

# Biofuels and Industrial Products from *Jatropha curcas*

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## 2.2 Lectin Activity in Toxic and Non-Toxic Varieties of *J. Curcas* Using a Latex Agglutination Test

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### Abstract

Lectin activity in toxic and non-toxic varieties of *Jatropha curcas* seed meal was investigated using a latex agglutination method. The glycoprotein (ovalbumin) was adsorbed onto the latex beads. The agglutination test was carried out in the presence of Ca<sup>2+</sup>, Mn<sup>2+</sup> and Mg<sup>2+</sup> ions. Mn<sup>2+</sup> ion was found to be the best. A concentration of 0.286 mM of Mn<sup>2+</sup> ion was maintained in the assay mixture. Lectin activity when expressed as reciprocal of the minimum quantity (in mg) of *Jatropha* meal per ml of the assay mixture which produced agglutination was  $2.88 \pm 0.57$  and  $1.71 \pm 0.00$  (mean  $\pm$  sd) for the toxic and the non-toxic varieties respectively, which did not differ significantly ( $P > 0.05$ ). Both toxic and non-toxic varieties were subjected to heat treatments: i) moist heat (MH) treatment (66 % moisture) at 100°C and 121°C for 20, 40 and 60 min and 10, 20, 30 and 40 min respectively, and ii) dry heat (DH) treatment at 130 and 160°C for 20, 40 and 60 min. The MH treatment at 100°C and DH treatments at 130°C and 160°C for 60 min did not inactivate lectin in both varieties. Agglutination of the latex beads was observed at 10 and 20 min of MH treatment at 121°C, however agglutination was not observed after 30 min. These results suggest that in *J. curcas* meal: i) MH treatment is more effective than DH treatment in inactivating lectins, ii) lectins can be completely inactivated using MH treatment at 121°C for 30 min, and iii) lectins probably are not the toxic principle in *Jatropha* meal.

### Introduction

*Jatropha curcas*, a multipurpose tree of significant economic importance belongs to the Euphorbiaceae family. The plant is widely distributed in wild or semi-cultivated in Central and South America, Africa, India and South East Asia [8, 9]. The seed weighs about 0.75 g and the kernel represents about 65 % of the seed weight. Reports on the chemical composition of the kernel revealed protein and lipid contents of 27 - 32 % and 58 - 60 % respectively [15, 2]. Besides being a source of oil *Jatropha* also provides a meal which may serve as a highly nutritious and economical protein supplement in animal feed if the toxins are removed [6]. *Jatropha* meal (fully degreased) has a protein content of between 53 - 58 % CP. The protein content of the seeds and cake therefore shows potential nutritional value of the seeds to man and livestock.

Several studies with animals [1, 4, 5, 11, 10, 15] have shown that the seeds are toxic. The meal has also been found to be toxic to fish [6]. This has therefore restricted its use as a food or feed source. The toxic and / or irritant compounds isolated so far from *Jatropha* seeds include  $\beta$ -D-glucosides of sitosterol [7], curcin [12, 17, 20, 21] flavonoids vitexine and isovitexine [19] and the 12-deoxyl-16-hydroxyphorbol [3]. The toxicity of *J. curcas* is considered to be caused by a lectin - curcin [9, 12, 20].

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Hitherto, studies carried out on lectins of *Jatropha* [2, 9, 12] have used one or another haemagglutination assay. This study attempts for the first time to use the latex agglutination test [13] to detect lectin activity of *Jatropha* meal. The objectives of this study are to quantify lectin activity in toxic and non-toxic varieties of *J. curcas* using the latex agglutination method and to determine heat treatment conditions for inactivation of lectins.

## Materials and Methods

### *Jatropha* meal samples

*Jatropha* meal samples from the toxic (Cape Verde) and non-toxic (Mexico) varieties were used for the study. The chemical composition of these two varieties is available in the report of [6].

### Heat treatment

**Moist Heat (MH) treatment.** Meal samples (4 g) of both varieties were weighed (in duplicates) into 50 ml capacity beakers and 8 ml distilled water was added to bring the moisture level to 66 %. The contents were made into a paste using a glass rod. The beakers were covered with aluminium foil and placed in a waterbath (100°C), or in an autoclave (121°C). The treatment times were 20, 40 and 60 min at 100°C and 10, 20, 30 and 40 min at 121°C. The samples were allowed to cool in a dessicator, placed in a freezer (6 h) and freeze-dried.

**Dry Heat (DH) treatment.** Meal samples (4 g) of both varieties were weighed into beakers and subjected to heat treatment at 130°C and 160°C for 20, 40 and 60 min in an oven.

### Latex beads preparation

The method of [13] was used to adsorb ovalbumin onto latex beads.

### Preparation of meal extract for the agglutination assay

The meal (0.5 g) was weighed in a 50 ml capacity polypropylene centrifuge tube (Greiner Labortechnik, Solingen, Germany) and then 10 ml of 0.9 % NaCl solution were added. The content was homogenised using an ultra-turrax (20,000 rpm) for 5 min (2 × 2.5 min) with intermittent cooling by keeping the tube in an iced water bath. The tubes were then centrifuged at 3,500 × g for 10 min, collected the supernatants into eppendorf cups, and centrifuged a second time at 9500 × g for 5 min. The supernatants were collected.

