synthesis causing death when administered in quantities of micrograms. In feeding experiments Böhme [3] determined the minimal lethal dose of *Jatropha* seeds for three different animal species (Tab. 1).

Tab. 1: Minimal lethal dose of *Jatropha curcas* seeds [2]

Animal	Amount of seeds fed		Estimated Curcin Intake	Death
	g/kg	g total	mg total	day
Sheep	7.4	67	460	9
Goat	1.5	8	55	12
Calf	3.0	36	248	12

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Until now about 60 phorbol esters have been isolated from circa twenty different plants, all belonging to the families *Euphorbiaceae* and *Thymelaeaceae* [4]. The irritant and cocarcinogenic effects of these esters are due to activation of protein kinase C (PKC), a key enzyme in cellular growth and signal transduction. The seed oil of *Jatropha curcas* contains besides DHPB [5] (Fig. 1) three other phorbol esters [6].

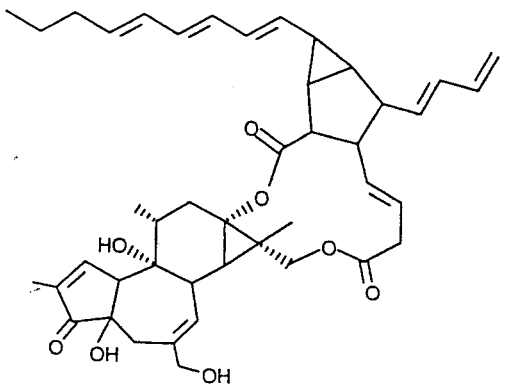

Fig. 1: DHPB (the 4'-[12',14'-butadienyl]-6'-[16',18',20'-nonatrienyl]-bicyclo [3.1.0]hexane-2'-[carboxylic acid]-3'-[8'-butenoic acid-10']diester of 12-deoxy-16-hydroxyphorbol), a phorbol ester isolated from the seeds of *Jatropha curcas* L. [4]

Further toxic or antinutritional components found in *Jatropha curcas* seeds include saponins, phytates, a trypsin inhibitor [7], a β-glucuronidase inhibitor, a xanthine oxidase inhibitor [8] as well as curcanoleic acid, a fatty acid similar to ricinoleic acid [2].

Although phorbol esters are said to be highly unstable and to auto-oxidise under normal storing conditions [9], they seem to be stabilised by some other components of the *Jatropha* oil. While most of the other toxic and antinutritional substances can be destroyed by heat treatment, phorbol esters seem to withstand even boiling of the oil.

The press cake remaining after the oil extraction is rich in crude protein (50 to 58 % depending on the residual oil) and could be used in human and animal nutrition - after appropriate detoxification. The oil of *Jatropha curcas* seeds contains a high percentage of unsaturated fatty acids (77.3 % for the Cabo Verde variety grown in Nicaragua [9]), the main fatty acids are oleic (44.7 %), linoleic (31.4 %) and palmitic acid (15.1 %) [10]. Thus the oil would make a high quality edible oil, if the toxin problem could be solved.

In this work feeding experiments with fish were carried out to determine the toxicity of the aqueous extract of the seeds, the residue of this extraction, the heat treated seed meal and the phorbol ester fraction in order to find a way to detoxify the oil as well as the press cake.

Materials and Meth

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Feeding experiment

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Materials and Methods

Seeds, press cake and raw oil

Two varieties of *Jatropha curcas* L. were examined. Both varieties, Nicaragua and Cabo Verde, were grown near León (Nicaragua). After harvesting, the seeds were sun dried and decorticated using a husker developed in our laboratories. The oil was expressed using an expeller.

Treatment of the raw oil

The raw oil was centrifuged (10 min at 25°C and 5000 rpm), heated to 70°C and mixed with the appropriate amount of 2M NaOH to derivatize the free fatty acids. After cooling and separation of the layers, the oil was centrifuged again and washed with distilled H_2O. Finally charcoal was added, the mixture was stirred for half an hour and filtered through glass wool resulting in a clear, yellow liquid.

Extraction of phorbol ester fractions

Ground seeds or press cake were stirred over night with the double amount (w/w) of methanol / water (3 : 1). The oil was extracted at least five times either with methanol or with methanol / water (3 : 1). When using only methanol the corresponding amount of water was added after the extraction. These solutions were extracted according to a modified version of the method described by Bauer et al. [11], shown in Fig. 2.

Aqueous extraction

The decorticated ground seeds were extracted with distilled H_2O for 24 hours at room temperature. The mixture was filtered, the residue dried at 70°C and the solution lyophilised using a Lyovac GT 2 system.

Heat treatment

The seed meal was degreased with petrol ether in a soxhlet extraction, dried, boiled with the double amount of water (w/w) for half an hour and washed several times with warm water.

NaOH treatment

The water extract or the phorbol ester fraction was stirred for half an hour with half the amount of hot NaOH solution (3 %).

Feeding experiments

Three different fish species were housed in two different aquariums and kept on commercial fish fodder for four days. After this time one group received the test diet, whereas the other (control) was continuously kept on commercial feed.

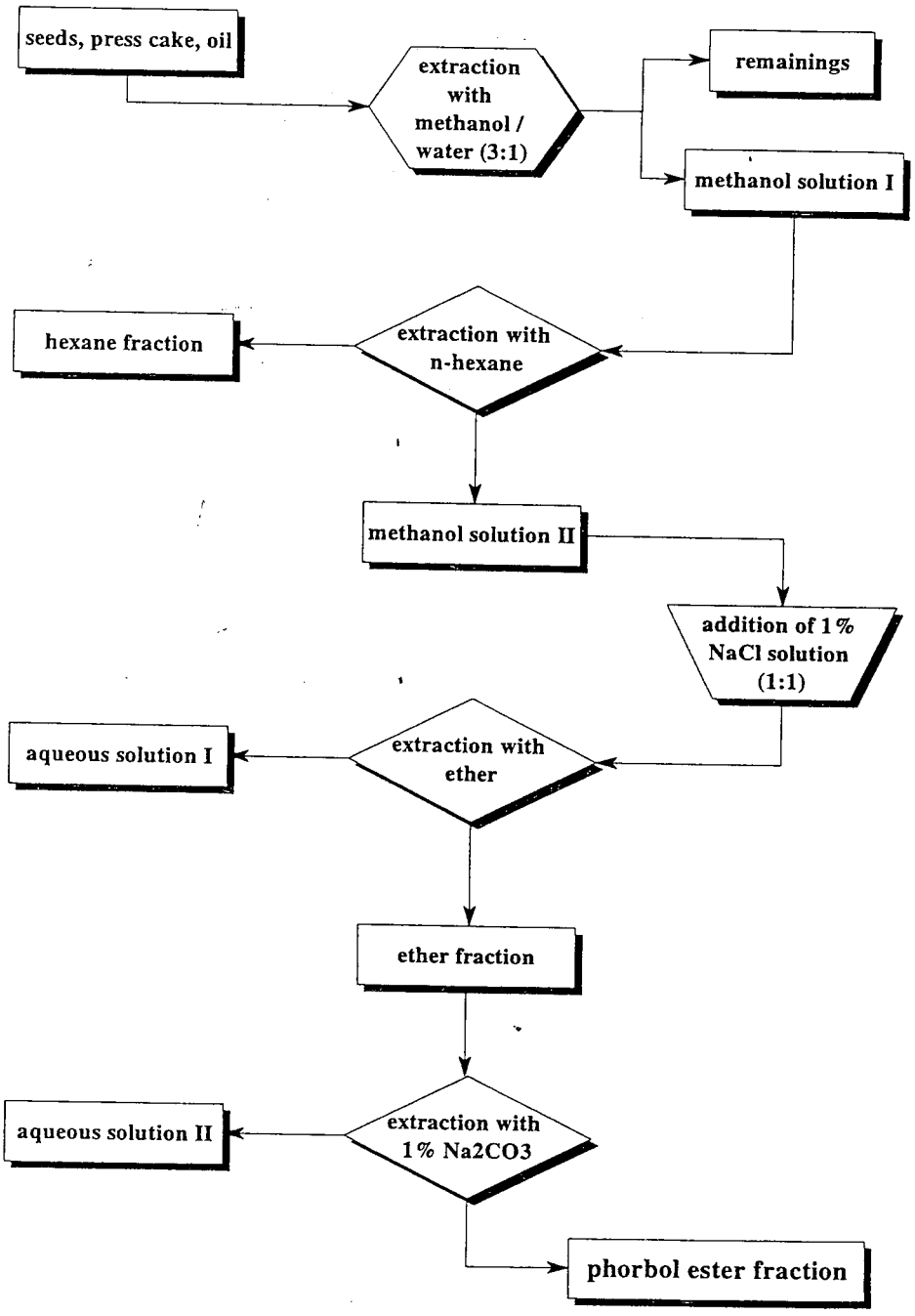

Fig. 2: Extraction scheme for the phorbol ester fractions

Results and Discussio

The fish fed with the intoxication. Nevertheless some substances giving the

Feeding of the freeze d showed loss of appetite, si the extract mortality was intoxication.

Moist heat treatment a reduced the number of d showed loss of appetite an

Feeding the fish with t signs of severe disorientat treatment had almost no ef

Tab. 2 shows a summar of *Jatropha curcas* seeds. are highly toxic to fish. Th with hot alkali, whereas th solution with hot alkali.

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Tab. 2: Sur *Jatropha cu*