### Latex agglutination assay

In round bottom wells of microtiter plates (Greiner Labortechnik, Solingen, Germany) 15 µl of the latex beads were mixed with an equal volume of the meal extract. The plates were gently shaken at room temperature for 2 - 4 h. The sedimentation pattern which indicated agglutination of the beads was a uniform circular clump at the bottom of the well, while a negative pattern (indicating no agglutination) was a suspended form similar to that in the blank.

The agglutination test was also carried out in the presence of Ca<sup>2+</sup>, Mn<sup>2+</sup> and Mg<sup>2+</sup> ions at a concentration of 0.286 mM. The latex agglutination assay finally used for studies reported in this communication was comprised of: 10 µl of Mn<sup>2+</sup> ions (1.15 mM to give a final concentration of -0.286 mM in the assay) added first to the microtiter plate, followed by 15 µl of the latex beads and finally 15 µl of the meal extract. The plates were treated in a manner mentioned above and read after 2 - 4 h. Lectin activity was expressed as reciprocal of the minimum quantity (in mg) of *J. curcas* meal per ml of the assay which produced agglutination. All data obtained were analysed statistically using the Studentized t-test [22].

## Results and Discussion

In this study the glycoprotein ovalbumin was used for coating the latex beads by physical adsorption. This protein was chosen because of its low cost and easy availability. Feeding studies conducted in our laboratory using rats and fish have established that the meal from the seeds obtained from Mexico is non-toxic and that from Cape Verde is toxic [6]. For both the toxic (Cape Verde) and non-toxic (Mexico) meals agglutination was not observed at a concentration of 18.75 mg/ml of the assay. When the assay was carried out in the presence of 0.286 mM of  $\text{Ca}^{2+}$  or  $\text{Mn}^{2+}$  or  $\text{Mg}^{2+}$  ions, agglutination was observed in the toxic and non-toxic varieties (Tab. 1). Amongst the three ions tested,  $\text{Mn}^{2+}$  was found to have the highest affinity and therefore was used in further assays. The lectin activity was  $2.88 \pm 0.57$  and  $1.71 \pm 0.00$  (means  $\pm$  sd) for the toxic and non-toxic varieties, respectively, and this did not differ significantly ( $P > 0.05$ ).

Tab. 1: Lectin activity of toxic and non-toxic *J. curcas* meal in presence of 0.286 mM ions ( $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Mg}^{2+}$ )

| Metal ions       | Lectin activity*   |                    |
|------------------|--------------------|--------------------|
|                  | Toxic (Cape Verde) | Non-toxic (Mexico) |
| $\text{Ca}^{2+}$ | 0.43               | 0.43               |
| $\text{Mn}^{2+}$ | 1.71               | 1.71               |
| $\text{Mg}^{2+}$ | 0.11               | 0.11               |

\*Expressed as reciprocal of the minimum quantity (in mg) of *J. curcas* meal per ml of the assay which produced agglutination.

In the heat treated samples, results showed that MH treatment at 100°C for 20, 40 and 60 min and DH treatment at 130°C and 160°C for 20, 40 and 60 min did not decrease the activity of lectin in both the toxic and non-toxic varieties (results not shown). However, for samples obtained after MH treatment at 121°C for 10, 20, 30 and 40 min agglutination was observed at 10 and 20 min. Lectin was inactivated with the MH treatment at 121°C for 30 min for both the varieties (Tab. 2).

Tab. 2: Lectin activity in the moist heat (MH) treated *Jatropha* meal samples (66 % moisture, 121°C)

| Time (min) | Lectin activity*   |                    |
|------------|--------------------|--------------------|
|            | Toxic (Cape Verde) | Non-toxic (Mexico) |
| 0          | 2.88               | 1.71               |
| 10         | 0.43               | 0.43               |
| 20         | 0.43               | 0.43               |
| 30         | nd                 | nd                 |
| 40         | nd                 | nd                 |

nd - not detected;

\* expressed as reciprocal of the minimum quantity (in mg) of *J. curcas* meal per ml of the assay which produced agglutination

Lectins are known to be heat labile and their activity could decrease by heat treatment [16]. In an earlier study Aderibigbe et al. [2] found increase in lectin activity following heat treatment which was attributed to some artefacts. It may be noted that in that study metal ions were not used. In the presence of metal ions ( $Mn^{2+}$ ) the artefacts seem to disappear.

The results suggest that lectin activity is similar in both toxic and non-toxic varieties and therefore toxicity of *J. curcas* meal cannot be attributed to lectin. Presence of lectin might aggravate the toxicity. The MH treatments are more effective than DH treatments in inactivating lectins of *J. curcas* meals. Lectins of both toxic and non-toxic varieties can be completely inactivated by MH treatment (66 % moisture, 121°C for 30 min).

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