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Conclusions

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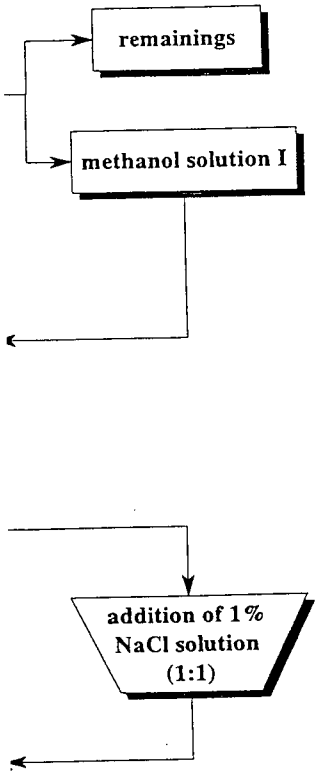

r fractions

Results and Discussion

The fish fed with the dried residue of the water extraction did not show any signs of intoxication. Nevertheless they seemed to highly dislike the residue's taste, which might be due to some substances giving the nut and the press cake a distasteful flavour.

Feeding of the freeze dried aqueous extract led to 100 % mortality within four days. The fish showed loss of appetite, signs of disorientation and lesions of the skin. After NaOH treatment of the extract mortality was only 30 %. Nevertheless, the surviving fish showed slight signs of intoxication.

Moist heat treatment and thoroughly washing of the degreased seed meal with warm water reduced the number of deaths occurring in the test group to 50 %. Again the surviving fish showed loss of appetite and slight disorientation.

Feeding the fish with the phorbol ester fraction they died within the first two hours showing signs of severe disorientation and alternating phases of hyperactivity and immobility. Hot NaOH treatment had almost no effect on the toxicity of this fraction.

Tab. 2 shows a summary of the feeding experiment results obtained with the different fractions of *Jatropha curcas* seeds. It can be assumed that the phorbol ester fraction and thus the seed oil are highly toxic to fish. The toxicity of the phorbol ester fraction cannot be destroyed by treatment with hot alkali, whereas the toxic principle of the aqueous extract can be destroyed by treating the solution with hot alkali.

The toxicity of the degreased press cake can be reduced by moist heat treatment and washing with water. Further experiments have to be carried out, whether the residues of the seed oil or the not completely destroyed lectin cause the remaining toxic effects.

Tab. 2: Summary of feeding trial results obtained with different fractions of *Jatropha curcas* seeds

Fraction	fish	dead	
	#	#	%
aqueous extraction residue (seeds)	5	0	0
aqueous extract (seeds)	5	5	100
aqueous extract, NaOH - treated (seeds)	3	1	33
seed meal, moist heat	4	2	50
phorbol ester (PE) fraction	4	4	100
PE fraction, NaOH - treated	5	4	80

Conclusions

The feeding experiments showed, that there is still a long way to go before the oil can be used as edible oil or the press cake can be utilised in human or animal nutrition. Obviously the lectin is destroyed during expression of the seed oil. The phorbol esters seem to be highly stabilised, so neither the temperatures nor the pressure common in press processes can destroy these compounds. Thus not only the oil is toxic, but also the press cake itself, due to a certain percentage of not expressed oil. Extraction of the phorbol esters with methanol can not be seen as a practical solution, not only because of the problematic use of methanol in technical processes, but mainly because of the vast number of extraction steps necessary to remove the phorbol esters completely.

Acknowledgements

This work was supported by Sucher & Holzer and the Austrian Government.

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4.4 Detoxificatio Experiments

H. Gross, G. Foidl, N

[1]Sucher & Holzer Austr Nicaragua

Abstract

At laboratory scale (to remove curcin an Feeding fish on heat additional extraction concentrate containing the ethanol extracted Feeding neutralised *J.* not provoke any signs

Introducción

La *Jatropha curca* puede servir, por su La harina de *J. curca* ser previamente dest componentes tóxicos contiene mayormente emplear solamente, sí

Por tanto se hace s y un tratamiento adec

Material y Método

La torta residual s manejo en las etapas

En un recipiente a un tiempo de cocción Luego la masa húme liofilizador. Una vez consistencia porosa. de la torta, mediante guardando la relación 74°C y una vez seca, en las fórmulas alime de acuarios.

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4.4 Detoxification of *J. curcas* Press Cake and Oil and Feeding Experiments on Fish and Mice

H. Gross, G. Foidl, N. Foidl

[1]Sucher & Holzer Austria / Universidad Nacional de Ingenieria, Departamento de Biomasa, Managua, Nicaragua

Abstract

At laboratory scale detoxification treatments were carried out on *J. curcas* press cake and oil (to remove curcin and phorbolic esters), followed by feeding experiments on fish and mice. Feeding fish on heat-treated only press cake, resulted in 100 % mortality, however after an additional extraction with ethanol (92 %) they grew without problems. When feeding mice on a concentrate containing *J. curcas* meal as protein source, the best results also were obtained with the ethanol extracted meal, but the mice were growing slower than the control group fed on soya. Feeding neutralised *J. curcas* oil lead to 100 % mortality, whereas the ethanol extracted one did not provoke any signs of intoxication.

Introducción

La *Jatropha curcas*, además de ser una fuente de aceite, genera también una harina la cual puede servir, por su alto contenido nutritivo, como suplemento proteínico en nutrición animal. La harina de *J. curcas* no puede emplearse como un componente en alimentos para animales sin ser previamente destoxificada, ya que contiene varias substancias tóxicas. Los principales componentes tóxicos de la harina son: la curcina, una toxoalbúmina y ésteres forbólicos. El aceite contiene mayormente ésteres forbólicos, es decir la harina y el aceite de *J. curcas* se pueden emplear solamente, sí las toxinas que estos contienen logran ser eliminadas.

Por tanto se hace sumamente necesario, determinar un proceso de destoxificación de la harina y un tratamiento adecuado al aceite, a fin de garantizar la eliminación del contenido de toxinas.

Material y Método

La torta residual se hace pasar por un molino, para así obtener, una harina fina y de mejor manejo en las etapas de la destoxificación.

En un recipiente a presión (pressure cooker), con una relación de harina-agua de 1 : 10 y con un tiempo de cocción igual a 1.5 horas, se efectúa la primer etapa con el fin de destruir la curcina. Luego la masa húmeda obtenida, se congela para realizarle un posterior secado en frío en el liofilizador. Una vez seca completamente la torta, se observa de un color amarillento y de consistencia porosa. Empleando una relación 1 : 10 de harina-etanol (92 %), se realiza el lavado de la torta, mediante agitación y centrifugado, siendo necesarios como mínimo 4 lavados y guardando la relación harina - alcohol. Como etapa final se coloca en el horno para ser secada a 74°C y una vez seca, se encuentra lista para ser integrada como el componente rico en proteínas, en las fórmulas alimenticias para ratones blancos y mezclarse con alimento comercial para peces de acuarios.

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En los ensayos se emplean, peces como indicadores y ratones blancos para los diferentes ensayos, y éstos se observan separadamente de acuerdo al tratamiento efectuado a la harina y al aceite de *J. curcas*.

Tab. 1: Formulación alimenticia para ratones blancos

	Grupo testigo	Grupo de ensayo
harina de soya	21.80 %	19.00 %
vitaminas y minerales	9.00 %	9.00 %
aceite de maíz	8.00 %	8.00 %
almidones	61.20 %	64.00 %
	Grupo aceite *J. curcas* neutralizado	Grupo aceite *J. curcas* neutralizado-lavado
harina de soya	21.80 %	21.80 %
vitaminas y minerales	9.00 %	9.00 %
aceite neutralizado	8.00 %	8.00 %
almidones	61.20 %	61.20 %

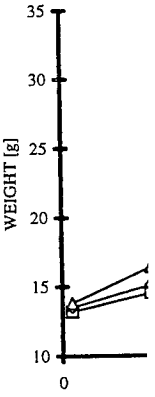
En las últimas fórmulas se mantienen los porcentajes, pero se sustituye al aceite de maíz por aceite de *J. curcas*, al cual se le realizan diferentes tratamientos. Los ratones blancos, empleados como sujetos de ensayo, se adquieren en tiendas del mercado local.

Resultados y Discusión

Para controlar el grado de destoxificación de la harina de *J. curcas*, se usaron inicialmente peces como indicadores. Estos se alimentaron con una mezcla de harina, previamente calentada y alimento comercial para peces, en proporciones 50 : 50 y el resultado fue 100 % de mortalidad en un total de cuatro días. Cuando la harina fue extraída con alcohol de 80 %, se obtuvo un 60 % de mortalidad en un período de ocho a doce días. Sin embargo, al usar harina extraída con alcohol de 92 %, los peces se alimentaron, crecieron y reprodujeron sin problemas.

Paralelamente, se alimentaron ratones blancos con una fórmula de concentrado que contiene, harina de *J. curcas* como fuente de proteínas. Los mejores resultados se obtienen de la harina extraída con alcohol de 92 %. Obsérvese la Fig. 1, en la primera etapa del ensayo, es decir, durante los primeros 25 días aproximadamente, en la fase de crecimiento primario; las diferencias entre el grupo control y los diferentes ensayos es mínima. No así, en la siguiente fase del experimento, donde el peso adquirido por los ratones alimentados con harina de *J. curcas* extraída con etanol de 80 %, muestran mayor diferencia que el grupo alimentado con harina extraída con etanol de 92 %, con respecto al grupo control. Estas diferencias se obedecen, al hecho de que, en la harina tratada con etanol de 80 %, la cantidad aún presente de ésteres forbólicos es mayor, que la que se encuentra en la harina tratada con etanol de 92 %, es decir para la remoción de estos ésteres forbólicos se debe emplear etanol con alta concentración alcohólica.

Los ratones alimentados con fórmula concentrada, conteniendo aceite de *J. curcas* neutralizado, perdieron rápidamente peso y todos murieron en un total de diez días.



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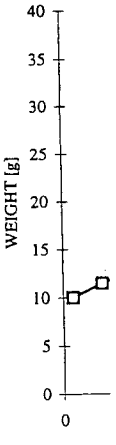

Fig. 2: Experiments

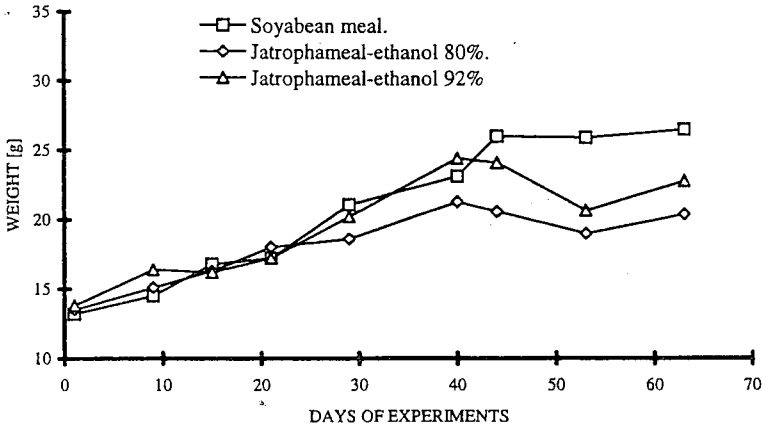

Fig. 1: Experiments with *Jatropha* meal extracted with ethanol of (80 %) and (92 %)

El grupo ensayo 1 que se presenta en la Fig. 2, muestra menor desarrollo que el grupo ensayo 2, nótese también que éste último no refleja una diferencia significativa comparado con el grupo control. Este desarrollo nouniforme puede ser debido a que los ratones empleados en los diferentes ensayos, no proceden de familias puras.

Los ratones alimentados con fórmula conteniendo aceite de *J. curcas* neutralizado y extraído con alcohol de 92 %, crecieron normalmente. Como se observa en la Fig. 2.

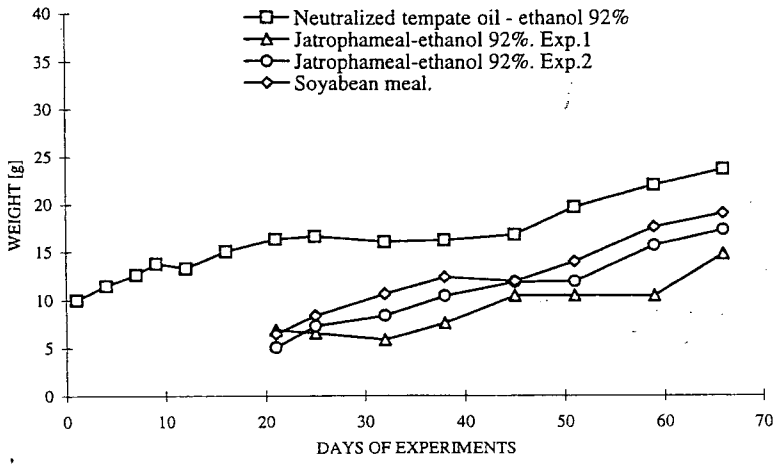

Fig. 2: Experiments with neutralized *J. curcas* oil and *Jatropha* meal extracted with ethanol (92 %)

Conclusiones

Al aplicar a la harina de *J. curcas* mezclada con agua, solamente tratamiento a temperaturas de ebullición, no se logra destruir el efecto nocivo de los componentes tóxicos. El procedimiento óptimo de destoxificación de la harina de *J. curcas* es:

Molino → Cocción (Pressure cooker), 1.5 horas → Congelamiento → Liofilización → Extracción con etanol (92 %) → Centrifugación → Horno T = 74°C.

El desarrollo y crecimiento de los ratones se ve poco afectado, como lo muestra la Fig. 1, si en la extracción se emplea etanol de 92 % en comparación con el grupo control. Al aceite de *J. curcas* que recibe un tratamiento de neutralizado, aunque se logra disminuirle el grado de toxicidad, el contenido residual de componentes tóxicos, se determinó es letal al emplearse este aceite tratado, como componente alimenticio integrándole al concentrado para los ratones.

El aceite de *J. curcas* neutralizado y extraído con etanol de 92 % y posteriormente empleado en la fórmula alimenticia, no genera ningún tipo de complicación en el desarrollo de los ratones, ya que estos crecen normalmente. De ésta manera se logra remover hasta un 98 % del contenido de toxinas. Ver Fig. 2.

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